Role of KCNQ1 in the Cell Swelling–Induced Enhancement of the Slowly Activating Delayed Rectifier K⁺ Current

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Abstract: Cell swelling enhances a slowly activating delayed rectifier K⁺ current (I_{Ks}) in cardiac cells. This investigation was undertaken to determine which of the two structural units reconstituting the I_{Ks} channel, KCNQ1 (KvLQT1) and KCNE1 (minK/IsK), plays a key role in the cell swelling–induced I_{Ks} enhancement and to dissect a possible involvement of tyrosine phosphorylation therein. KCNQ1 was transiently expressed alone or together with KCNE1 in a heterologous mammalian cell line. Two distinct whole-cell membrane currents were separately observed during the exposure of transfected cells to various degrees of hyposmotic solutions. A hyposmotic challenge (0.7 times control osmolarity) resulted in about a twofold increase not only in the heteromeric KCNQ1/KCNE1, but also in the homomeric KCNQ1 channel currents. There was no

significant difference in the incremental ratio of current amplitude in response to hyposmotic stress between the two KCNQ1-related currents, and the cells expressing the heteromeric channels swelled less than those with the homomeric channels or without the exogenous ones. The cell swelling–induced I_{Ks} enhancement was not affected by a protein tyrosine kinase (PTK) inhibitor, by genistein (50 μ M), or by an inhibitor of phosphotyrosine phosphatase (PTP), orthovanadate (500 μ M), or a nonhydrolyzable ATP analogue, AMP-PNP (5 mM). Taken together, it is very likely that KCNQ1 might primarily participate in the I_{Ks} enhancement by osmotic cell swelling. The obligatory dependence of the I_{Ks} augmentation on PTK activity remained to be demonstrated, at least, in this expression system. [Japanese Journal of Physiology, 52, 31–39, 2002]

Key words: cell swelling, slowly activating delayed rectifier K⁺ current (I_{Ks}), KCNQ1, KCNE1, tyrosine phosphorylation.

Cell swelling alters the electrophysiological properties in cardiac myocytes [1]. Previous studies reported that an exposure of cardiac cells to anisomolar solutions led to the shortening of action potential duration (APD) in bullfrog and guinea pig ventricles [2, 3]. Although many ion conductances contribute to cardiac action potentials, APD is pivotally determined by a variety of transient or sustained K⁺ currents [4]. The ionic basis of the changes in APD, however, has yet to be fully understood.

To date, experimental studies using patch-clamp techniques have shown that hyposmotically induced

cell swelling in isolated cardiomyocytes modulates several different ion channels and transporters, including the swelling-induced Cl[−] channel [5], the K_{ATP} channel [6], the Na⁺/K⁺ pump [7], and the Na⁺/Ca²⁺ exchanger [8]. In particular, present interest in the cell swelling–induced enhancement of the delayed rectifier K⁺ current (I_K) was triggered from the observations of Sasaki *et al.* [9, 10] that in guinea pig ventricular myocytes, hyposmotic stress stimulates its activity. On the basis of the kinetic and pharmacological properties of I_K , it can be separated into two components in different cardiomyocytes of various species: a rapidly

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activating component (I_{Kr}) and a more slowly activating component (I_{Ks}) [11, 12]. In this connection, Rees *et al.* have elegantly demonstrated that in guinea pig ventricular myocytes, I_{Ks} is potentiated, but I_{Kr} is inhibited by cell swelling, indicating that it has differential effects on these two components [13].

Recently, a pioneering study that defined the genetic defects behind the long QT syndrome type 1 (LQT1) has facilitated the identification of the molecules responsible for I_{Ks} [14]. It is now accepted that the two structurally different membrane proteins, the pore-forming α -subunit KCNQ1 and the regulatory β -subunit KCNE1, reconstitute the I_{Ks} channel [15, 16]. Thus the present study was initiated to determine which of the two distinct subunits plays a key role in the cell swelling-induced I_{Ks} enhancement.

Given the range of regulatory factors that can modulate the activity of I_{Ks} , including protein kinase A (PKA), protein kinase C (PKC) [17], and intracellular Ca^{2+} [18], little has been unraveled about underlying mechanisms, whereby osmotic cell swelling activates the I_{Ks} channel. Noticeably, Zhou *et al.* suggested that in canine ventricular myocytes, protein tyrosine kinase (PTK) participated in the I_{Ks} enhancement by cell swelling [19], though it remains unanswered whether the channel itself or an intermediate substrate in the regulatory pathway is phosphorylated. Therefore we further attempt to dissect a possible involvement of tyrosine phosphorylation and dephosphorylation as the major mechanism in the I_{Ks} augmentation.

