Osmotic modulation of slowly activating $I_{Ks}$ in guinea-pig ventricular myocytes

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Aims

The aims of the study were to determine the effects of anisosmotic bathing solution on selected properties of $I_{Ks}$, the slowly activating delayed-rectifier $K^+$ current important for repolarization of the action potential in cardiac cells.

Methods and results

Guinea-pig ventricular myocytes were voltage-clamped using either the ruptured-patch or perforated-patch technique, and the amplitude, time course, and voltage dependence of $I_{Ks}$ were determined before [isosmotic (1T)] and during superfusion of hyposmotic (<1T) or hyperosmotic (>1T) bathing solution. Hyposmotic solution increased the amplitude of $I_{Ks}$, and hyperosmotic solution decreased it. Anisosmotic-induced changes in $I_{Ks}$ amplitude were complete in 2–5 min, well-maintained, reversible, and not accompanied by significant changes in $I_{Ks}$ time course and voltage dependence. There was little difference in the results obtained with the ruptured-patch technique and those obtained with the perforated-patch technique. The amplitude of $I_{Ks}$ was sensitive to small (±10%) changes in osmolality, maximally increased by hyposmotic solution with $T < 0.7$, and strongly decreased by hyperosmotic solution with $T > 1.5$. Experimental data on a plot of relative (1T = 1.0) $I_{Ks}$ amplitude vs. the reciprocal of relative osmolality are well-described by a Hill equation that has a lower asymptote of 0.0, an upper asymptote of 2.0, and a slope factor of 1.87 ± 0.07.

Conclusion

Modulation of $I_{Ks}$ amplitude by anisosmotic solution is independent of patch configuration, unaccompanied by changes in current gating, and well-described by a Hill dose–response relation that predicts relatively strong responses of $I_{Ks}$ to small perturbations in external osmolality.

Keywords

Myocytes • Slowly activating ($I_{Ks}$) • Hyperosmotic solution • Hyposmotic solution • ($I_{Ks}$–osmolality relationship

1. Introduction

Myocytes in the heart may swell under a variety of conditions, including ischaemia-reperfusion,1–3 hypothermic cardioplegia,4 and adaptive cardiac remodelling.5 Conversely, myocytes in the heart likely shrink during hypernatraemia,6 intermittent ischaemia,7 and hyperosmotic fluid resuscitation.8,9 As evaluated by the responses of isolated myocytes to replacement of isosmotic bathing solution by anisosmotic solution, osmotic swelling and shrinkage affect myocyte electrical activity by altering the concentrations of intracellular ions and modifying the activities of ion pathways in the cell membrane.10–15 Prominent amongst these pathways is the slowly activating delayed-rectifier $K^+$ channel16 whose molecular correlate is the α- and β-subunit complex KCNQ1/KCNE1.17 The current carried by this channel, $I_{Ks}$, is activated at membrane potentials similar to those attained during the plateau phase of the cardiac action potential.16 $I_{Ks}$ promotes repolarization of the cardiac action potential,18–20 and contributes to repolarization reserve.21

The directional effects of anisosmotic bathing solution on the amplitude of ventricular $I_{Ks}$ are well established: hyposmotic solution increases the amplitude,10,12,13,22 and hyperosmotic solution decreases it.11,23 However, many aspects of the response of $I_{Ks}$ to anisosmotic solution remain to be clarified. In the present study, we provide information on the time course and reversibility of osmolality-induced changes in the amplitude of $I_{Ks}$ in guinea-pig ventricular myocytes, compare results obtained with ruptured-patch and perforated-patch voltage-clamp techniques, evaluate whether osmolality-induced changes in $I_{Ks}$ amplitude are accompanied by changes in current kinetics, and determine the relation between relative $I_{Ks}$ amplitude and relative osmolality.
2. Methods

