Cell Swelling Has Differential Effects on the Rapid and Slow Components of Delayed Rectifier Potassium Current in Guinea Pig Cardiac Myocytes

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ABSTRACT Cell swelling has been shown to cause activation of a variety of cardiac sarcolemmal ionic conductances including potassium channels. The aim of this study was to investigate the effect of swelling on the two subtypes of delayed rectifier potassium current (Iₖᵣ and Iₖₛ) in single guinea pig myocytes using the whole-cell configuration of the patch clamp technique. When the holding potential was set at -40 mV and stepped to +40 mV for 1 s under isoosmotic conditions (300 mOsm) a delayed rectifier current (Iₖ) was activated (0.86 ± 0.05 nA; n = 43). Switching to a hypoosmotic solution (200 mOsm) caused a rapid increase in Iₖ to a mean value of 1.43 ± 0.10 nA (p < 0.05; n = 43). The effect of swelling on the two subtypes of Iₖ was studied by analysis of deactivating tail currents using an envelope of tails protocol (stepping from -40 to +40 mV for 18 different pulse durations between 50 ms and 2.9 s; n = 16). Swelling caused a decrease in current amplitude measured at the end of the pulse (and Iₖᵣ) at short durations (< 150 ms) however, when the pulse duration was >1 s swelling caused a significant increase in current. Using a pulse protocol to measure Iₖᵣ with minimal contamination by Iₖₛ (voltage step from -40 to -10 mV for 250 ms) a 50-100 pA current was elicited which could be completely blocked by dofetilide (0.2 µM; n = 3). Introduction of hypoosmotic solution caused a significant decrease in Iₖᵣ and when dofetilide (0.2 or 1.0 µM) was introduced the current remaining was decreased further (p < 0.05; n = 5), but was not completely blocked, thus suggesting that swelling had decreased the ability of dofetilide to block Iₖᵣ. Similar results were obtained over a range of dofetilide concentrations and with a second Iₖᵣ blocker, La³⁺. In Ca²⁺-free external solutions, pulsing to -10 mV for 500 ms to measure Iₖᵣ in the absence of Iₖₛ, and to +60 mV for 5 s (with 0.2 µM dofetilide) to evoke only Iₖₛ, it was clear that swelling significantly increased Iₖᵣ (pulse and tail currents) and decreased Iₖₛ. In addition, when measured using the perforated patch

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method, swelling modulated $I_{K}$ and $I_{K0}$ in a similar fashion. We conclude that swelling has differential effects on the subtypes of the classical cardiac $I_{K}$, which may have important implications in our understanding of the mechanisms underlying ischaemia- and reperfusion-induced arrhythmogenesis.

INTRODUCTION

In general, mammalian cells cannot withstand differences in osmolality between the intracellular and extracellular spaces, as water moves rapidly across the plasma-lemma leading to cell shrinkage or swelling. Swelling of cardiac myocytes occurs during acute myocardial ischaemia (Tranum-Jensen, Jarne, Fiolet, Krieger, D'Aloncourt, and Durrer, 1981) and this is exacerbated after reperfusion (Jennings, Schaper, Hill, Steenbergen, and Reimer, 1985). During the ischaemic period there is an intracellular accumulation of metabolites, such as lactate, which leads to an increase in tissue osmolality (Tranum-Jensen et al., 1981), so that when reperfusion occurs it is accompanied by an inward movement of water, increasing cell volume. A number of potassium ($K^{+}$) channels, which are the focus of this paper, have been shown to be modulated by cell swelling/stretch. Swelling or stretching ventricular myocytes has been shown to increase delayed rectifier $K^{+}$ current ($I_{K}$; Sasaki, Mitsuiye, and Noma, 1992 and Sasaki, Mitsuiye, Wang, and Noma, 1994). Van Wagner (1993) has demonstrated that in rat atrial myocytes the ATP-dependent $K^{+}$ channel ($I_{KATP}$) was activated by cell stretch (applying negative pressure via the pipette) and by osmotic cell swelling. Thus, it appears that $K^{+}$ channels, which play an important role in the repolarisation of the action potential and in maintaining resting membrane potential, are perturbed by changes in cell volume, perhaps contributing to arrhythmogenesis.