METHODS

Expression constructs for KCNQ1 and KCNE1. Both expression vectors of pCI-KCNQ1, which contains a full-length human KCNQ1 cDNA (GenBank AF000571) in pCI vector (Promega, Madison, WI, USA), and pIRES/CD8-KCNE1, which carries a human KCNE1 cDNA (GenBank M26685) in pIRES/CD8 vector, were generously provided by Dr. J. Barhanin (Institut de Pharmacologie Moléculaire et Cellulaire, CNRS, France). The restriction fragment *NheI*–*XmaI* containing the KCNQ1 coding region was subcloned into pIRES2/EGFP plasmid (Clontech, Palo Alto, CA, USA) for its sole expression study.

Cell culture and electrophysiological recordings. COS7 cells were seeded at a density of 3×10^4 cells per 35 mm petri dish, then cultured for 12 to 24 h before transfection in Dulbecco's modified Eagle medium (Gibco-BRL, Rockville, MD, USA) supplemented with 10% fetal bovine serum (Gibco-BRL). The cells were transiently transfected with KCNQ1 alone or together with KCNE1, using the

LipofectAMINE method according to the manufacturer's instructions (Gibco-BRL). The amount of the plasmids employed here was 1 μ g of pIRES2/EGFP-KCNQ1 for the homomeric KCNQ1 channel and 1 μ g of pIRES/CD8-KCNE1 for the reconstitution of the heteromeric KCNQ1/KCNE1 (I_{Ks}) channel. For electrophysiological experiments 24 to 48 h later, transfected cells were easily visualized with fluorescence in expression experiments for KCNQ1 alone and further selected by decoration with anti-CD8 antibody-coated beads (Dynabeads CD8, Dynal Biotech., Norway) in coexpression with KCNE1.

The tight-seal, whole-cell, voltage-clamp configuration of the patch-clamp technique was used for electrophysiological recordings. Pipettes were prepared from thin-walled borosilicate glass capillaries (Narishige, Tokyo, Japan) on a Narishige PP83 electrode puller (Narishige). The resistances of the pipettes ranged from 3.0 to 5.0 M Ω when filled with the pipette solutions described below. A tight seal between the cell membrane and the pipette tip (seal resistance, >5 G Ω) was achieved by a gradual application of light suction. After the formation of a tight seal, the membrane under the pipette tip was ruptured by a further increase in suction, to form the whole cell-recording configuration. An Axopatch 200A amplifier (Axon Instruments, Foster City, CA, USA) was used for voltage clamping. Commercial software from Axon Instruments (pCLAMP 6) was used for the generation of voltage-clamp protocols, data acquisition, and data analysis. Data were filtered at 1 kHz by a filter incorporated in the Axopatch 200A amplifier and stored on the hard disk of an IBM-compatible computer (IBM, Tokyo, Japan) for later analysis. The liquid junction potential between the pipette solution and the external one was not corrected for any membrane potential recordings. An exchange of external solutions was performed by switching the perfusates at the inlet of the recording chamber, and the time for a total exchange of solutions in the chamber was ~ 1 min. All experiments were conducted at $37 \pm 1^\circ\text{C}$.

During these experiments, the patch-clamped COS7 cells displayed a concomitant increase in background membrane conductance that was manifested as an outward shift of the instantaneous current level during a hypotonic challenge. Plasmid control-transfected cells produced only a small amplitude of the background conductance in response to depolarizing voltage pulses under the control condition, and superfusion with hypotonic solutions was accompanied by a gradual increase in a time-independent, outward conductance on step depolarization. Because it was negligibly small in current amplitude (~ 0.3 nA), in this

study the changes of those time-dependent currents were estimated as the difference in current amplitude between their values measured at the start and at the end of depolarizing pulses.

The changes of the cell volume during the osmotic cell swelling were approximated by geometric measurements [20]. According to previous studies [20, 21], we measured the cell area under the microscope as an approximation of the cell volume. The image of the single transfected cell under the whole cell voltage clamp was recorded with a video recorder, and the cell area was measured on a computer by the use of software of the NIH image (NIH, Bethesda, MD, USA).