2.1 Myocyte preparation

The investigation conforms with the ‘Guide for the Care and Use of Laboratory Animals’ published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Hearts were quickly removed from guinea-pigs (250–300 g) killed by cervical dislocation in accord with Canadian and Dalhousie University regulations on animal experimentation. The hearts were mounted on a Langendorff column, and perfused (37 °C) through the coronary artery for 10–15 min. The perfusate (pH 7.4) contained (in mM) NaCl 140, KCl 5, MgCl2 1.2, glucose 10, HEPES 5, and 0.08–0.12 mg/mL collagenase (Yakult Pharmaceutical Co., Tokyo, Japan). On completion of collagenase digestion, the heart tissue was minced and myocytes were dispersed in storage solution that contained KOH 80, KCl 30, KH2PO4 30, MgSO4 3, glutamic acid 50, taurine 20, glucose 10, EGTA 0.5, and HEPES 10 (pH 7.4 with KOH). For experiments, a few drops of the cell suspension were placed in a 0.3-mL chamber mounted on the stage of an inverted microscope, and the chamber was perfused (≈3 mL/min) with bathing solution (36 °C).

2.2 Electrophysiology

Myocytes were voltage-clamped using either the conventional ruptured-patch method or the nystatin perforated-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 solution. Liquid junction potentials between external and pipette-filling solutions were offset during data analysis. In the ruptured-patch experiments, the series resistance ($R_s$) ranged between 4 and 8 MΩ and was compensated by 60–80%. In the perforated-patch experiments, $R_s$ monitored after seal formation declined to 8–25 MΩ within 10–20 min, and experimental observations began when $R_s$ was stable over a 10-min period. $R_s$ compensation reduced uncompensated resistance to <10 MΩ (typically 2–6 MΩ). Membrane currents were recorded with an EPC-9 amplifier (HEKA Electronics, Mahone Bay, NS, Canada), low-pass filtered (3 kHz), and digitized with Pulse software (HEKA Electronics) (sampling rate 12 kHz). Data files were converted from Pulse to Axon (Molecular Devices, Sunnyvale, CA, USA) format, and analyzed with Clampfit electrophysiology software (Molecular Devices).

2.3 Superfusates and pipette solutions

Isosmotic 1T solution contained (in mM) NaCl 70, MgCl2 1, CdCl2 0.2, glucose 10, mannitol 140, and HEPES 5 (pH 7.4) (calculated osmolarity ≈295 mosmol/L). Hyposmotic solutions were made by lowering the mannitol in the 1T solution, and hyperosmotic solutions by adding suitable amounts of mannitol (e.g. by 30, 150, 300, and 450 mM for 1.1T, 1.5T, 2.2T, and 2.8T, respectively). Osmolarity was verified using a freezing point depression osmometer (Osmette II, Precision Systems, Natick, MA, USA). Thiopentone sodium (Abbott Laboratories, Saint Laurent, QC, Canada) and E4031 (Tocris Bioscience, Ellisville, MO, USA) were dissolved in the bathing solution.

Pipettes used in the ruptured-patch experiments were filled with solution that contained (in mM) KCl 30, potassium aspartate 110, MgCl2 5, and HEPES 5 (pH 7.2 with KOH). Nystatin (Sigma-Aldrich Co., Oakville, ON, Canada) was added at final concentration of 50–100 μg/mL.

2.4 Statistics

Statistical data are expressed as means ± SEM. Comparisons were made by using Student’s t-test unless otherwise specified. Differences were considered to be significant when $P < 0.05$.

3. Results

Membrane currents were recorded under experimental conditions (K+-free, Ca2+-free, low-Na+, Cd2+ external solution; Na+-free, and Ca2+-free pipette solution) that minimized Na+- and Ca2+-dependent currents and minimized contributions of rapidly activating $I_{Kr}$ to delayed-rectifier K+-currents.16,24 Myocytes in perforated-patch or ruptured-patch configuration were regularly pulsed with 0.5- or 1-s steps from holding potential near −40 mV to a test potential near +60 mV every 10 or 20 s to monitor the amplitude of the tail current elicited on repolarization. Regular pulsing was interrupted as required for application of longer duration test pulses and/or determination of isochronal current–voltage ($I–V$) relations.