The aim of the present study was to investigate further the effect of cell swelling on $I_{K}$ and in particular to investigate the effect of this intervention on the two subtypes of $I_{K}$. Noble and Tsien (1969) were the first to speculate that $I_{K}$ may consist of two components which they termed $I_{K1}$ and $I_{K2}$. This was confirmed, pharmacologically and on the basis of channel kinetics, in guinea pig ventricular myocytes (Sanguinetti and Jurkiewicz, 1990a). Two components have been identified, one which is rapidly activated ($I_{K1}$) and one which is activated more slowly ($I_{K2}$). $I_{K}$ activates over the voltage range −40 to 0 mV and is sensitive to blockade by drugs such as E-4031. $I_{K1}$ activation occurs over a voltage range typical of the classically described cardiac $I_{K}$ (at potentials more positive than −10 mV) and is not blocked by E-4031 (Sanguinetti and Jurkiewicz, 1990a). In the present study we have made use of dofetilide which has been shown to be a potent and highly selective blocker of $I_{K}$, with no effect on $I_{K1}$ (Jurkiewicz and Sanguinetti, 1993), $La^{3+}$ (Sanguinetti and Jurkiewicz, 1990b) and UK696,914 which appears to block $I_{K2}$ and $I_{K}$ (Gwilt, Dalrymple, Burges, Blackburn, Dickinson, Cross, and Higgins, 1991) to investigate the effect of cell swelling on the two subtypes of $I_{K}$. We have also used the envelope of tails test, which is a powerful technique for discriminating between the two $I_{K}$ subtypes in the absence of any other currents.

This work has been presented in part at the following meetings: "Ion Channels in the Heart" held at Tokyo Women's Medical College, Japan, July 1994, and the Brit-
ish Pharmacological Society meeting in Sunderland, UK, September, 1994 (Rees, Yoshida, Vandenberg, Twist, and Powell, 1994; Rees, Yoshida, Twist, Vandenberg, and Powell, 1995).

METHODS

Single ventricular myocytes were isolated from adult guinea pig hearts (Olac, UK) using an enzymatic dissociation technique as described previously in detail (Powell, Terrar, and Twist, 1980). Briefly, hearts were perfused in the Langendorff mode with nominally Ca²⁺-free Tyrode for 5 min. Then perfusion was continued with solution containing 1 mg/ml collagenase and 0.1 mg/ml protease. After a further 10 min, the heart was removed from the perfusion apparatus and roughly chopped. The tissue was then incubated at 37°C for 20 min with enzyme-containing solution. Finally the tissue was centrifuged twice (22 g for 1 min) to remove all of the enzyme-containing solution and resuspended in culture medium.

An aliquot of cells was allowed to settle on a cover slip in a chamber mounted above an inverted microscope (Diaphot, Nikon, Japan). Myocytes were then superfused (~4 ml/min) with an isosmotic solution containing (in millimolar), 140.0 NaCl, 5.4 KCl, 1.8 CaCl₂, 0.5 MgCl₂, 0.33 NaH₂PO₄, 5.5 glucose and 5.0 HEPES (pH 7.4 with NaOH; ~35°C). The composition of the hypoosmotic solution was identical except for a reduced NaCl concentration (100 mM). Osmolality of the isosmotic and hypoosmotic solutions, measured using a freezing point depression osmometer (Automatic Osmometer, Camlab, UK) were ~300 and ~200 mOsm, respectively. To exclude the possibility that any swelling-induced changes in current might occur as a result of changes in extracellular sodium (Na⁺) or chloride (Cl⁻), experiments were also performed in which osmolality was altered by switching from an isosmotic solution containing 100 mM NaCl plus 80 mM sucrose (300 mOsm) to 100 mM NaCl without sucrose (200 mOsm). Previous work from our laboratory has shown that superfusing cells with hypoosmotic solution rapidly causes cell swelling which is clearly observable using bright-field light microscopy (see Vandenberg, Yoshida, Kirk, and Powell, 1994).