Solutions and drugs. The composition of the 70% hyposmotic solution was (in mM) NaCl 100, KCl 5.4, CaCl_2 2, MgCl_2 1, and HEPES 5, and the pH was adjusted to pH 7.4 with NaOH. The control (isosmotic) solution with comparable ionic compositions was prepared by supplementing the 70% hyposmotic medium with 80 mM mannitol. The 80 and 90% hyposmotic solutions were prepared by adding each amount of mannitol to the 70% hyposmotic solution, 26.7 and 53.3 mM, respectively. The pipette solution contained (in mM) KCl 140, MgCl_2 2, EGTA 5, HEPES 10, and Mg-ATP 5 (pH 7.3 with KOH). In some experiments, genistein (50 μM , Sigma, St. Louis, MO, USA), forskolin (10 μM , Sigma), and IBMX (3-isobutyl-1-methylxanthine) (100 μM , Sigma) were employed in the external solutions, and sodium orthovanadate (500 μM , Wako, Osaka, Japan) was included in the pipette solution. In other experiments, AMP-PNP (5 mM, Sigma) was substituted for Mg-ATP in the pipette solution.

Statistical analysis. Numerical data are presented as means $\pm$ SEM (for n observations). Comparisons are made by using the Student's t -test and analysis of variance (ANOVA). Statistical differences were considered significant when $p < 0.05$.

RESULTS

Cell swelling enhances the homomeric KCNQ1 channel current

Cell swelling during superfusion with hyposmotic solutions increases I_{Ks} in guinea pig [10, 13, 22] and canine [19] ventricular myocytes. We first focused on the effect of hyposmotic stress on the homomeric KCNQ1 channel and compared it with that on the heteromeric KCNQ1/KCNE1 (I_{Ks}) channel. The representative whole-cell membrane current recordings were obtained from cells transfected with both KCNQ1 and KCNE1 (Fig. 1A and B), or KCNQ1 alone (Fig. 1E and F), initially when superfused with the control

solution (Fig. 1A and E), and subsequently while challenged with the 70% hyposmotic medium (Fig. 1B and F).

As previously reported [15, 16], the heteromeric KCNQ1/KCNE1 channel generated a slowly activating and noninactivating K^+ current, which is typical of cardiac I_{Ks} (Fig. 1A). In contrast, the homomeric KCNQ1 channel yielded a rapidly activating outward current much smaller in amplitude than that seen with a co-expression of KCNQ1 and KCNE1 (Fig. 1E; note that the scales for time and current amplitude are different between the upper and lower panels). Like the behavior of native I_{Ks} in cardiac cells, the time-dependent outward currents of the heteromeric KCNQ1/KCNE1 channel increased during the hyposmotic challenge (Fig. 1B). The noticeable finding was that the same hyposmotic stress equally induced a comparable manner of augmentation in the homomeric KCNQ1 channel current (Fig. 1F).

The averaged steady-state I - V relationships for the two KCNQ1-related currents in the multiple cells are illustrated in Fig. 1C and G. As for the heteromeric KCNQ1/KCNE1 channel, the respective normalized activation curves fitted to Boltzmann's equation obtained in the control and hyposmotic conditions were almost superimposed (Fig. 1D).

To confirm the close relationship between osmotic cell swelling and the resultant enhancement of the currents for the heteromeric KCNQ1/KCNE1 and the homomeric KCNQ1 channels, we separately examined the effects of various degrees of hyposmotic challenge on (1) the cell volume and (2) the two KCNQ1-related currents. Figure 2A shows the relationship between the hyposmotic stress and the corresponding incremental ratio of the cell volume. The magnitude of cell swelling was smaller in transfected cells expressing the heteromeric KCNQ1/KCNE1 channels than in those with the homomeric KCNQ1 channels or without exogenous channels in the 3 different hyposmotic conditions. There was no significant difference in the incremental ratio of cell volume between transfected cells with the homomeric KCNQ1 channels and those without exogenous ones.

Figure 2B summarizes the relations between various hyposmotic stress and the corresponding percent increase of current amplitude in the two KCNQ1-related channels measured at +50 mV during superfusion with each of the hyposmotic solutions. The greater hyposmotic stress resulted in the greater increase in each of the two currents, and there was no significant difference in incremental percentage of current amplitude between the two currents.