3.1 Modulation of $I_{Kr}$ by hyposmotic bathing solution

Figure 1 depicts the changes that occurred in the amplitudes of tail currents recorded during representative experiments on myocytes in the perforated-patch configuration. The myocytes were equilibrated with isosmotic 1T bathing solution, and then superfused with hyposmotic 0.9T, 0.8T, or 0.55T bathing solution. The amplitudes of tail currents elicited after 1-s pulses to +60 mV began to increase shortly after admission of hyposmotic solution, and reached new stable levels 1–4 min later (Figure 1A–C). The percentage increase in amplitude was near 25% in the experiment with 0.9T solution.
45% with 0.8T solution, and 95% with 0.55T solution. \( I_{KS} \) blocker thopentone (150 \( \mu \)M) was applied during the experiment with 0.55T solution to ascertain that the tail current being measured was exclusively or almost exclusively contributed by \( I_{KS} \). Addition of thopentone after attainment of steady-state hyposmotic stimulation reduced the tail current by more than 90% (Figure 1C). Overall, the degree of inhibition (93 \( \pm \) 5%: \( n = 4 \)) was similar to that observed under isosmotic conditions (91 \( \pm \) 4%: \( n = 5 \)).

The results shown in Figure 2 provide further detail on the effects of hyposmotic solution on delayed-rectifier \( K^+ \) current in guinea-pig ventricular myocytes. The current records in Figure 2A were obtained during an experiment on a ruptured-patch myocyte that was equilibrated in isosmotic 1T bathing solution, superfused with 0.75T hyposmotic solution for 7 min, and then superfused with 0.75T solution that contained 10 \( \mu \)M E4031 for an additional 7 min. The records indicate that superfusion with 0.75T solution caused marked increases in the amplitudes of time-dependent currents activated by 2-s depolarizations from \(-40 \) mV to potentials between 0 and \(+80 \) mV, as well as in the amplitudes of the corresponding time-dependent tail currents elicited by repolarizations to \(-40 \) mV. The hyposmotic-induced increases were well-maintained during the subsequent treatment with selective \( I_{KS} \) blocker E4031. Similar results in other experiments with 0.6T–0.75T solution and 3–10 \( \mu \)M E4031 (\( n = 7 \), data not shown), as well as the findings with thopentone (see above), indicate that the current components under investigation can be attributed to \( I_{KS} \).

The effects of hyposmotic solution on the voltage dependence of \( I_{KS} \) were investigated by measuring the amplitudes of tail currents recorded before (control 1T) and during steady-state stimulation of \( I_{KS} \) by hyposmotic solution. The tail currents were elicited on repolarizations from 2-s \( I_{KS} \)-activating pulses applied from holding potential \(-40 \) mV to potentials between \(-30 \) and \(+90 \) mV, and the results obtained in a set of eight experiments with 0.6T solution are shown in Figure 2B. The Boltzmann function \( I/I_{\text{max}} = 1/(1 + \exp[(V_{0.5} - V)/S]) \) fitted to the 1T tail current data has a half-voltage (\( V_{0.5} \)) of \(+18.7 \pm 0.6 \) mV and a slope factor (\( S \)) of 15.4 \( \pm \) 0.4 mV, values that are in good accord with those reported in earlier studies on \( I_{KS} \) in guinea-pig ventricular myocytes (e.g. \( V_{0.5} \) and \( S \) values of \(+15.7 \) and 12.7 mV\(^{16} \), \(+20.1 \) and 14.5 mV\(^{20} \) and \(+23.7 \) and 19.6 mV\(^{21} \)). After attainment of steady-state stimulation with 0.6T solution, the \( V_{0.5} \) and \( S \) values were unchanged at \(+17.1 \pm 1.5 \) and 15.9 \( \pm \) 1.3 mV, respectively (Figure 2B).

The time course data in Figure 1 and the current records in Figure 3A illustrate that the stimulation of \( I_{KS} \) by hyposmotic bathing solution was a relatively rapid, fully reversible phenomenon that was independent of the voltage-clamp technique employed. Support for this characterization is provided by data obtained in two large sets of experiments with hyposmotic solution. In the first of these, 36 ruptured-patch myocytes were equilibrated in control 1T solution, superfused with 0.75T solution for 431 \( \pm \) 22 s, and then recovered in 1T solution for 5–7 min. The amplitude of the \( I_{KS} \) tail elicited on repolarization from \(+60 \) mV reached a steady-state level of 172.7 \( \pm \) 3.6% of control 1T amplitude after 206 \( \pm \) 12 s superfusion with hyposmotic solution, and declined to 98.3 \( \pm \) 3.1% of control amplitude after readmission of 1T solution (Figure 3C).