ᵢₗ was recorded using the whole-cell configuration of the patch clamp technique (Hamill, Marty, Neher, Sakmann, and Sigworth, 1981) using an Axopatch-1C amplifier (Axon Instruments, USA). Pipettes with resistances of 1-3 MΩ, pulled using a two stage electrode puller (Narishige, Japan), were filled with internal solution containing (in millimolar), 140 K-aspartate, 5 MgCl₂, 5 K₄ATP, 10 EGTA and 5 HEPES (pH 7.4 with KOH). As no internal Na⁺ or Ca²⁺ was provided by the pipette solution Na⁺-K⁺ pump or Na⁺-Ca²⁺ exchange activity should be minimal. Also participation of the Na⁺-dependent K⁺ channel can be excluded, since it is only activated by high concentrations of internal Na⁺ (>20 mM; Kameyama, Kakei, Sato, Shibasaki, Matsuda, and Irisawa, 1984). The holding potential was set at −40 mV (thus, inactivating fast inward Na⁺ current) and after waiting for a period of 5–10 min (to ensure adequate cell dialysis), 𝐈ₗ was measured during a 1 s depolarizing step to +40 mV (sampling frequency 0.5 kHz). Series resistance was compensated electronically by ~70%. 𝐈ₗ was monitored for 10–15 min in ventricular myocytes superfused with isosmotic solution. After a stable baseline current had been established, the superfusate was switched from an isosmotic to a hypoosmotic solution. Exposure to hypoosmotic solution was maintained for <2 min, until any current changes had reached a plateau. The current-voltage (I-V) relationship before and after switching to hypoosmotic solution were obtained by stepping between −80 and +60 mV in 10 mV steps. The effect of hypoosmotic solution on 𝐈ₗ was also measured by analysis of tail currents using an envelope of tails protocol (Noble and Tsien, 1969). Tail currents were evoked by stepping from −40 to +40 mV for varying durations (range: 50 ms to 2.9 s, sampling frequency: 2.5 kHz and 0.5 kHz after 400 ms). The Ca²⁺ antagonist nisoldipine (0.3 μM) was included in solutions to ensure that 𝐈ₓ does not interfere with 𝐈ₗ tail currents measured after short pulses. The time constant of deactivation of tail currents (τ) was found by an exponential fit
$I(t) = A \exp\left(-\frac{t-k}{\tau}\right) + c$ obtained using the Chebyshev transformation (pClamp6) of the experimental record measured over a period of 1 s from the peak of the tail current.

In some experiments, whole-cell membrane currents were recorded using the perforated patch technique (Horn and Marty, 1988) as described by Rae, Cooper, Gates, and Watsky (1991) with 240 µg/ml amphotericin B (from a stock solution of 60 mg/ml in dry DMSO) added to the standard internal pipette solution (see above). Once a tight seal had formed between the cell and the pipette, tip suction was released and time was allowed for the patch to permeabilize. Access resistance was monitored by applying repetitive −10 mV voltage pulses from a holding potential of −80 mV and experiments were started once this reached values <30 MΩ. Series resistance compensation (up to ~70%) was applied. Under these conditions, current amplitudes were found to be stable over the experimental period.

To characterize further the current changes which occurred on switching solutions, two drugs were used which selectively block $I_K$ (UK66,914 (Gwilt et al., 1991) which blocks both subtypes (see Results section) and dovetilide which blocks $I_{Ca}$ selectively (Gwilt, Arrowsmith, Blackburn, Burges, Cross, Dalrymple, and Higgins, 1990; Jurkiewicz and Sanguinetti, 1993). UK66, 914 was used at a concentration of 10 µM, based on our own data (see below) and on information from Gwilt et al., (1991), who showed that at this concentration the drug prolongs action potential duration (an index of $K^+$ channel block) without affecting conduction time, maximum rate of depolarisation or action potential amplitude (indicating a lack of effect on the rapid inward $Na^+$ current). Also, from voltage clamp studies in guinea pig ventricular myocytes, UK66,914 at >2 µM blocks $I_{Na}$ fully without affecting $I_K$ or $Ca^{2+}$ current (Gwilt et al., 1991). Dofetilide was used at a concentration of 0.2 µM based on data from Gwilt et al., (1990) showing that this concentration of dovetilide blocks $I_K$ completely without affecting $I_{Na}$ or $Ca^{2+}$ current. These results have been confirmed more recently by Jurkiewicz and Sanguinetti, (1993), who performed a full concentration-response study for dovetilide in guinea pig ventricular myocytes and found that at 0.2 µM dovetilide blocked 85-90% of $I_K$ (which we have also confirmed, see below).

Data (current, voltage and temperature) were monitored as the experiment progressed on a pen recorder (model RS3200, Gould, UK) and current data were stored for further analysis on DAT tape (model DTC 1000ES, Sony) and on the hard disk of a computer (486D/33, Dell). Data was analyzed using software custom written in Quick Basic or using pClamp6.1 (Clampex for data acquisition and Clampfit for data analysis).