Fig. 1. Cell swelling enhances not only the heteromeric KCNQ1/KCNE1 channel current, but also the homomeric KCNQ1 channel current in COS7 cells. The currents for the heteromeric KCNQ1/KCNE1 channel (**A** and **B**) and the homomeric KCNQ1 channel (**E** and **F**) were recorded under the control solution (**A** and **E**) and during superfusion with the 70% hypotonic medium (**B** and **F**). Depolarizing test pulses of different durations (2 or 0.5 s) were applied in 10 mV increments from -30 to $+40$ mV, followed by a return to -30 mV. In multiple transfected cells, the averaged steady-state I - V relations for the former (**C**) and the

latter (**G**) were measured from -30 to $+60$ mV under the control solution (open symbols) and the 70% hypotonic medium (closed symbols). To determine the voltage-dependence of activation for the heteromeric KCNQ1/KCNE1 channel, the deactivating tail currents were used for analysis, and data points were fitted to a Boltzmann distribution using calculated maximum values (**D**). The half-activation voltage $V_{0.5}$ and slope factor V_s are 35.8 ± 1.7 mV, 13.7 ± 0.5 mV in the control condition and 32.6 ± 1.5 mV, 13.4 ± 0.3 mV in the 70% hypotonicity ($p=0.15$, $p=0.72$, respectively; $n=14$).

Is tyrosine phosphorylation a key step in the I_{Ks} enhancement by cell swelling?

As stated earlier, an activity of PTK has been suggested to be responsible for the cell swelling-induced enhancement of I_{Ks} in canine ventricular myocytes [19]. Thus we employed pharmacological maneuvers that could modulate a putative PTK-mediated pathway pertaining to the I_{Ks} enhancement and thereby attempted to determine whether tyrosine phosphorylation and dephosphorylation are key steps in the underlying mechanism.

At first, a broad spectrum PTK inhibitor, genistein, was used in this expression system as examined in canine ventricular myocytes [19]. Figure 3A illustrates a time course of changes in current amplitude of the heteromeric KCNQ1/KCNE1 channel measured at the end of 1-s test pulses at 0 mV. The exposure of a transfected cell expressing the reconstituted I_{Ks} channel to the 70% hypotonic medium (HYPO) rapidly elicited about a twofold increase in the I_{Ks} current (Fig. 3A-a). Switching back to the control solution readily reduced it to its previous level, although there was a slight cur-

rent rundown (Fig. 3A-b). A subsequent application of $50 \mu\text{M}$ genistein resulted in a further reduction in amplitude (Fig. 3A-c). In the continuous presence of the PTK inhibitor; however, a re-exposure to the 70% hypotonic medium unaffectedly augmented the I_{Ks} current (Fig. 3A-d). After being superfused with the control solution, a washout of genistein allowed it to regain the current level comparable to that in the absence of the drug (Fig. 3A-e). A challenge with the 70% hypotonic medium was repeatedly able to produce about a twofold increase in the I_{Ks} current (Fig. 3A-f).

If tyrosine phosphorylation is a key step in the cell swelling-induced I_{Ks} enhancement, the rapid reversibility of its amplitude upon removal of the hypotonic challenge (e.g., see Fig. 3A, a $\rightarrow$ b) might be accounted for by a counteracting process of tyrosine dephosphorylation by PTP. On this assumption, the cell swelling-induced augmentation of the I_{Ks} current might be sustained as long as a concomitant activity of PTP is suppressed by its potent inhibitor, orthovanadate.

A **B**

Fig. 2. Effects of various degrees of hyposmotic stress on the cell area and the two KCNQ1-related currents. The relationships between the various degrees of hyposmotic stress and the cell area are shown in the cells transfected with plasmid control ($n=10$), KCNQ1 alone ($n=8$), or both KCNQ1 and KCNE1 ($n=9$) (**A**). The corresponding enhancement of the currents induced by the respective hyposmotic challenge are compared between the heteromeric KCNQ1/KCNE1 (90%, $n=23$; 80%, $n=13$; 70%, $n=10$) and the homomeric KCNQ1 channels (90%, $n=19$; 80%, $n=14$; 70%, $n=8$) (**B**). The time-dependent currents were measured as the difference at the start and the end of 2-s test pulses at +50 mV from a holding potential of -70 mV before and during superfusion with various hyposmotic solutions, and the incremental magnitude is shown as a percentage.