We evaluated whether patch-clamp technique played a role in the effect of hyposmotic solution on \( I_{KS} \) amplitude by determining the responses of \( I_{KS} \) tails to solutions whose osmolarities fell within one of three test ranges (0.85–0.97T, 0.7–0.75T, and 0.5–0.55T). It is evident from the data in Figure 3D that the degree of stimulation of \( I_{KS} \) increased with the severity of the hyposmotic treatment (\( P < 0.001 \), two-way ANOVA), and was not related to the use of one clamp technique over the other (\( P > 0.5 \), two-way ANOVA).

The effects of hyposmotic solution on the time course of \( I_{KS} \) activating during a depolarizing pulse were evaluated by fitting double exponential functions to records of \( I_{KS} \) elicited by 2-s depolarizations to \(+60 \) mV. In eight ruptured-patch myocytes equilibrated with 1T solution and then superfused with 0.6T solution for 7 min, the time constants of the fitting functions were 162 \( \pm \) 28 and 1475 \( \pm \) 205 ms (1T) and 171 \( \pm \) 43 and 1578 \( \pm \) 165 ms (0.6T) (no significant difference). The time course of deactivation of tail currents at \(-40 \) mV in these myocytes was also fitted with double exponential functions. The time constants after equilibration with 1T solution were 130 \( \pm \) 11 and 483 \( \pm \) 31 ms, values that are in satisfactory agreement with recent measurements on \( I_{KS} \) tails in guinea-pig ventricular myocytes (161 \( \pm \) 10 and 601 \( \pm \) 101 ms at \(-40 \) mV).\(^{21} \) After attainment of steady-state stimulation of \( I_{KS} \) with 0.6T solution, the time constants were 139 \( \pm \) 12 and 504 \( \pm \) 26 ms, i.e. not significantly different than the 1T values. Time constants were also unaffected in experiments with 0.7T and 0.8T solutions (data not shown).

### 3.2 Modulation of \( I_{KS} \) by hyposmotic dialysate

It seems highly likely that the stimulation of \( I_{KS} \) by hyposmotic bathing solution was initiated by generation of an osmotic imbalance (external osmolality \textless internal osmolality) rather than by hyposmotic bathing solution per se. To ascertain that this was the case, an osmotic imbalance was generated by dialyzing myocytes with hyposmotic pipette solution while they were being superfused with 1T bathing solution. Patch breakthrough was invariably followed by a run-up in the amplitude of the \( I_{KS} \) tail elicited on repolarization from \(+60 \) mV. The run-ups began almost immediately, and continued for several minutes. Thereafter, the enhanced amplitude of the \( I_{KS} \) tail was well-maintained. To gauge the degree of stimulation of \( I_{KS} \) induced by the hyposmotic pipette solution, the amplitude of the \( I_{KS} \) tail during steady-state stimulation (5 min post-patch breakthrough: 1T bathing solution) was compared with a baseline amplitude (the amplitude measured 5 min after switching from 1T bathing solution to one whose osmolality matched that of the hyposmotic pipette solution). The amplitude during steady-state stimulation was 75 \( \pm \) 5% larger than the baseline amplitude in experiments with 1.5T pipette solution (\( n = 4 \)), and 121 \( \pm \) 11% larger in experiments with 2.2T pipette solution (\( n = 16 \)) (both \( P < 0.001 \)).

### 3.3 Modulation of \( I_{KS} \) by hyposmotic bathing solution

Myocytes dialyzed with standard pipette solution were equilibrated with 1T bathing solution, and then superfused with a solution whose osmolality ranged from 1.1T to 2.8T. As illustrated by results obtained from experiments on ruptured-patch myocytes (Figure 4), superfusion with hyposmotic solution induced rapid
decreases in the amplitudes of $I_{Ks}$ on depolarization and $I_{Ks}$ tail on repolarization. The severity of the decreases increased with the osmolarity of the solution, being about 25% of control 1T amplitude for 1.2T solution, 60% for 1.5T solution, and 80% for 2.8T solution (Figure 4A–C). The effects of 1.1T–1.8T solutions were readily reversed upon readmission of 1T solution (Figure 4A), whereas the effects of higher tonicity solutions were less commonly fully reversible (data not shown).

