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Inward-rectifier K^+ Current in Guinea-pig Ventricular Myocytes Exposed to Hyperosmotic Solutions

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Abstract. Superfusion of heart cells with hyperosmotic solution causes cell shrinkage and inhibition of membrane ionic currents, including delayed-rectifer K^+ currents. To determine whether osmotic shrinkage also inhibits inwardly-rectifying K^+ current (I_{K1}), guinea-pig ventricular myocytes in the perforated-patch or ruptured-patch configuration were superfused with a Tyrode's solution whose osmolarity (T) relative to isosmotic ($1T$) solution was increased to 1.3–2.2T by addition of sucrose. Hyperosmotic superfusate caused a rapid shrinkage that was accompanied by a negative shift in the reversal potential of Ba^{2+}-sensitive I_{K1}, an increase in the amplitude of outward I_{K1}, and a steepening of the slope of the inward I_{K1}-voltage (V) relation. The magnitude of these effects increased with external osmolarity. To evaluate the underlying changes in chord conductance (G_{K1}) and rectification, G_{K1}-V data were fitted with Boltzmann functions to determine maximal G_{K1} (G_{K1}max) and voltage at one-half G_{K1}max ($V_{0.5}$). Superfusion with hyperosmotic sucrose solutions led to significant increases in G_{K1}max (e.g., 28 ± 2% with 1.8T), and significant negative shifts in $V_{0.5}$ (e.g., −6.7 ± 0.6 mV with 1.8T). Data from myocytes investigated under hyperosmotic conditions that do not induce shrinkage indicate that G_{K1}max and $V_{0.5}$ were insensitive to hyperosmotic stress per se but sensitive to elevation of intracellular K^+. We conclude that the effects of hyperosmotic sucrose solutions on I_{K1} are related to shrinkage-induced concentrating of intracellular K^+.

Key words: Cell volume — Cardiac I_{K1} — K^+ conductance — Dimethylsulfoxide — Intracellular K^+

Introduction

Superfusion of heart cells with anisosmotic solution induces either a loss or a gain of cell water, with resultant changes in the concentrations of intracellular ions and activities of membrane transporters (Fozzard & Lee, 1976; Allen & Smith, 1987; Drewnowska & Baumgarten, 1991; Rasmusson, Davis & Lieberman, 1993; Whalley et al., 1993; Wright et al., 1995; Ogura, You & McDonald, 1997). Based on observations from noncardiac cells, it seems likely that changes in heart cell volume also affect the cytoskeleton and intracellular enzymatic pathways (Sarkadi & Parker, 1991; McCarty & O'Neil, 1992; Nelson et al., 1996; Lang et al., 1998; Kapus et al., 1999; Pedersen, Mills & Hoffmann, 1999). Consequently, it is not surprising that superfusion with anisosmotic solutions affects membrane currents in heart cells. In general, hyposmotic superfusate has stimulatory effects, including activation/stimulation of Cl^- current (I_{Cl}) (Hume et al., 2000), non-selective cation current (Kim & Fu, 1993), ATP-sensitive K^+ current (Van Wagoner, 1993), inwardly-rectifying cation current (Clemo & Baumgarten, 1997), Ca^{2+} current (I_{Ca}) (Matsuda et al., 1996; Pascarel, Brette & Le Guennec, 2001), and slow delayed-rectifier K^+ current (I_{Ks}) (Rees et al., 1995; Kocic, Hirano & Hiraoka, 2001). Conversely, hyperosmotic solution causes deactivation/inhibition of I_{Cl} (Clemo, Stambler & Baumgarten, 1999), I_{Ca} (Ogura et al., 1997), I_{Ks} (Ogura et al., 2003) and rapid delayed-rectifier K^+ current (I_{Kr}) (Ogura et al., 2003).

An interesting aspect of the foregoing findings on the effects of cell volume on delayed-rectifier

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K^+ currents is the apparent lack of correlation between changes in intracellular K^+ and changes in current magnitudes, i.e., cell swelling (and consequent dilution of intracellular K^+) stimulates the currents, whereas cell shrinkage (and consequent concentrating of intracellular K^+) inhibits them. Whether changes in cell volume have qualitatively similar effects on classical inward-rectifying K^+ current (I_{K1}) is unclear. This current, which is important for setting the resting potential, promoting repolarization, and modulating the excitability of cardiac cells (Noble, 1979; Ibarra, Morley & Delmar, 1991; Lopatin & Nichols, 2001), was little affected by osmotic swelling of guinea-pig (Sasaki, Mitsuiye & Noma, 1992; Sasaki et al., 1994; Rees et al, 1995) and rabbit (Clemo & Baumgarten, 1997) ventricular myocytes. However, the consequences of osmotic shrinkage have not been reported.

The objective of the present study was to evaluate whether I_{K1} is modified when guinea-pig ventricular myocytes are superfused with solutions whose osmolarity relative to standard isosmotic solution (1*T*) ranged from 1.3*T* to 2.2*T*. The results indicate that hyperosmotic superfusion has stimulatory effects that appear to be primarily mediated by shrinkage-induced elevation of intracellular K^+ concentration ($[K^+]_i$).

Materials and Methods

Myocyte Preparation

Adult guinea-pigs (250–300 g) were killed by cervical dislocation in accord with national and local regulations on animal experimentation. Hearts were quickly removed, mounted on a Langendorff column, and perfused (37°C) through the coronary artery for 10–15 min. The perfusate contained (in mM) NaCl 140, KCl 5, $MgCl_2$ 1.2, glucose 10, and *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) 5 (pH 7.4), as well as 0.08–0.12 mg/ml of collagenase (Yakult Pharmaceutical Co., Tokyo, Japan). Myocytes were dispersed in a high-K^+, nutrient-supplemented storage solution that contained KOH 80, KCl 30, KH_2PO_4 30, $MgSO_4$ 3, glutamic acid 50, taurine 20, glucose 20, ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (EGTA) 0.5, and HEPES 10 (pH 7.4 with KOH), and stored at 22°C. For experiments, a few drops of the cell suspension were placed in a 0.3-ml chamber mounted on the stage of an inverted phase-contrast microscope, and the chamber was perfused with a cell bathing solution (*see below*) at a flow rate near 3 ml/min. Only rod-shaped quiescent cells with smooth contours were selected for study.