As shown in Fig. 3B, however, intrapipette 500 μ M orthovanadate failed to sustain the hyposmotically induced increase of the current obtained during the 70% hyposmotic challenge. In contrast, its PKA-dependent augmentation lasted over 4 min even after a washout of two PKA activators, forskolin (10 μ M) and IBMX (100 μ M) (to the right of Fig. 3B). As previously re-

ported [23], the latter part of the observation warranted that this concentration of orthovanadate was substantially effective in inhibiting not only PTP, but also phosphoserine/threonine phosphatases. As summarized in Fig. 3C, neither genistein nor orthovanadate appeared to affect the I_{Ks} enhancement by osmotic cell swelling.

From the above results, the cell swelling-induced I_{Ks} enhancement appeared very unlikely to depend on PTK activity. Since PTKs do not efficiently utilize hydrolyzable nucleotide triphosphates other than ATP as a phosphate donor for phosphorylation [24], we finally examined the effect of AMP-PNP, a nonhydrolyzable ATP analog that acts as a competitive inhibitor against ATP-dependent enzymes.

Figure 4A depicts a time course of changes in the current amplitude of the reconstituted I_{Ks} channel measured at the end of 1-s test potential of 0 mV in the continuous presence of 5 mM AMP-PNP. The changes were observed initially in response to PKA stimulation and subsequently during a superfusion with the 70% hyposmotic medium. The PKA activators produced only a slight increase in the I_{Ks} current (to the left of Fig. 4A), indicating that AMP-PNP mostly blocked the PKA-dependent I_{Ks} augmentation (see Fig. 3B). In contrast, in accordance with the experiments in Fig. 3 the 70% hyposmotic challenge was seen to elicit about a twofold increase in the current despite the presence of this compound (to the right of Fig. 4A). Figure 4B summarizes the differential effects of AMP-PNP on the PKA-dependent and the cell swelling-induced I_{Ks} enhancement, suggesting that the I_{Ks} enhancement by cell swelling might not be associated with ATP-dependent phosphorylation.

DISCUSSION

Role of KCNQ1 in the cell swelling-induced I_{Ks} enhancement. The initial goal of this study was to determine which of the two structural units of the I_{Ks} channel plays a key role in the cell swelling-induced I_{Ks} enhancement. We have shown that osmotic cell swelling augments the heteromeric KCNQ1/KCNE1 channel current. This phenomenon is in agreement with the behavior of native I_{Ks} observed in guinea pig and canine ventricular myocytes [10, 13, 19, 22], though the cellular background is quite different between cultured heterologous cells and isolated cardiomyocytes. It remains unclear whether the phenomenon is ubiquitously seen in whatever type of cells the I_{Ks} channel exists; however, it would appear unlikely that intracellular components specific to cardiomyocytes are necessary for the I_{Ks} regulation. It is

interesting that hyposmotic stress was equally capable of enhancing the homomeric KCNQ1 channel current. There was no significant difference in the incremental ratio of current amplitude in response to various hyposmotic challenges between the heteromeric KCNQ1/KCNE1 and homomeric KCNQ1 channel currents. Our data suggest that KCNQ1 might primarily participate in the cell swelling-induced I_{Ks} enhancement.

In the present study, we further explored a possible involvement of tyrosine phosphorylation and dephosphorylation in the cell swelling-induced I_{Ks} enhancement in a heterologous expression system. The tyrosine kinase inhibitor genistein (50 μ M) [25] diminished the baseline amplitude of the I_{Ks} current; still it

failed to completely abolish augmentation of the I_{Ks} by osmotic cell swelling (Fig. 3A and C). Our observations were apparently in contrast with those of Zhou *et al.* that the cell swelling-induced enhancement of canine I_{Ks} was totally blocked by the PTK inhibitor at the same concentration as the one tested in our experiments [19]. These results, however, might be due to differences in the expression system examined (cloned or native), experimental conditions, and perhaps species (human or canine). It is noteworthy that since genistein can reduce I_{Ks} activity independently of PTK in guinea pig ventricular myocytes [26], the additional action of the PTK inhibitor might have led to the apparent conflict of these results. The drug-induced suppression of the I_{Ks} in our preparations (Fig. 3A) was like that seen in canine ventricular myocytes [19]. To settle this issue, it might be helpful to examine the effects of other equally potent PTK inhibitors on the I_{Ks} enhancement.