All results are expressed as mean ± SEM. Statistical significance on applying the $t$ test for paired data was taken at the $p < 0.05$ level.

**Materials**

Glass for pipettes (thin wall, 1.5 mm diam) was supplied by World Precision Instruments, Florida.

The collagenase (Worthington, type I) used for cell isolations was obtained from Lorne Laboratories, UK and protease (type XIV) was obtained from Sigma Chemical Co. (St. Louis, MO). All salts plus nifedipine, $LaCl_3$ and amphotericin B were obtained from Sigma Chemical Co. Nifedipine and $La^{3+}$ were dissolved in water and stored as 1 mM stock solutions. Amphotericin B was dissolved in DMSO and diluted for use in internal solution. Since it is photosensitive the usual precautions were taken to avoid exposure to light.

UK66,914 and dovetilide (previously known as UK68,798) were gifts from Pfizer Central Research, Kent, UK. Both drugs were dissolved in acidified 50% (vol/vol) ethanol/50% (vol/vol) water and were stored as 1 mM stock solutions. Nisoldipine was a gift from Bayer, Berkshire, UK. It was dissolved in ethanol and stored as a 1 mM stock (wrapped in aluminium foil as it is light sensitive). For preparation of stock and salt solutions ultra-pure water from a reverse osmosis system was used (Millipore Ltd., UK).
RESULTS

Effect of Swelling on \( I_K \) in Ventricular Cells

A representative current record from a single ventricular myocyte voltage clamped (whole-cell configuration) at \(-40 \text{ mV}\) and depolarized for 1 s to \(+40 \text{ mV}\) is shown in Fig. 1 A. This voltage step caused activation of an outward current with kinetics characteristic of the classical \( I_K \). Other ion transport mechanisms may be activated simultaneously by such a voltage step and would complicate interpretation of the results we have obtained, so participation of these other mechanisms must be ruled out. Activation of \( I_{K(ATP)} \) was minimized in the present experiments by inclusion of 5 mM ATP in the pipette solution and any contributions from \( \text{Na}^+\text{-K}^+ \) pump or \( \text{Na}^+\text{-Ca}^{2+} \) exchange were avoided by excluding both internal \( \text{Na}^+ \) and \( \text{Ca}^{2+} \) (EGTA was also included in the pipette to buffer endogenous \( \text{Ca}^{2+} \)). Activation of \( \text{Na}^+\text{-activated} K^+ \) current can be ruled out, because this current is only activated when \([\text{Na}^+]_i > 20 \text{ mM}\) (Kameyama et al., 1984). In 43 ventricular cells perfused with isoosmotic solution the mean amplitude of \( I_{K_0} \) measured at 0.95 s during a depolarizing pulse to \(+40 \text{ mV}\) from a holding potential of \(-40 \text{ mV}\), was 0.86 ± 0.05 nA. Switching to hypotonic solution caused an increase in \( I_K \) to a mean value of 1.43 ± 0.10 nA (\( p < 0.05 \) compared to control) which represents a 65.5 ± 8.2% increase. This current increase occurred rapidly (i.e., within ~60 s of changing solutions).

To ensure that the current changes recorded on switching from solution containing 140 mM NaCl to solution containing 100 mM NaCl were due to the resultant reduction in osmolality and not due to the alteration in external \( \text{Na}^+ \) or \( \text{Cl}^- \) concentrations, further experiments were conducted in which osmolality was reduced by removal of sucrose (i.e., switching from a solution containing 100 mM NaCl plus sucrose to a solution containing 100 mM NaCl without sucrose). In isoosmotic solution, current (at 0.95 s) was 0.57 ± 0.10 nA and switching to hypotonic solution caused an increase in current to 1.06 ± 0.19 nA (\( n = 10; \ p < 0.05 \)). Concomitant with this increase in current there was an inward shift in the holding current \( (I_H) \), such that in isoosmotic solution \( I_H \) was 0.57 ± 0.08 nA, vs 0.41 ± 0.07 nA in hypotonic solution (\( n = 10; \ p < 0.05 \)). When cells were held at a holding potential of 0 mV and pulsed for 1 s to +40 mV, there was still an increase in outward current on cell swelling (0.44 ± 0.06 nA to 0.75 ± 0.10 nA; \( n = 8, \ p < 0.05 \)) but no accompanying change of \( I_H \) (0.28 ± 0.02 nA vs 0.30 ± 0.02 nA; \( n = 8, \ p \text{ NS} \)). We have no clear explanation for the shift in \( I_H \) accompanying introduction of hypotonic solution (no alteration in \( I_H \) was observed when osmolality was decreased by removal of NaCl). One possible explanation, though speculative, is that switching to a hypotonic solution modulates a background conductance. The resulting change in \( I_H \) is observed in the sucrose removal protocol, but in the NaCl removal protocol it is masked by an opposing change in some \( \text{Na}^+ \) or \( \text{Cl}^- \)-dependent background conductance. Further experimentation will be necessary to investigate this point. However, overall swelling-induced changes in \( I_K \) were similar irrespective of the method used for altering osmolality.