Myocyte Volume

Cell images were recorded on videotape using a television system (series 67 camera system, Dagan Corp., Minneapolis, MN), and cell width and length were measured from replayed images as described previously (Ogura et al., 1997); the measurements were reproducible to <1%. Cell volume was calculated as length × (width)^2 on the assumption that there are proportional changes in cell width and thickness under anisosmotic conditions (Drewnowska & Baumgarten, 1991; Ogura et al., 1997).

Electrophysiology

Myocytes were voltage-clamped using either the nystatin perforated-patch method or the standard ruptured-patch method. Recording pipettes were fabricated from thick-walled borosilicate glass capillaries (H15/10/137, Jencons Scientific Ltd., Leighton Buzzard, UK), and had resistances of 2–3 MΩ when filled with dialysate. Liquid junction potentials between external and pipette-filling solutions were offset before formation of a gigaohm seal. In the perforated-patch experiments, series resistance (R_S) was monitored after seal formation. R_S declined to 8–25 MΩ within 10–20 min of seal formation, and experiments were initiated when R_S was stable over a 10-min period. R_S compensation was used in most of the experiments such that uncompensated resistance was reduced below 10 MΩ (typically 2–6 MΩ). In the ruptured-patch experiments, R_S ranged between 4–8 MΩ and was compensated by 60–80%. Membrane currents were recorded with an EPC-7 amplifier (Heka Elektronik, Lambrecht, Pfalz, Germany). The electrical signals were low-pass filtered at 3 kHz, and digitized with an A/D converter (Digidata 1200A, Axon Instruments, Foster City, CA) and pCLAMP software (Axon Instruments) at a sampling rate of 8–10 kHz prior to analysis. All experiments were conducted at 36°C.

Superfusates and Pipette Solutions

Isosmotic 1*T* solution contained (in mM) either NaCl 140 or N-methyl-D-glucamine (NMDG) Cl 140, KC1 5, $CaCl_2$ 1.8, $MgCl_2$ 1.2, glucose 10, and HEPES 5 (pH 7.4; ≈ 297 mOsmol/kg), as well as 1 mM Cd^{2+} to suppress Ca^{2+} and Na^+ currents, 3 μM E4031 (N-[4-[[1-[2-(6-methyl-2-pyridinil)ethyl]-4-piperidinyl]carbonyl]phenyl] methanesulfonamide dihydrochloride dihydrate) (Eisai, Tokyo, Japan) to suppress I_{Kr}, and 3 μM glibenclamide (Sigma-Aldrich, Oakville, ON, Canada) to suppress any ATP-sensitive K^+ current. Hyperosmotic solutions were made by adding appropriate amounts of sucrose to 1*T* solution, while maintaining 1*T*-constituent concentrations constant. Inclusion of 20 mM Cs^+ in some solutions was compensated by removal of 20 mM Na^+ or $NMDG^+$. Hyperosmotic DMSO solution was prepared by substituting dimethyl sulfoxide for 2% of the water in IT solution.

Pipettes used in the perforated-patch experiments were filled with solution that contained (in mM) KCl 30, potassium aspartate 110, $MgCl_2$ 5, and HEPES 5 (pH 7.2 with KOH). Nystatin (Sigma Chemical St. Louis, MO) was added to the pipette-filling solution from a stock solution (100 mg/ml in DMSO) to give a final concentration of 50–100 μg/ml. The nystatin stock solution was freshly prepared and used for up to 2 h. In the ruptured-patch experiments, the standard pipette solution contained (in mM) KC1 30, potassium aspartate 110, MgATP 5, EGTA 5, and HEPES 5 (pH 7.2 with KOH). The osmolalities of these solutions ranged between 288 and 300 mosmol/kg H_2O. High-K^+ pipette solution was prepared by addition of 140 mM potassium aspartate to standard pipette solution.

Statistics

Statistical data are expressed as means ± SEM; *n* represents the number of experiments. Comparisons were made by Student's *t*-test. Differences were considered significant when $P < 0.05$.