Orthovanadate (500 μ M), widely used to enhance tyrosine phosphorylation, did not disturb the rapid reversibility of the I_{Ks} upon the removal of a hyposmotic challenge (Fig. 3B and C). AMP-PNP (5 mM) clearly failed to block the I_{Ks} enhancement by osmotic cell swelling (Fig. 4). This argues against a possible role of protein kinases, including PTK, which require ATP for their action as a dominant regulator in the I_{Ks} augmentation. Although it may be premature to conclude that neither ongoing phosphorylation nor dephosphorylation is involved in the cell swelling-induced I_{Ks} enhancement, its obligatory dependence on PTK activity remained to be demonstrated, at least in our

Fig. 3. Neither inhibitor of PTK nor PTP affects the I_{Ks} enhancement by osmotic cell swelling. **A:** Time course of changes in the reconstituted I_{Ks} current amplitude during superfusion with the 70% hyposmotic medium (HYPO) in the absence or presence of 50 μ M genistein. See the text for the definition of letters a to f in this panel. **B:** Time course of changes in the current amplitude, initially during exposure to the 70% hyposmotic medium (HYPO), and subsequently during the application of two PKA activators, 10 μ M forskolin, and 100 μ M IBMX, in the presence of 500 μ M orthovanadate. In both A and B, the changes of time-dependent currents are represented by the difference in current amplitude between the 2 values measured at the start and end of 1-s depolarizing steps to 0 mV applied from a holding potential of -70 mV. The pulse step was repeated every 5 s. Periods of solution exchanges and an application of the drugs are denoted by horizontal bars. The definition of the letters a to e in this panel is also referred to the text. **C:** The effects of genistein and orthovanadate on the I_{Ks} enhancement by osmotic cell swelling are compared between the control condition (left bar, $n=11$), 50 μ M genistein (right bar, $n=10$), and 500 μ M orthovanadate (middle bar, $n=10$).

A **B**

Fig. 4. No ATP-dependent phosphorylation is required in the cell swelling-induced I_{Ks} enhancement. **A:**

A time course of changes in the I_{Ks} current is observed, initially during the application of the PKA activators, and subsequently, during exposure to the 70% hyposmotic medium (HYPO) in the presence of 5 mM AMP-PNP. The pulse protocol and recording intervals are the same as used in the experiment of Fig. 3, and the changes of time-dependent currents are represented by the difference in current amplitude between the 2 values measured at the start and end of depolarizing pulses. Horizontal bars denote the periods of exposure to the drugs and solution changes. The letters a to e in this panel are defined in the text. **B:** The effects of AMP-PNP on the I_{Ks} augmentation caused by PKA stimulation (forskolin+IBMX) in the presence of ATP (left bar, $n=13$), and AMP-PNP (second bar, $n=9$), or exposure to the 70% hyposmotic medium (HYPO) in the presence of ATP (third bar, $n=11$), and AMP-PNP (right bar, $n=16$).

preparations.

Physiological and pathological implications of the cell swelling-induced I_{Ks} enhancement in cardiac cells. As shown in the experiments of

Fig. 2A, the cells expressing the heteromeric KCNQ1/KCNE1 channel swelled less than those with the homomeric KCNQ1 channel or without exogenous channels. Several studies have shown that mammalian cardiac cells swell rapidly when exposed to hyposmotic media, but the reported data for volume regulation during a hyposmotic challenge in those cells are inconclusive [27]. Previous experiments in vestibular dark cells showed that the I_{sK} channel, a counterpart of the cardiac I_{Ks} channel, is volume-sensitive and that it might possibly be involved in the volume regulation of the cells during a hyposmotic challenge [28, 29]. In most cells, a regulatory decrease in cell volume is accomplished by the loss of potassium chloride released as inorganic osmolytes through separate potassium and chloride channels or the K^+-Cl^- cotransporter [30]. In the transfected cells examined here, the cell swelling-induced incremental percentage was not significantly different between the current for the heteromeric KCNQ1/KCNE1 channel and that for the homomeric KCNQ1 channel. Nevertheless, there was an even larger net K^+ efflux in the cells expressing the former than in those expressing the latter, as indicated in measured current amplitude. The I_{Ks} channel might therefore be contributory to the volume regulation.