The example records in Figure 4 indicate that hyperosmotic-induced decreases in $I_{Ks}$ amplitude were not accompanied by significant changes in the time courses of $I_{Ks}$ activating during depolarizing pulses and $I_{Ks}$ deactivating on repolarizations. In experiments on five ruptured-patch myocytes challenged with 2T solution, the time constants of double exponential functions fitted to $I_{Ks}$ activating at +60 mV were 181 ± 35 and 1310 ± 98 ms (1T) and 176 ± 29 and 1414 ± 168 ms (2T). In the same myocytes, the time constants of functions fitted to $I_{Ks}$ deactivating at −40 mV were 139 ± 13 and 456 ± 42 ms (1T) and 123 ± 17 and 423 ± 51 ms (2T).

The effects of hyperosmotic bathing solution on the amplitude and voltage dependence of $I_{Ks}$ were evaluated in a series of experiments on perforated-patch myocytes. The myocytes were depolarized with a sequence of 1-s pulses from −30 mV to potentials up to +60 mV just before and 5–7 min after switching from 1T solution to a test hyperosmotic solution. In agreement with the results obtained in experiments on ruptured-patch myocytes, superfusion of perforated-patch myocytes with hyperosmotic solution caused decreases in the amplitudes of activating and deactivating $I_{Ks}$ (Figure 5A). As indicated by the data shown in the $I_{Ks}$ tail-V plot of Figure 5B, the degree of inhibition increased with the severity of the hyperosmotic treatment. The amplitude of the $I_{Ks}$ tail elicited on repolarization from +60 mV declined to 72 ± 6% (n = 4), 28 ± 6% (n = 5), and 11 ± 3% (n = 4) of control 1T amplitude in experiments with 1.2T, 1.8T, and 2.8T solutions, respectively. Similar-sized percentage declines in the amplitudes of tail currents elicited after pulses to other test potentials suggested that these hyperosmotic treatments had little effect on the voltage dependence of $I_{Ks}$. Boltzmann fits to the 1T, 1.2T, 1.8T, and 2.8T $I_{Ks}$ tail data (Figure 5B) have the following $V_0.5$ and S values: +17.6 ± 0.8 and 13.6 ± 0.6 mV (1T), +16.2 ± 1.1 and 13.8 ± 0.8 mV (1.2T), +19.2 ± 2.4 and 14.2 ± 2.3 mV (1.8T), and +20.2 ± 6.5 and 13.0 ± 4.8 mV (2.8T). Statistical analysis (ANOVA, Dunnett’s multiple comparison test) indicates that neither $V_0.5$ nor S was affected by the superfusions with test hyperosmotic solutions.

### 3.4 Empirical relation between $I_{Ks}$ and external osmolarity

Figure 6 is a plot of relative (anisosmotic/isosmotic) $I_{Ks}$ amplitude vs. the reciprocal of relative external osmolarity. The data on the plot were obtained in experiments on myocytes that were either in the ruptured-patch or perforated-patch configuration. The myocytes were equilibrated in 1T bathing solution, and then superfused with one of 16 anisosmotic test solutions for 5–7 min. It is evident that
the modulation of $I_{Ks}$ amplitude is well-described by a dose–response function of the form $y = a_1 + (a_2 - a_1)/(1 + 10^{(\log_{10}(x) - \log_{10}(p))})$, where $a_1$ is a lower asymptote, $a_2$ an upper asymptote, $\log_{10}(x)$ the centre point, and $p$ a Hill slope factor. Since relative current amplitude is 1.0 at 1.0 on the osmolarity$^{-1}$ axis (Figure 6), $\log_{10}(x) = 1.0$; with $a_1$ set to 0, and $a_2$ set to 2.0, the best-fit value for $p$ is 1.87 ± 0.07.