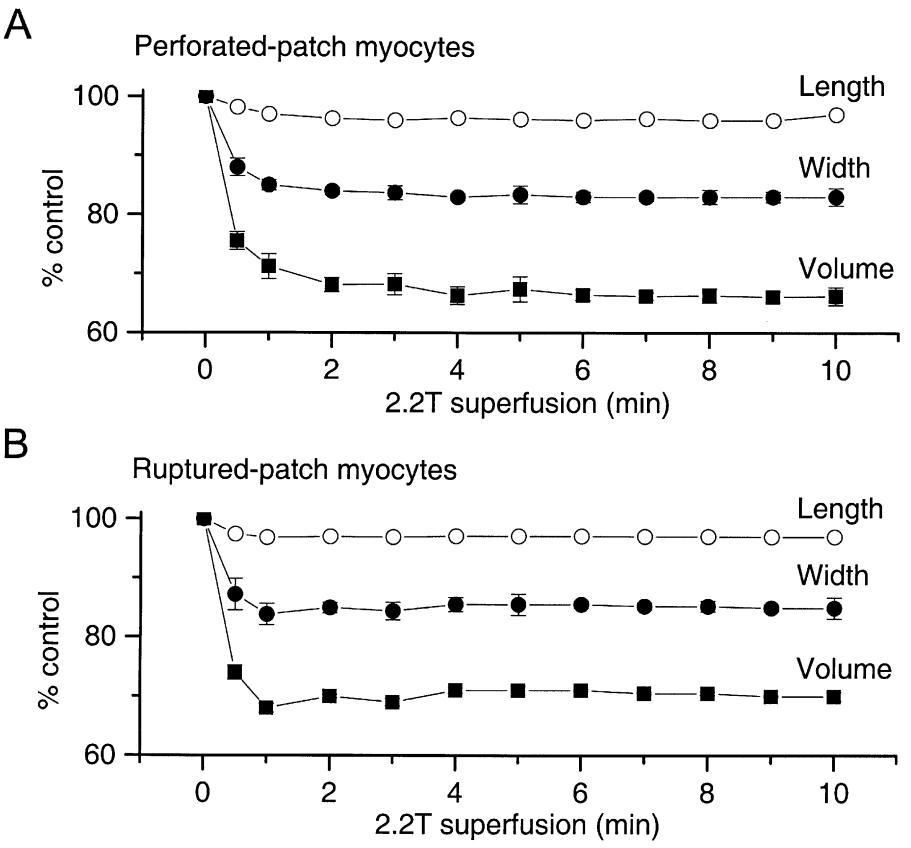

Fig. 1. Effects of hyperosmotic 2.2*T* superfusate on the dimensions and shell volumes of patched myocytes. The myocytes were depolarized from −90 mV to 0 mV for 200 ms at 0.2 Hz during supervision with control (1*T*) solution and then 2.2*T* solution for 10 min. Percentage changes in volume were calculated from percentage changes in length and width as explained under Methods. (*A*) Data from perforated-patch myocytes ($n = 8$). (*B*) Data from ruptured-patch myocytes ($n = 8$). Most of the SEM bars are hidden by the symbols.

Results

Shrinkage of Myocytes Exposed to Hyperosmotic Sucrose Solution

To determine the effects of hyperosmotic sucrose solution on the volume of myocytes configured for either perforated-patch or ruptured-patch voltage clamp, we estimated cell volume as length × (width)^2 from measurements of cell surface dimensions in accord with earlier studies (Roos, 1986; Drewnowska & Baumgarten, 1991; Ogura, Shuba & McDonald, 1995). There was little change ($\leq 2\%$) in volume when patched myocytes were superfused with control 1*T* solution for up to 30 min ($n = 10$ for each method) (*data not shown*), but rapid shrinkage when 1*T* solution was replaced by hyperosmotic solution. For example, exposure of perforated-patch myocytes to 2.2*T* solution caused a shrinkage to 67 ± 2% of control volume within 2 min ($n = 8$), primarily due to a decline in cell width (Fig. 1*A*). There was little further change over the next 8 min, consistent with the absence of a significant regulatory volume increase in mammalian ventricular myocytes (Suleymanian & Baumgarten, 1996; Sasaki et al., 1999). Similar results were obtained in experiments on ruptured-patch myocytes where exposure to 2.2*T* solution reduced cell volume to 69 ± 1% and 70 ± 2% ($n = 8$) of control volume after 2 and 10 min, respectively (Fig. 1*B*). Predictably, shrinkage was less severe during exposures to weaker hyperosmotic solutions (e.g., ruptured-patch myocytes: 81 ± 2% ($n = 8$) of control volume after 5 min in 1.5*T* solution).

I_{K1} in Perforated-Patch Myocytes Exposed to Hyperosmotic Sucrose Solution

The data in Fig. 2 provide an overview of the effects of hyperosmotic solutions on membrane currents in perforated-patch myocytes. Successive 5-min exposures to 1.5*T* and 2.2*T* solutions had a number of reversible effects on late current-voltage relationships over the range −110 to +40 mV (Fig. 2*A*). At positive potentials, there was marked inhibition of time-dependent I_{KS} (*see* inset), in agreement with earlier studies; at negative potentials, there were osmolarity-dependent (2.2*T* > 1.5*T*) increases in the amplitude of outward current, and negative shifts in the apparent reversal potential (E_{rev}). In five experiments of this type, the outward current at −60 mV increased by 35 ± 5% (1.5*T*) and 63 ± 9% (2.2*T*), and E_{rev} shifted by −4.1 ± 0.3 (1.5*T*) and −7.5 ± 0.7 mV (2.2*T*) ($P < 0.05$ versus 1*T* for all values).

It seemed quite likely that the hyperosmotic-induced increases in outward current at potentials between E_{rev} and −20 mV (Fig. 2*A*) were due to increases in the amplitude of inwardly-rectifying I_{K1}. Firstly,