Moreover, COS7 cells most likely have swelling-activated Cl^- channels [31]. In our experiments, the estimated equilibrium potentials for K^+ (E_K) and Cl^- (E_{Cl}) were about -87 and $+7$ mV, respectively. Assuming that their resting membrane potential ranges between these two values, the opening of swelling-activated Cl^- channels evokes an inward current as a result of the efflux of cytosolic Cl^- from the cells. Although this current could contribute to a depolarization of the cell membrane, it would be offset by the concurrent K^+ efflux through the heteromeric KCNQ1/KCNE1 channel that might re-establish the resting membrane potential.

The significance of cell swelling-induced I_{Ks} enhancement might be better understood under pathological conditions. During ischemia and reperfusion, cardiac cells have been shown to undergo significant swelling [32], which has marked effects on cardiac myocytes electrophysiology by modulating the function of several of sarcolemmal ion channels and transporters [1]. In particular, the activation of I_{Ks} would promote the repolarization of cell membrane and the shortening of the action potential duration, thereby resulting in a decrease in the refractory period, which can contribute to the genesis of, or facilitate the perpetuation of, reentrant arrhythmias [33]. This implication is of more clinical importance because KCNQ1 may represent the more specific molecular target of

the two subunits of the I_{Ks} channel for designing novel I_{Ks} selective antiarrhythmic agents that are more efficacious during myocardial ischemia and reperfusion.