To test the contribution that current through \( L\text{-type Ca}^{2+} \) channels \( (I_{Ca}) \) may
FIGURE 1. (A) Typical current recordings obtained from a guinea pig ventricular myocyte when the holding potential was stepped from −40 to +40 mV. Current observed in isoosmotic solution (140 mM NaCl; ○) and current activated after ~50 s exposure to hypoosmotic solution (100 mM NaCl; □). (B) Current-voltage relationship in isoosmotic (■) and in hypoosmotic solution (□) measured in a guinea pig ventricular cell. Currents were measured at the end of a 1-s pulse. In hypoosmotic solution there was a significant increase in the outward current flowing at potentials between +10 and +60 mV.

make to the effects described above, further experiments were performed in the presence of a Ca^{2+} antagonist and in the presence of Ca^{2+}-free external solutions. From Table I it can be seen that there was still a significant swelling-activated $I_K$ current in the presence of 0.3 μM nisoldipine or 2 μM nifedipine. Similarly, in the presence of Ni^{2+} (0.1 or 2 mM) which blocks $I_{Ca}$ and Na^{+}-Ca^{2+} exchange (Kimura, Miyamae, and Noma, 1987) there was still a substantial swelling-activated current. Another important finding from this study was that all of the Ca^{2+} antagonists used reduced $I_K$ under baseline conditions (see Table I). The sensitivity of $I_K$ to intracellular and extracellular Ca^{2+} has been investigated in single cardiac cells by others (Tohse, Kameyama, and Irisawa, 1987; Tohse, 1990; Sanguinetti and Jurkiewicz, 1992). When Ca^{2+}-free external solutions were used there was still a significant swelling-activated outward current (0.75 ± 0.06 nA in Ca^{2+}-free isoosmotic solution and 1.29 ± 0.08 nA in Ca^{2+}-free hypoosmotic solution; $n = 7$) indicating that the current activated by swelling is not dependent on external Ca^{2+}. 
TABLE I
The Effects of Ca2+ Antagonists on Baseline IK and on the Swelling-induced Current Measured at 0.95 s during a Depolarization from a Holding Potential of -40 to +40 mV in Guinea Pig Ventricular Myocytes

| Agent         | Iso + Ca2+ antagonist | Hypo + Ca2+ antagonist |
|---------------|-----------------------|------------------------|
| 2 mM Ni2+     | 1.04 ± 0.39 [4]       | 0.86 ± 0.28 [4]        |
| 0.1 mM Ni2+   | 0.95 ± 0.14 [8]       | 0.78 ± 0.11* [8]       |
| 2 μM nifedipine| 0.93 ± 0.15 [11]      | 0.81 ± 0.12* [11]      |
| 0.3 μM nisoldipine | 1.71 ± 0.30 [11]  | 1.50 ± 0.26* [11]      |

Iso = isosmotic. Hypo = hypoosmotic. Results are expressed as mean ± SEM [n].
* p < 0.05 when results are compared with control using a paired t test.
* p < 0.05 when results in hypoosmotic solution are compared with those in isosmotic solution.

The I-V relationships of membrane current, measured at the end of a 1-s depolarizing pulse over the range -80 to +60 mV, before and during swelling are illustrated in Fig. 1 B. Cell swelling caused an increase in outward current flowing at all membrane potentials in the range +10 to +60 mV, indicative of an increase in IK.