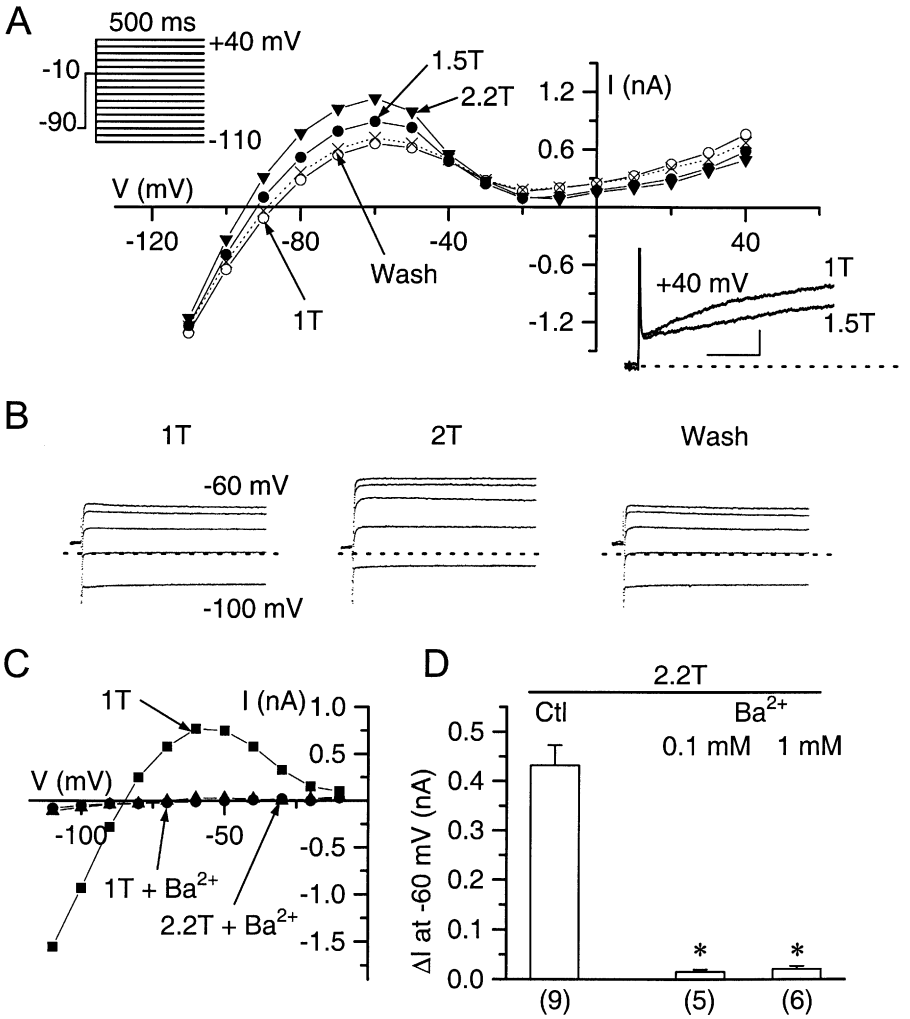

Fig. 2. Reversible effects of hyperosmotic solutions on membrane currents in perforated-patch myocytes. (*A*) *I-V* relationships obtained before (1*T*), during (1.5*T*, 2.2*T*) and after (*Wash*) successive 5-min exposures to 1.5*T* and 2.2*T* solutions. Currents were measured at the end of 500-ms pulses applied at 0.2 Hz (*see* schematic). *Inset*: superimposed records showing inhibition of time-dependent outward K^+ current (+40 mV) by 1.5*T* solution. The calibration bars indicate 100 ms and 0.2 nA. (*B*) Records of currents elicited by 200-ms pulses to potentials between −60 mV (*top records*) and −100 mV (*bottom records*). The records were obtained before (1*T*), during (2*T*), and 7 min after (*Wash*) a 5-min exposure to 2*T* solution. The dashed lines indicate zero-current levels. (*C, D*) Lack of effect of hyperosmotic 2.2*T* solution on membrane current in myocytes treated with Ba^{2+}. The example results in *C* indicate that the addition of 1 mM Ba^{2+} to 1*T* solution for 5 min (1*T* + Ba^{2+}) blocked almost all of the control (1*T*) inwardly-rectifying current, and that the continued presence of Ba^{2+} during the subsequent 5-min exposure to 2.2*T* solution prevented the changes normally associated with 2.2*T* exposure. The data summary in *D* indicates that the change in current (ΔI) at −60 mV induced by 5-min exposures to 2.2*T* solution was almost completely suppressed in the presence of 0.1 or 1 mM Ba^{2+}. *$P < 0.001$ versus Ba^{2+}-free control. Numbers of myocytes in parentheses.

the currents at these potentials were essentially time-independent (Fig. 2*B*), and secondly, they were sensitive to classical I_{K1} blocker, Ba^{2+}, i.e., $Ba^{2+} > 0.1$ mM not only suppressed almost all of the current between −110 and −20 mV under 1*T* conditions, it also prevented the changes in current normally induced by hyperosmotic 2.2*T* solution over this potential range (Fig. 2*C*, *D*). Similar antagonism of 2.2*T*-induced increases was observed ($n = 3$, *data not shown*) when external Cs^+ was present at a concentration (20 mM) known to block outward I_{K1} (Isenberg, 1976).

In nine additional experiments on perforated-patch myocytes, exposure to 2.2*T* solution shifted E_{rev} by −7.3 ± 0.5 mV ($P < 0.001$) and increased the amplitude of outward I_{K1} at −60 mV by 59 ± 7% ($P < 0.01$). Although the 2.2*T*-induced shift in E_{rev} resulted in a ca. 25% increase in the driving force on K^+ ions at −60 mV, this increase was not large enough to explain the magnitude of the increase in I_{K1} at that potential. Therefore, it seemed likely that increases in inward-rectifier channel conductance were also involved in the effects of hyperosmotic solution. To estimate these, we determined the slope of the *I-V*