4. Discussion

4.1 Effects of hyposmotic swelling on $I_{Ks}$

The effects of hyposmotic external solution on $I_{Ks}$ amplitude in this study can be compared with results obtained in a number of earlier studies. Sasaki et al. found that 0.7T solution increased the amplitude of total delayed-rectifier K$^+$ current ($I_{Ks}$) at +60 mV (primarily $I_{Ks}$) in guinea-pig ventricular myocytes by 70–77%. Both larger (136% for $I_{Ks}^R$) and smaller (39% for $I_{Ks}^P$, 41% for $I_{Ks}^L$) stimulations of current by 0.66T–0.78T solutions have been reported in subsequent studies on these myocytes. In the present study, 0.75T and 0.7T solutions increased the amplitude of $I_{Ks}$ by amounts (74 ± 5 and 77 ± 9%, respectively) not unlike the midpoint of the earlier results. There are no earlier reports on the responses of guinea-pig ventricular $I_{Ks}$ to more severe hyposmotic stress, but the 101 ± 5% increase in current amplitude induced by 0.5T solution in the present study is similar to the 105 ± 43% increase induced by 0.56T solution in canine ventricular myocytes. In regard to time required for attainment of steady-state stimulation of $I_{Ks}$ in guinea-pig ventricular myocytes exposed to 0.55T–0.7T solution, the ca. 3 min determined here is several times longer than that observed in one earlier study, and several times shorter than that in another.

We evaluated whether the stimulation of ventricular $I_{Ks}$ amplitude by hyposmotic solution was accompanied by changes in the time courses of activating $I_{Ks}$ and deactivating $I_{Ks}$ by fitting records of $I_{Ks}$ activating at +60 mV and $I_{Ks}$ deactivating at −40 mV with double exponential functions. The outcome was that stimulation induced by replacement of 1T solution with 0.6T–0.75T solution had no significant effect on the time constants of the functions. Of interest in regard to this finding are reports indicating lack of significant effects of 0.75T hyposmotic solution on the time courses of activating $I_{Ks}$ in guinea-pig ventricular myocytes, activating and deactivating KCNQ1 channel current in Xenopus oocytes and COS-7 cells, and deactivating $I_{Ks}$ in guinea-pig atrial myocytes.

We measured the amplitudes of $I_{Ks}$ tails elicited on repolarizations from test potentials up to +90 mV, and fitted the data with the Boltzmann function to obtain estimates of the $V_{0.5}$ and slope factor of the voltage dependence of $I_{Ks}$ activation. It would not have been surprising to have found that the voltage dependence was...
4.2 Effects of hyperosmotic bathing solution on $I_{KS}$

The amplitude of the $I_{KS}$ tail on repolarization from +60 mV declined to $83 \pm 4, 52 \pm 3, 44 \pm 4, 19 \pm 5$, and $11 \pm 3$% of control 1T value when myocytes were exposed to 1.1T, 1.3T, 1.5T, 2.2T, and 2.8T solutions, respectively. These results may be compared with those reported in earlier studies on guinea-pig ventricular myocytes: 1.3T solution decreased the amplitude of fully activated $I_{K}$ (+60 mV) to 65% control, and 1.5T and 2.2T solutions decreased the amplitude of the $I_{KS}$ tail to 47 and 18% control, respectively.

Replacement of 1T bathing solution by 1.2T, 1.8T, or 2.8T solution had no significant effect on the Boltzmann parameters. These findings support and extend earlier observations related to the effects of hyperosmotic solution on the voltage dependence of $I_{KS}$. Hyposmotic 0.73T–0.78T solution had little effect on the $V_{0.5}$ and slope of ventricular $I_{KS}$ tail-V relations, and little effect on the voltage dependence of activating KCNQ1 channel current.