Effects of Cell Swelling on IKr and IKs Measured Using the Envelope of Tails Test
To determine whether cell swelling activated the rapid, the slow, or both components of IK, we investigated the effect of swelling on whole-cell current using an envelope of tails protocol, which measures the deactivation of outward current activated during a depolarising step (Noble and Tsien, 1969). This test predicts that if IK is the result of conductance through a single channel type, then the magnitude of tail current (IKtail) after a pulse of variable duration should increase in parallel with the current during the pulse, i.e., the ratio of IKtail/IK should remain constant irrespective of pulse duration (Sanguinetti and Jurkiewicz, 1990a). An additional benefit of using tail currents as a measure of IK is that one can further reduce the possibility that other currents may contribute to the current measured, as in general the deactivation kinetics of other currents such as ICa are much faster than the kinetics of IK. Cells (n = 16) were voltage clamped at -40 mV and depolarized to +40 mV for various durations between 50 ms and 2.9 s (see Fig. 2 A). The time course of deactivation of the tail current was best fitted by a mono-exponential curve. Switching to hypoosmotic solution caused alterations in the time course of current decay (τ) as shown in Fig. 2 B. During short pulses hypoosmotic solution lengthened τ, whereas at longer pulses hypoosmotic solution shortened τ. Over the middle range of pulse durations, when the contributions that IKr and IKs make to total current would be expected to be similar, there was no effect on τ.

Swelling also had differential effects on the amplitude of the current, depending on the duration of the depolarization pulse (see Fig. 3 A). Swelling caused a decrease in current amplitude measured at the end of the pulse at short pulse durations (< 150 ms) but for longer pulses (> 1 s) swelling caused a significant increase.
FIGURE 2. (A) Typical records from experiments in which an envelope of tails test was performed in isoosmotic (●) and hypoosmotic (○) solutions. Sample records are shown for pulses of 200 and 650 ms, and 2.9-s durations (see Methods). In total 18 different pulse durations were used. (B) Kinetics of tail current decay after depolarizing pulses of various durations (n = 16). Decay was mono-exponential and was fitted using Chebyshev's fit (Clampfit, pClamp6). Isoosmotic results are represented by (■) and hypoosmotic results are represented by (□). Switching to hypoosmotic solution caused a significant increase in τ when pulse duration was short (<50 ms). However, when pulse duration was longer (>1 s) swelling caused a significant decrease in τ. *p < 0.05 level when the t test for paired data was applied.

in current. Similar results were obtained when I_{K_{tail}} was measured i.e., a decrease in amplitude during swelling for short pulses and an increase at longer pulses (Fig. 3 B). The data has also been expressed as the ratio of I_{K_{tail}}/I_{K} (see Fig. 3 C). This method of analysis minimizes any cell to cell variability when raw current amplitudes are measured and allows us to predict whether the current recorded is the result of conductance through a single channel subtype or through multiple channel subtypes (Sanguinetti and Jurkiewicz, 1990a). Values of I_{K_{tail}}/I_{K} varied with the pulse duration, indicating that multiple channel subtypes are involved, consistent with the results of Sanguinetti and Jurkiewicz (1990a). Cell swelling caused a significant increase in the ratio at short pulse durations, but had no effect at durations >350 ms. Thus, it seems that at longer pulse durations I_{K_{tail}} and I_{K} are increasing in parallel, whereas at short pulses I_{K} decreases more than I_{K_{tail}} leading to a significant increase in the current ratio. These results suggest that cell swelling causes a decrease in I_{K} (the major current activated at short pulses) and an increase in I_{K_{tail}} (the dominant current at longer pulses).

Effect of Selective I_{K} Blockers on Swelling-induced Current

In a series of experiments, the effects of swelling on the two I_{K} subtypes have been investigated using two I_{K} blockers, UK69,614 and dofetilide, to help dissect out the individual components. The results are summarized in Table II. Using a pulse protocol identical to that applied previously (depolarization to +40 mV for 1 s from a hold-
Effect of Cell Swelling on \( I_{Ks} \) and \( I_{Kr} \)

The effect of cell swelling on \( I_{Kr} \) was studied using the envelope of tails protocol, since this allows better separation of the two components of \( I_{Kr} \). Dofetilide decreased the current at short pulses only (i.e., the current which had already been decreased by cell swelling; \( n = 4 \); see Fig. 4) and had no effect on current at longer pulses (current continued to increase in amplitude). These results are consistent with dofetilide being a selective blocker of \( I_{Kr} \).