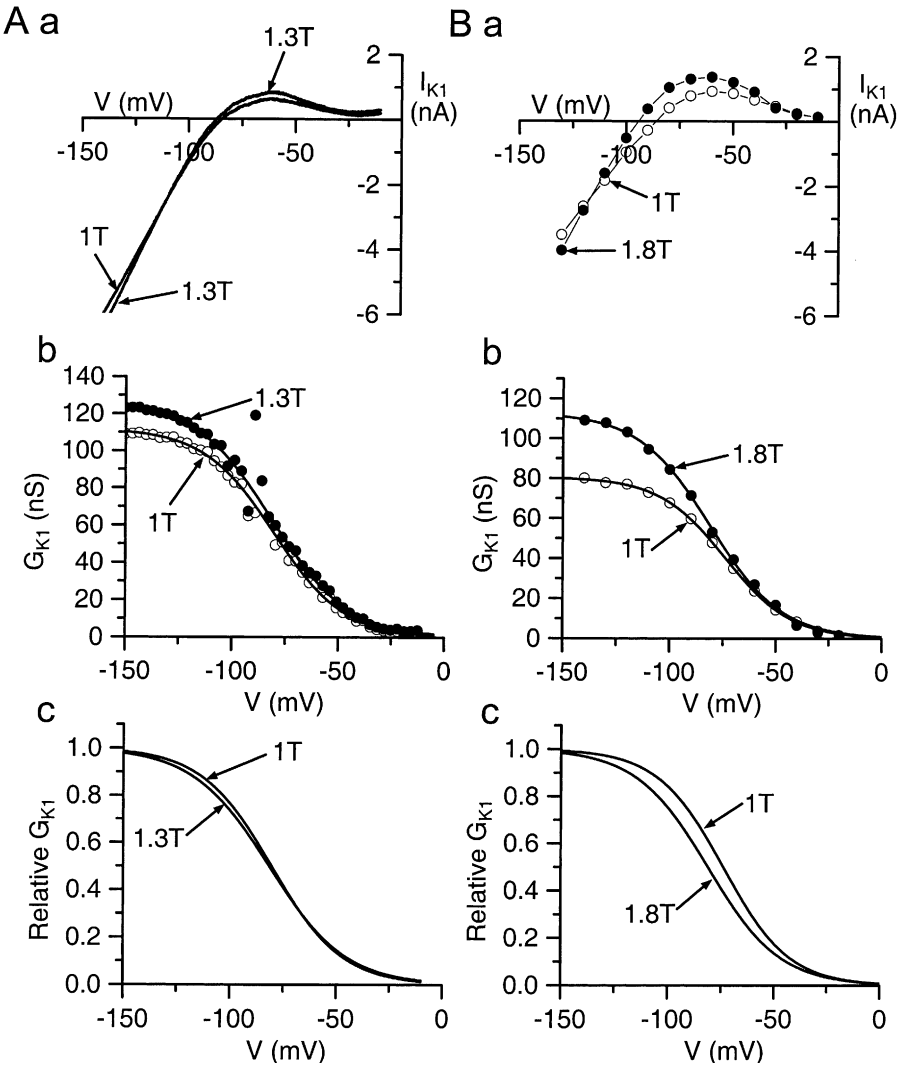

Fig. 3. Effects of hyperosmotic solutions on I_{K1} and (chord) G_{K1} in ruptured-patch myocytes. All solutions were $NMDG^+$-based and contained 100 μM Gd^{3+}. (*A*) Effects of 1.3*T* solution on (*a*) I_{K1}-*V* (inward current chopped for presentation purposes), (*b*) G_{K1}-*V* and (*c*) normalized (G_{K1}max = 1.00) G_{K1}-*V*. Currents were elicited by depolarizing ramps (100 mV/s) from −150 mV, and chord G_{K1} was obtained by dividing the current amplitude by the driving force, $V - E_{rev}$. The single Boltzmann function fitting the G_{K1}-*V* data has the following values: control 1*T* (G_{K1}max, 112 nS; $V_{0.5}$, −70.1 mV; and slope factor of −16.8 mV), 1.3T (G_{K1}max, 127 nS; $V_{0.5}$, −71.6 mV; and slope factor, −17.6 mV). (*B*) Effects of 1.8*T* solution. Currents were elicited by 200-ms pulses from holding potential −80 mV. The function fitting the G_{K1}-*V* data has the following values: control 1*T* (G_{K1}max, 81 nS; $V_{0.5}$, −74.3 mV; and slope factor, −16.1 mV), 1.8*T* (G_{K1}max, 113 nS, $V_{0.5}$, −80.7 mV, and slope factor, −16.0 mV).

relation (−110 to −100 mV) before and 5 min after exposure to hyperosmotic solution. The slope increased by a significant ($P < 0.05$–0.01) 15 ± 3% ($n = 10$), 31 ± 7% ($n = 8$), and 39 ± 9% ($n = 9$) during superfusion with 1.5, 1.8, and 2.2*T* solution, respectively. In four of the myocytes exposed to 1.8*T*, enhanced slope conductance was unaffected by 5-min applications of Gd^{3+} (100 μM), an inhibitor of nonselective cation conductance (Clemo & Baumgarten, 1997; Hu & Sachs, 1997) (*see* also below).

I_{K1} in Ruptured-Patch Myocytes Exposed to Hyperosmotic Sucrose Solutions

The effects of hyperosmotic sucrose solutions on I_{K1} in ruptured-patch myocytes were examined by applying sequences of 200-ms pulses from the prepulse potential of −40 mV, or by imposing ramp depolarizations from −150 mV. The myocytes were generally held at −80 mV, and superfused with $NMDG^+$-based solutions that in some series (1.3*T*, 1.8*T*) also contained 100 μM Gd^{3+}. In control trials ($n = 4$), this concentration of Gd^{3+} had little or no effect on I_{K1} in myocytes superfused with 1*T* solution, a result that is in agreement with earlier findings on guinea-pig ventricular myocytes (Pascarel et al., 1998).

The results of representative experiments with 1.3*T* and 1.8*T* solutions are shown in Fig. 3. As in the experiments on perforated-patch myocytes, the hyperosmotic solutions shifted E_{rev} to more negative potentials, enhanced outward I_{K1} at −60 mV, and increased the slope of the inward limb of *I-V* relationships. Each of these effects was smaller with 1.3*T* solution than with 1.8*T* solution (Fig. 3*Aa* and *Ba*). Chord conductance (G_{K1}) was calculated as $I_{K1}/(V - E_{rev})$, and the data from the representative experiments are shown in Fig. 3*Ab,c* and *Bb,c*. Both the control and hyperosmotic data are well described by single Boltzmann functions, with the fits to the hyperosmotic data indicating higher maximal G_{K1} (G_{K1}max) and negative shifts in the voltage at half-maximal G_{K1} ($V_{0.5}$). For example, the fit to the 1.8*T*