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REFERENCES

- Vandenberg JI, Rees SA, Wright AR, and Powell T: Cell swelling and ion transport pathways in cardiac myocytes. *Cardiovasc Res* 32: 85–97, 1996
- Kawata H, Kawagoe K, and Tateyama I: Effects of osmolarity change on the excitation contraction coupling of bullfrog ventricle. *Jpn J Physiol* 24: 587–603, 1974
- Ehara T and Hasegawa J-I: Effects of hypertonic solution on action potential and input resistance in the guinea-pig ventricular muscle. *Jpn J Physiol* 33: 151–167, 1983
- DeFelice LJ, Goolsby WN, and Mazzanti M: Potassium channels and the repolarization of cardiac cells. *Ann N Y Acad Sci* 588: 174–184, 1990
- Tseng GN: Cell swelling increases membrane conductance of canine cardiac cells: evidence for a volume-sensitive Cl channel. *Am J Physiol* 262: C1056–C1068, 1992
- Van Wagoner DR: Mechanosensitive gating of atrial ATP-sensitive potassium channels. *Circ Res* 72: 973–983, 1993
- Whalley DW, Hool LC, Ten Eick RE, and Rasmussen HH: Effect of osmotic swelling and shrinkage on Na^+ - K^+ pump activity in mammalian cardiac myocytes. *Am J Physiol* 265: C1201–C1210, 1993
- Wright AR, Rees SA, Vandenberg JI, Twist VW, and Powell T: Extracellular osmotic pressure modulates sodium-calcium exchange in isolated guinea-pig ventricular myocytes. *J Physiol (Lond)* 488: 293–301, 1995
- Sasaki N, Mitsuiye T, and Noma A: Effects of mechanical stretch on membrane currents of single ventricular myocytes of guinea-pig heart. *Jpn J Physiol* 42: 957–970, 1992
- Sasaki N, Mitsuiye T, Wang Z, and Noma A: Increase of the delayed rectifier K^+ and Na^+ - K^+ pump currents by hypotonic solutions in guinea pig cardiac myocytes. *Circ Res* 75: 887–895, 1994
- Sanguinetti MC and Jurkiewicz NK: Two components of cardiac delayed rectifier K^+ current. Differential sensitivity to block by class III antiarrhythmic agents. *J Gen Physiol* 96: 195–215, 1990
- Horie M, Hayashi S, and Kawai C: Two types of delayed rectifying K^+ channels in atrial cells of guinea pig heart. *Jpn J Physiol* 40: 479–490, 1990
- Rees SA, Vandenberg JI, Wright AR, Yoshida A, and Powell T: Cell swelling has differential effects on the rapid and slow components of delayed rectifier potassium current in guinea pig cardiac myocytes. *J Gen Physiol* 106: 1151–1170, 1995
- Wang Q, Curran ME, Splawski I, Burn TC, Millholland JM, VanRaay TJ, Shen J, Timothy KW, Vincent GM, de Jager T, Schwartz PJ, Towbin JA, Moss AJ, Atkinson DL, Landes GM, Connors TD, and Keating MT: Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. *Nat Genet* 12: 17–23, 1996
- Barhanin J, Lesage F, Guillemare E, Fink M, Lazdunski M, and Romey G: KVLQT1 and IsK (minK) proteins associate to form the I_{Ks} cardiac potassium current. *Nature* 384: 78–80, 1996
- Sanguinetti MC, Curran ME, Zou A, Shen J, Spector PS, Atkinson DL, and Keating MT: Coassembly of KVLQT1 and minK (IsK) proteins to form cardiac I_{Ks} potassium channel. *Nature* 384: 80–83, 1996
- Lo CF and Numann R: Independent and exclusive modulation of cardiac delayed rectifying K^+ current by protein kinase C and protein kinase A. *Circ Res* 83: 995–1002, 1998
- Busch AE and Maylie J: Effects of calcium buffers and temperature on the receptor-mediated regulation of the slowly activating potassium current (I_{Ks}) in guinea pig cardiac myocytes. *Cell Physiol Biochem* 4: 1–9, 1994
- Zhou YY, Yao JA, and Tseng GN: Role of tyrosine kinase activity in cardiac slow delayed rectifier channel modulation by cell swelling. *Pflügers Arch* 433: 750–757, 1997
- Drewnowska K and Baumgarten CM: Regulation of cellular volume in rabbit ventricular myocytes: bumetanide, chlorothiazide, and ouabain. *Am J Physiol* 260: C122–C131, 1991
- Wang Z, Mitsuiye A, and Noma A: Cell distension-induced increase of the delayed rectifier K^+ current in guinea pig ventricular myocytes. *Circ Res* 78: 466–474, 1996
- Groh WJ, Gibson KJ, and Maylie JG: Hypotonic-induced stretch counteracts the efficacy of the class III antiarrhythmic agent E-4031 in guinea pig myocytes. *Cardiovasc Res* 31: 237–245, 1996
- Zhou SS, Takai A, Tominaga M, and Okada Y: Phosphatase-mediated enhancement of cardiac cAMP-activated Cl^- conductance by a Cl^- channel blocker, anthracene-9-carboxylate. *Circ Res* 81: 219–228, 1997
- Hunter T and Cooper JA: Protein-tyrosine kinases. *Annu Rev Biochem* 54: 897–930, 1985
- Akiyama T and Ogawara H: Use and specificity of genistein as inhibitor of protein-tyrosine kinases. *Methods Enzymol* 201: 362–370, 1991
- Washizuka T, Horie M, Obayashi K, and Sasayama S: Genistein inhibits slow component delayed-rectifier K currents via a tyrosine kinase-independent pathway. *J Mol Cell Cardiol* 30: 2577–2590, 1998
- Deaton LE: Comparative aspects of cellular-volume regulation in cardiomyocytes. *Physiol Zool* 70: 379–390, 1997
- Wangemann P, Liu J, Shen Z, Shipley A, and Marcus DC: Hypo-osmotic challenge stimulates transepithelial K^+ secretion and activates apical IsK channel in vestibular dark cells. *J Membr Biol* 147: 263–273, 1995
- Shiga N and Wangemann P: Ion selectivity of volume regulatory mechanisms present during a hypoosmotic challenge in vestibular dark cells. *Biochim Biophys*

- Acta 1240: 48–54, 1995
30. O'Neill WC: Physiological significance of volume-regulatory transporters. *Am J Physiol* 276: C995–C1011, 1999
 31. Ishii T, Hashimoto T, and Ohmori H: Hypotonic stimulation induced Ca^{2+} release from IP_3 -sensitive internal stores in a green monkey kidney cell line. *J Physiol (Lond)* 493: 371–384, 1996
 32. Garcia-Dorado D and Oliveras J: Myocardial oedema: a preventable cause of reperfusion injury? *Cardiovasc Res* 27: 1555–1563, 1993
 33. Wit AL and Coromilas J: Role of alterations in refractoriness and conduction in the genesis of reentrant arrhythmias. Implications for antiarrhythmic effects of class III drugs. *Am J Cardiol* 72: 3F–12F, 1993