4.3 Empirical relation between $I_{KS}$ amplitude and external osmolality

To our knowledge, this is the first study that fully captures the modulation of cardiac $I_{KS}$ by anisomotic bathing solution. The extent of the modulation of the amplitude of the current was particularly striking. To quantify it, we measured the steady-state amplitude of the $I_{KS}$ tail under anisomotic conditions, normalized it by reference to the amplitude under control isosmotic conditions, and plotted the resultant relative $I_{KS}$ amplitude against the reciprocal of relative external osmolality. As shown in Figure 6, the data are well-described by a dose–response function that approaches zero amplitude at low osmolality $^{-1}$ and two times isomotic amplitude at high osmolality $^{-1}$. There are several interesting issues that arise in connection with these data. The first concerns the results obtained with the perforated-patch technique and those obtained with the ruptured-patch technique. It is evident from these (see also Figure 3) that the type of patch was not a significant factor in the response of $I_{KS}$ to anisomotic solution. This was neither an expected nor unexpected outcome because although patch type had little influence on the stimulation of guinea-pig ventricular $I_{KS}$ by 0.67T solution, it had a pronounced one on the development of swelling-activated Cl$^-$ current in chick heart cells.

From our results, we conclude that any changes in cytoplasmic composition particular to application of the ruptured-patch technique in the present study had little bearing on the responses of $I_{KS}$.
A second issue that arises concerns the degree to which $I_{Ks}$ tail amplitude may have been affected by swelling/shrinkage-induced changes in $K^+$ driving force as a consequence of changes in the concentration of intracellular $K^+$. These changes will have been more pronounced, the more severe the anisometric challenge. Any lowering of the driving force on $K^+$ ions due to swelling-induced lowering of intracellular $K^+$ will have subtracted from the ‘true’ increase in $I_{Ks}$ tail amplitude induced by hyposmotic solution, whereas any raising of driving force due to shrinkage-induced elevation of intracellular $K^+$ will have subtracted from the ‘true’ decrease in $I_{Ks}$ amplitude induced by hyperosmotic solution. In regard to the function fitting the data, the net effect would have been to reduce the slope and reduce the maximum. An upper estimate of the reduction in the maximum can be obtained by supposing that (i) the concentration of extracellular $K^+$ during superfusion with $K^+$-free solution was 0.01 mM, and (ii) pronounced hyposmotic swelling lowered intracellular $K^+$ concentration from 140 to 70 mM. With these suppositions, the driving force at tail-recording potential $-40$ mV would have declined from $\approx 213$ to $\approx 195$ mV (or $\approx 8.6\%$). One reason for suggesting that the latter is an upper estimate of the percentage reduction in maximum is that the scenario being considered fails to take account of the strong likelihood that early rapid dilution of cytoplasmic $K^+$ caused by rapid osmotic movement of water is quickly moderated/partially reversed by diffusion of $K^+$ from the patch pipette. All in all, the relative $I_{Ks}$ amplitude-osmolarity$^{-1}$ relationship depicted in Figure 6 appears to be a good reflection of the osmosensitivity of whole-cell $K_s$-channel conductance.

Classically, cell volume is the parameter that is plotted against relative osmolarity$^{-1}$ (van’t Hoff plot), and the Y intercept of the regression line fitted to the data is taken as an estimate of the cell volume that is osmotically inactive. Studies on ventricular myocytes indicate that the osmotically inactive volume is $\approx 30\%$. A novel finding in the present study is that the amplitude of $I_{Ks}$ approached zero with diminishing osmolarity$^{-1}$. An implication of the finding is that $I_{Ks}$ likely responds to changes in osmotically active volume rather than total volume. However, the nonlinearity of the $I_{Ks}$ amplitude–osmolarity$^{-1}$ relationship indicates that osmoregulation of whole-cell $I_{Ks}$ is more complex than change in current proportional to change in osmotically active myocyte volume. Possible links between the two include membrane tension, cytoskeleton organization, and/or concentrations of intracellular moieties.

The $I_{Ks}$–osmolarity$^{-1}$ function should prove useful in models that project the responses of ventricular myocytes to osmotic disturbances, and in comparisons with the responses of other membrane currents. A feature of the function is its near-linearity between 0.75$^{-1}$ and 1.25$^{-1}$ (i.e. between 1.33T and 0.8T). Over this range, the amplitude of $I_{Ks}$ changes by a factor of three. While it is clear that myocytes would rarely utilize more than a small fraction of such capacity for compensatory elevation/reduction of $K_s$-mediated $K^+$ flux, it is instructive that extracellular osmolarity in the heart may decline to $<70\%$ of intracellular osmolarity during post-ischaemic reperefusion, and increase by 10–20% during hypernatremia and up to 45% during acute severe hyperglycaemia.

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