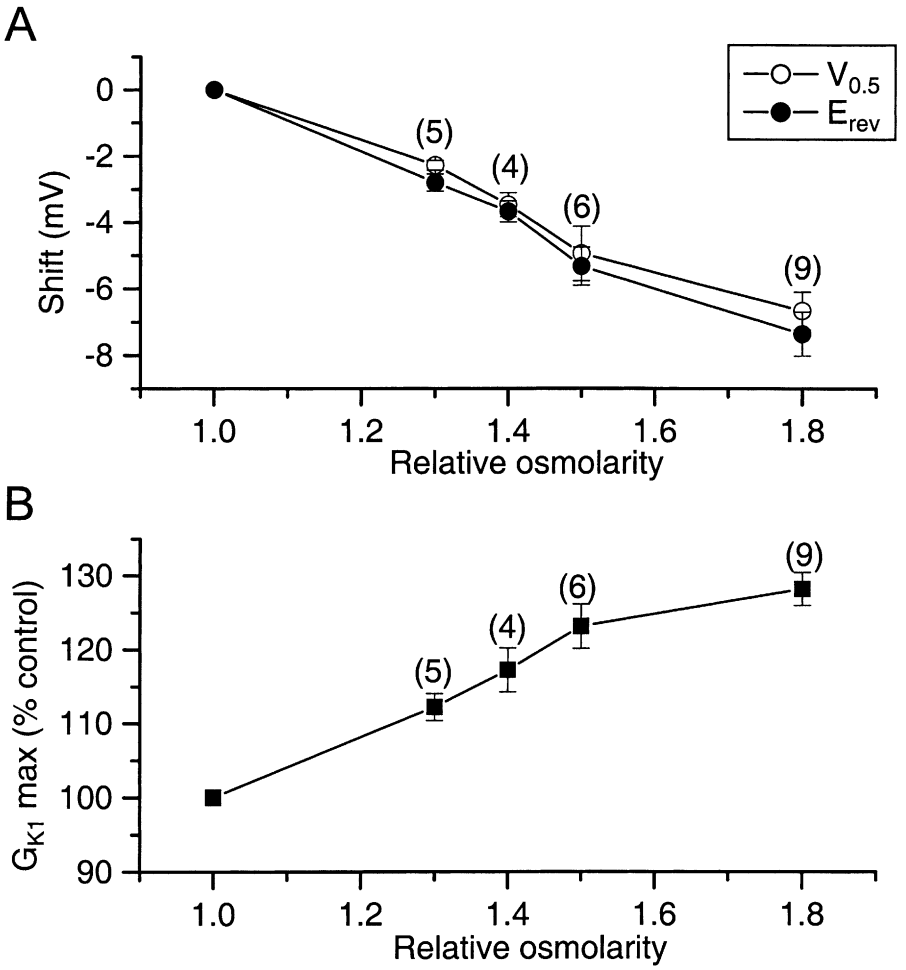

Fig. 4. Summary of changes in E_{rev} and G_{K1}-V parameters induced by exposure of ruptured-patch myocytes to hyperosmotic solutions. All superfusates were $NMDG^+$-based, and those used in the $1.3T$ and $1.8T$ series contained 100 μM Gd^{3+}. Myocytes were exposed to a single hyperosmotic solution, and measurements were taken just before and 3–5 min after the intervention. (*A*) Changes in E_{rev} and $V_{0.5}$. Numbers of myocytes in parentheses. $P < 0.01$–0.001 (versus $1T$) for all data points. (*B*) G_{K1}max (% control) in the same myocytes as in *A*. $P < 0.05$–0.01 (versus $1T$) for all data points.

data has G_{K1}max of 115 nS (versus control 81 nS), an unchanged slope, and a $V_{0.5}$ that is shifted by −6.4 mV.

A summary of the changes in E_{rev} and conductance parameters induced by hyperosmotic solutions is provided in Fig. 4. The shifts in E_{rev} ranged from −2.8 ± 0.3 mV ($n = 5$) ($1.3T$ solution) to −7.3 ± 0.7 mV ($n = 9$) ($1.8T$ solution), and the corresponding shifts in $V_{0.5}$ ranged from −1.8 ± 0.2 mV to −6.7 ± 0.6 mV ($P < 0.01$ versus $1T$ for all values) (Fig. 4*A*). The degree of enhancement of G_{K1}max was also dependent on the osmotic strength of the superfusate; it increased by 12 ± 2% with $1.3T$ solution, 23 ± 3% with $1.5T$, and 28 ± 2% with $1.8T$ ($P < 0.05$ −0.001) (Fig. 4*B*).

Effects of Elevated $[K^+]_i$ on I_{K1}

Assuming that E_{rev} (I_{K1}) ≈ E_K in guinea-pig ventricular myocytes, and that $[K^+]_i \approx$ [dialysate K^+] (140 mM) under isosmotic conditions, we calculate that average $[K^+]_i$ in ruptured-patch myocytes exposed to $1.8T$ solution was ≈ 180 mM. This concentration is considerably lower than that expected from the shrinkage of myocyte shell volume, i.e., assuming an osmotically-inactive fraction of 34% (Drewnowska & Baumgarten, 1991), $[K^+]_i$ should have increased to ≈ 250 mM. We attribute the discrepancy between experimental and theoretical estimates to diffusion of K^+ from the cytoplasm to the pipette.

To obtain information on the role of elevated $[K^+]_i$ in hyperosmotic-induced changes in G_{K1}max and $V_{0.5}$, we measured I_{K1} in myocytes that were dialyzed with high (240 mM) K^+ solution. Since this dialysate was hyperosmotic, the myocytes were superfused with $1T$ solution for <1 min following patch breakthrough, and then with a series of hyperosmotic ($1.2T$, $1.4T$, etc.) solutions to maintain osmotic balance as cell dialysis progressed. Although this experimental design was not ideal, it nevertheless resulted in predictable, consistent findings. First, delaying the introduction of hyperosmotic superfusate caused cell swelling and a marked increase in delayed-rectifier I_{Ks}, effects that confirmed diffusion of high-K^+ dialysate from the pipette to the cell membrane. Second, introduction of hyperosmotic superfusate reversed/prevented the latter two effects, indicating satisfactory osmotic balance.