Since the envelope of tails experiments demonstrated that hypoosmotic stress decreased \( I_{Kr} \) at short pulse durations (when \( I_{Kr} \) is dominant) but increased \( I_{Kr} \) at longer durations (when \( I_{Kr} \) is the major current; see Fig. 3, A and B), further experiments were undertaken to study the effect of cell swelling on \( I_{Kr} \) under conditions in which \( I_{Kr} \) was minimized. This was achieved by depolarizing to \(-10 \) mV from a holding potential of \(-40 \) mV for a short period of time (250 ms; see Jurkiewicz and Sanguinetti, 1993). Using this pulse protocol a 50–100 pA current was elicited after 250 ms and tail currents were very small and often difficult to measure with any accuracy. When the solution was switched from isosmotic solution to isosmotic solution plus dofetilide (0.2 \( \mu \)M; \( n = 3 \)) the current was almost completely abolished (Fig. 5 A). Pretreatment of cells with dofetilide also prevented any change in current when cells were swollen. In a separate series of cells (\( n = 5 \); Fig. 5 B) introduction of hypoosmotic solution caused a significant decrease in \( I_{Kr} \) (26.3 \( \pm \) 8.4%) and when dofetilide (0.2 \( \mu \)M) was introduced (in the presence of hypoosmotic solution) the current remaining was decreased by a further 47.0 \( \pm \) 10.4% \( (p < 0.05) \), but was not completely abolished even when dofetilide was administered at 1.0 \( \mu \)M. From these data it would seem that swelling ventricular cells alters the ability of dofetilide to block \( I_{Kr} \).

To investigate this point further, experiments were undertaken using a wider range of concentrations of dofetilide. \( I_{Kr} \) was elicited using an identical protocol to that described above (voltage step to \(-10 \) mV from a holding potential of \(-40 \) mV for 250 ms). It can be seen from Fig. 6 that over the concentration range 10 nM to 10 \( \mu \)M dofetilide is an effective and potent blocker of \( I_{Kr} \) in nonswollen myocytes. However, when cells were swollen by reducing the osmolality of the bathing solution, this concentration-response relationship was altered such that higher concentrations of dofetilide were required to block \( I_{Kr} \) (Fig. 6). Specifically, under isosmotic conditions 0.2 \( \mu \)M dofetilide blocked 88.3 \( \pm \) 7.6% of \( I_{Kr} \) \( (n = 7) \) whilst the same concentration produced only 47.0 \( \pm \) 10.4% block \( (n = 5) \) when applied during a hypoosmotic challenge. During cell swelling 10 \( \mu \)M dofetilide blocked \( I_{Kr} \) to the same degree as 0.2 \( \mu \)M under isosmotic conditions (see Fig. 6). This apparent antagonism may indicate that there is competition between the mediator(s) of the swelling-induced decrease in \( I_{Kr} \) and dofetilide. However, at present we have no evidence as to how cell swelling alters the ability of dofetilide to block \( I_{Kr} \).

Effect of Swelling on \( I_{Kr} \) in the Absence of \( I_{Kr} \)

Although our results suggest strongly that cell swelling has differential effects on \( I_{Kr} \) and \( I_{Kr} \) further confirmation of this finding was achieved using experimental conditions under which \( I_{Kr} \) or \( I_{Kr} \) can be measured in isolation. Sanguinetti and Jurk-
iewicz (1992) and Jurkiewicz and Sanguinetti (1993) have shown that in Ca²⁺-free solution activation of $I_K$ is shifted to more negative potentials, whereas the activation of $I_K$ is shifted to more positive potentials. We exploited this observation to measure $I_K$ in the absence of $I_K$ by depolarising cells to $-10$ mV from a holding potential of $-40$ mV for $500$ ms in Ca²⁺-free solutions. Under these conditions $I_K$ was decreased significantly by swelling from $59.0 \pm 12.2$ pA to $47.9 \pm 10.5$ pA ($n = 6$, $p < 0.05$) representing a reduction of $20.9 \pm 7.3\%$. $I_K$ tail currents were also decreased by $69.4 \pm 16.9\%$ in six cells, from $29.8 \pm 7.9$ pA under isoosmotic conditions to $11.4 \pm 7.9$ pA under hypoosmotic conditions ($p < 0.05$). When this experimental
Cell swelling had differential effects on current depending on the pulse duration: switching to an hypoosmotic solution caused a significant decrease in current amplitude when pulse duration was short (<150 ms), but a significant increase when pulse duration was longer (>900 ms).

\[ *p < 0.05 \text{ level (t test for paired data).} \]

On switching to an hypoosmotic solution there was a decrease in current amplitude when pulse duration was short (<50 ms) and a significant increase when pulse duration was longer (>450 ms).