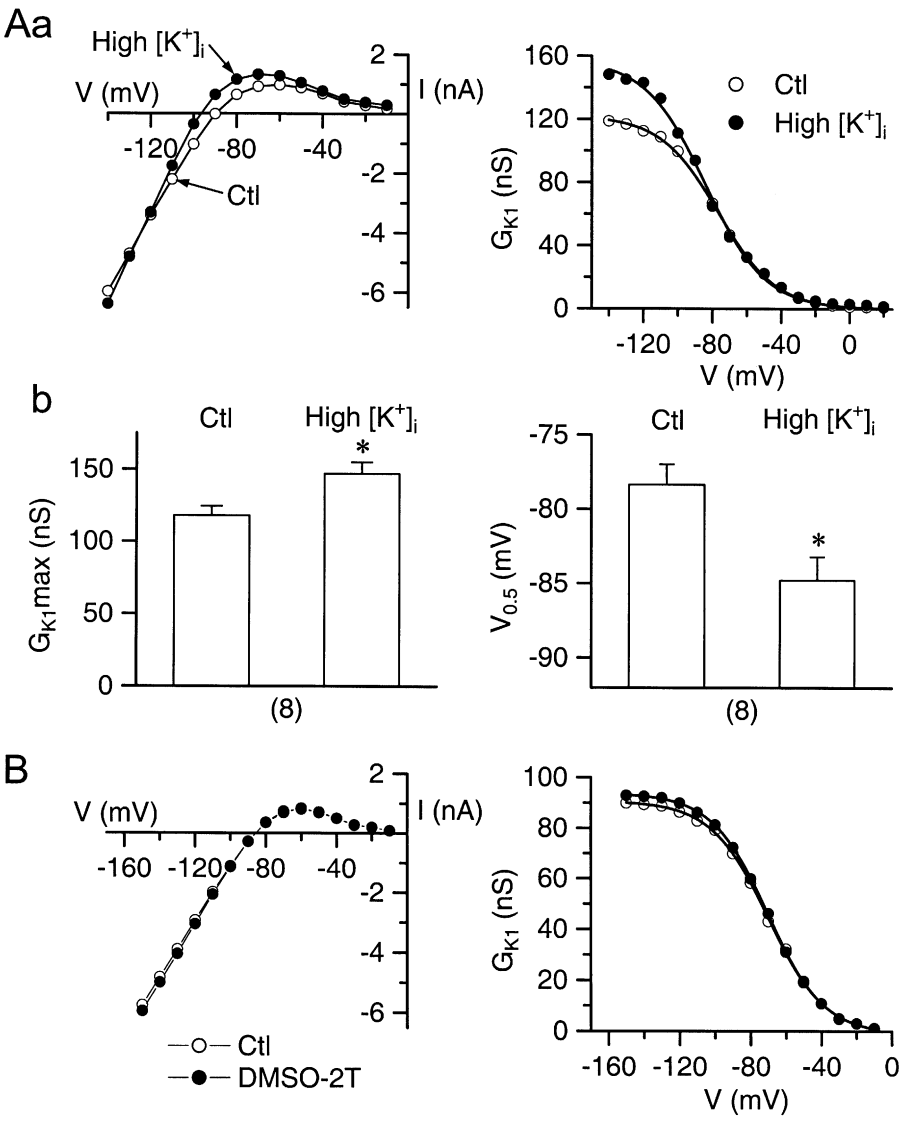

Fig. 5. I_{K1} and G_{K1} in hyperosmotic-stressed ruptured-patch myocytes that had near-normal volume and either elevated or normal $[K^+]_i$. The myocytes were superfused with $NMDG^+$-based solutions that contained 100 μM Gd^{3+}. *I-V* relationships were obtained by using a voltage command that started at −140 mV and stepped the voltage by 10 mV every 200 ms. These *I-V* runs were applied every 20 s. (*A*) Effects of high-$[K^+]_i$. Myocytes were dialyzed with 240 mM K^+ pipette solution, and superfused with a series of hyperosmotic solutions (1.2*T*, 1.4*T*, etc.) to maintain osmotic balance, (*a*) Data obtained from a representative myocyte. *Left*: end-of-step I_{K1}-*V* relationships determined shortly after patch breakthrough (*Ctl*) and 4 min later (*high* $[K^+]_i$). Right: G_{K1}-*V* relationships. The control curve has G_{K1}max of 122 nS, slope of −16.8 mV, and $V_{0.5}$ of −76.6 mV; the high-$[K^+]_i$ curve has G_{K1}max of 157 nS, slope of −17.1 mV, and $V_{0.5}$ of −84.0 mV. (*b*) Summary of G_{K1} parameters determined in 8 test myocytes. Control data were obtained from the first post-patch *I-V* run, and high-$[K^+]_i$ data from the earliest *I-V* run that indicated a shift in E_{rev} of −6 to −8 mV (mean shift: −7.1 ± 0.3 mV). *$P < 0.001$ versus control. (*B*) Lack of effect of a 7-min exposure to DMSO-2*T* solution on the I_{K1}-*V* and G_{K1}-*V* relationships in a representative myocyte.

Application of the high-$[K^+]_i$ protocol to myocytes resulted in time-dependent shifts in E_{rev} and increases in outward I_{K1} and slope conductance below E_{rev}. To quantify changes in conductance parameters, I_{K1}-*V* and G_{K1}-V relationships were determined at two times, shortly after patch breakthrough (control) and after E_{rev} had shifted by approximately −7 mV (high $[K^+]_i$). Analysis of G_{K1} data from eight test myocytes indicates that G_{K1}max increased from control 118 ± 6 nS to 147 ± 8 nS ($P < 0.001$) after a −7.1 ± 0.3 shift in E_{rev}, and that these changes were accompanied by a −6.4 ± 0.6 mV ($P < 0.001$) shift in $V_{0.5}$ (Fig. 5*A*).

Effects of Non-Shrinkage-inducing Hyperosmotic Solution on I_{K1}

For comparison with the results obtained from the preceding experiments, we determined the effects of a hyperosmotic challenge that was designed to induce

little change in either the volume or $[K^+]_i$ of ruptured-patch myocytes. In brief, myocytes dialyzed with standard K^+ pipette solution were exposed to a superfusate whose osmotic strength had been raised to $2T$ by addition of membrane-permeable DMSO (2% v.v.). The exposures to DMSO-$2T$ solution had relatively small effects on the shell volume of myocytes clamped at −90 mV (reduction to 96 ± 2% control, $n = 8$), and little or no effect on I_{K1}-V and G_{K1}-V relationships in myocytes pulsed to potentials between −10 and −150 mV (e.g., Fig. 5*B*). In the latter experiments ($n = 5$), DMSO-$2T$ solution changed G_{K1}max by an insignificant −2.4 ± 3.2%, and changed $V_{0.5}$ by an insignificant −0.4 ± 0.9 mV.

Discussion

We have investigated the effects of osmotic cell shrinkage on I_{K1} in guinea-pig ventricular myocytes and found that it shifted E_{rev} to a more negative voltage, increased the amplitude of outward-directed current, and steepened the inward limb of the I_{K1}-V relationship. Analysis of G_{K1}-V data indicated that osmotic shrinkage caused an increase in G_{K1}max and a negative shift in the rectification parameter $V_{0.5}$. We discuss whether measurements of I_{K1} are likely to have been affected by overlapping currents, compare our results with earlier published findings, and consider possible mechanisms responsible for shrinkage-induced changes in conductance and rectification.

Possible Interference by Non-I_{K1} Osmosensitive Currents

Our conclusions on the effects of hyperosmotic conditions on the inward-rectifier are based on the premise that any I_{K1}-overlapping K^+, Na^+ and/or Cl^- current, whether osmosensitive or not, was of negligible amplitude and consequence. The K^+ currents that might overlap I_{K1} are ATP-sensitive K^+ current ($I_{K,ATP}$) and tandem-pore (TREK, TRAAK) current. Any $I_{K,ATP}$ must have been very small because the pipette solution contained high (5 mM) ATP and the bathing solutions contained 3 μM glibenclamide. Likewise, any tandem-pore current must have been relatively small because tandem-pore current is relatively insensitive to external Ba^{2+} (Lesage & Lazdunski, 2000) which, at 0.1–1 mM, blocked almost all of the membrane current present at ≤−20 mV (Fig. 3).

A non-selective cation current that is activated by osmotic shrinkage has been identified in a number of cell types (Nelson et al., 1996; Koch & Korbmacher, 1999, 2000). However, it is unlikely that this or other non-selective cation current interfered with measurement of I_{K1} because our results were unaffected by inclusion of Gd^{3+} and replacement of external Na^+ with poorly-permeant $NMDG^+$. Similarly, I_{K1} measurements were not compromised by an inwardly-rectifying Cl^- current present in some cardiomyocytes (Duan et al., 2000) because that current, which is fully blocked by 0.3 mM Cd^{2+} (Duan et al., 2000), must have been negligible in the Cd^{2+} (1 mM)-treated myocytes investigated here.

Comparison with Previous Findings

To our knowledge, there have been no previous studies on the effects of osmotic shrinkage on I_{K1}. However, there have been two studies on the effects of osmotic shrinkage on the inward-rectifier K^+ pathway in skeletal muscle fibers. In the first of these, Standen and Stanfield (1980) found that superfusion of fibers with $2T$ solution resulted in a 28% increase in maximal chord conductance; in the second, a similar hyperosmotic challenge had no significant effect on chord conductance (Leech & Stanfield, 1981). A point of agreement between the two studies was that osmotic shrinkage did not alter the voltage dependence of chord conductance. By contrast, shrinkage of ventricular myocytes induced a negative shift in the G_{K1}-V relationship. Whether this difference reflects a cell-type difference, or perhaps an experimental difference (the skeletal muscle fibers were superfused with a high-K^+ solution), is not clear.

Possible Mechanisms Involved in Shrinkage-induced Changes in I_{K1}

Exposure of myocytes to hyperosmotic sucrose solution results in a loss of cell water and resultant concentrating of cytoplasmic constituents that might have direct or indirect effects on I_{K1}. Amongst the latter, we discount a role for MgATP, Na^+, Ca^{2+}, and Mg^{2+} for the following reasons: (i) MgATP was already in good supply prior to shrinkage; (ii) Na^+ is likely to have been very low both before and after shrinkage; (iii) any elevation of Mg^{2+} caused by shrinkage will have been inhibitory (Vandenberg, 1987; Martin, Koumi & Ten Eick, 1995); and (iv) the concentration of Ca^{2+} is likely to have been orders lower in (EGTA-dialyzed) ruptured-patch myocytes than in perforated-patch myocytes, and yet I_{K1} responses were similar in the two groups of myocytes. In contrast to the foregoing, a role for shrinkage-induced concentrating of intracellular K^+ is supported by the finding that non-shrinkage elevation of $[K^+]_i$ via dialysis with high-K^+ solution had effects on I_{K1} that were similar to those caused by shrinkage. To the best of our knowledge, there are no earlier data available for comparison with the results obtained with high-$[K^+]_i$ dialysate. However, there have been several pertinent studies on the effects of low-K^+ dialysates under isosmotic conditions, and the results from

these provide qualitative support for the concept that $[K^+]_i$ is a determinant of G_{K1}max. For example, Saigusa and Matsuda (1988) reported that maximal slope conductance in guinea-pig ventricular myocytes was decreased by 30% when dialysate K^+ was lowered from 150 to 75 mM, and Hagiwara and Yoshii (1979) found a similar decrease in the inward-rectifier conductance of starfish egg cell membrane when perfusate K^+ was lowered from 270 to 125 mM. Although the foregoing reductions in slope conductance were not accompanied by significant shifts in $V_{0.5}$, Cohen et al. (1989) have reported that both $V_{0.5}$ and E_{rev} shifted to less negative potentials when Purkinje fiber myocytes were dialyzed with low-K^+ solution.

The mechanisms underlying the modulation of G_{K1}max and $V_{0.5}$ by $[K^+]_i$ are unclear. However, in regard to G_{K1}max, it may be pertinent that calculations using E_{rev}-estimated elevation of $[K^+]_i$ (1.8*T*) and the constant field equation predict an increase of 30%, a value that is in good agreement with the experimentally determined increase of 28%. In regard to $V_{0.5}$, Watanabe et al. (1991) have calculated that increases in $[K^+]_i$ raise the concentration of free spermine in the cytoplasm. Since voltage-dependent block by positively-charged spermine is the principal mechanism responsible for inward-rectification in heart cells (Lopatin & Nichols, 2001), a $[K^+]_i$-mediated increase in spermine concentration could account for the $[K^+]_i$-induced negative shift in the $V_{0.5}$ of the G_{K1}-V relationship.

Regardless of the mechanism(s) involved in the postulated $[K^+]_i$-mediated changes of I_{K1} in shrunken myocytes, the question arises as to why the opposite changes have not been observed in studies on osmotically swollen myocytes (*see* Introduction for citations). One possibility is that swelling may cause activation of a K^+-selective, inwardly-rectifying cation conductance (Clemo & Baumgarten, 1997) that could obscure a decrease in I_{K1}. A second is that diffusion of K^+ from the patch pipette may offset the dilution of intracellular K^+ caused by the movement of water into the cell. For example, Zhou et al. (1997) reported that neither E_{rev} nor I_{K1}-*V* was affected by hyposmotic (0.55*T*) swelling of canine ventricular myocytes to 133% of control volume.

In summary, we have shown that osmotic shrinkage of isolated ventricular myocytes causes an increase in G_{K1}. To the extent that a similar response occurs in vivo during episodes of ischemia-reperfusion (Askenasy & Navon, 1997) or elevated plasma tonicity (e.g., Kramer et al., 1989), the increase in G_{K1} would accelerate phase-4 repolarization and antagonize the generation of proarrhythmic afterdepolarizations.

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