\[ *p < 0.05 \text{ level (t test for paired data).} \]

Switching to hypoosmotic solution caused an increase in the ratio over short pulse durations (<350 ms) due to the fact that \( I_K \) (mainly \( I_w \) at these short durations) decreased more than \( I_{cal} \).

\[ *p < 0.05 \text{ level (t test for paired data).} \]

**TABLE II**

| Agent     | Iso   | Hypo  | Hypo + drug |
|-----------|-------|-------|-------------|
|           | nA    | nA    | nA          |
| UK66,914  | 0.76 ± 0.10 | 1.10 ± 0.13* | 0.79 ± 0.14† |
| (n = 14)  |       |       |             |
| Dofetilide| 1.02 ± 0.08 | 1.69 ± 0.18* | 1.50 ± 0.16† |
| (n = 5)   |       |       |             |

Iso = isoosmotic. Hypo = hypoosmotic. Results are expressed as mean ± SEM.

*\( p < 0.05 \) when compared with control using a paired t test.

†\( p < 0.05 \) when compared with hypoosmotic solution alone (paired t test).
Figure 4. (A) Ratio of $I_{Na1}/I_{K}$ measured after pulses of various durations between 50 ms and 2.9 s, in the presence of isoosmotic (●), hypoosmotic (○), and hypoosmotic solution containing 0.2 μM dofetilide (□) in four cells. (B) Current amplitude at the end of depolarizing pulses measured in
protocol was repeated in the presence of 30 μM tetrodotoxin (to block any possible noninactivating component of Na⁺ current; see Brown, Lee, and Powell, 1981) \( I_K \) was still decreased by cell swelling from 127.5 ± 12.5 pA to 95.0 ± 16.0 pA \((n = 2, p < 0.05)\).

Given that we were measuring \( I_K \) under conditions where contamination by \( I_Na \) is minimal, this allowed us to investigate the selectivity of UK66,914. \( I_K \) was again evoked by a voltage step from −40 to −10 mV for 500 ms under Ca²⁺-free condi-

the same cells. (C) Tail current amplitudes \((I_{hadj})\) measured in the same cells. For each panel, measurements between 0 and 1 s have been expanded and placed in an insert on the right hand side of the main diagram. Dofetilide decreased \( I_{hadj} \) at short pulses only, resulting in a decrease in \( I_{hadj}:I_K \). It had no effect on \( I_{hadj} \), \( I_Na \) or on \( I_{hadj}:I_Na \) at longer pulses, in fact current continued to increase in amplitude.

*Represent significance at the \( p < 0.05 \) level for hypoosmotic solution with dofetilide vs hypoosmotic solution alone (t test for paired data). For clarity significance for isoosmotic group vs hypoosmotic group is not indicated (this is shown in Fig. 3).
UK66,914 (10 μM) significantly decreased $I_{Kr}$ measured at the end of the clamp step (96.6 ± 32.9 pA vs 48.8 ± 21.1 pA, $n = 5$, $p < 0.05$) as well as the resulting tail current (36.8 ± 9.4 pA vs −7.7 ± 13.3 pA, $n = 5$, $p < 0.05$).

In addition to allowing us direct examination of the effects of UK66,914 on $I_{Kr}$, we were also able to address the question of whether the loss of effectiveness of dofetilide when cells are swollen was peculiar to this $I_{Kr}$ blocker, by using the trivalent cation La$^{3+}$ (30 μM). At this concentration La$^{3+}$ has been shown to block $I_{Kr}$ (Sanguinetti and Jurkiewicz, 1990b; Delpón, Valenzuela, Pérez, Casis, and Tamargo, 1995). The results are shown in Fig. 7. In five cells, under isoosmotic Ca$^{2+}$-free conditions, La$^{3+}$ significantly reduced $I_{Kr}$ (measured using the protocol described above) from 76.2 ± 14.4 pA to 46.0 ± 20.8 pA (a 52.4 ± 19.8% reduction). When cells were swollen ($n = 6$), La$^{3+}$ was less effective than under isoosmotic conditions, with $I_{Kr}$ being reduced from 47.9 ± 10.5 pA to 34.9 ± 12.6 pA (i.e., a reduction of 29.4 ± 15.4%). Effects on tail currents reflected a similar diminution of drug effectiveness at blocking $I_{Kr}$ in swollen vs nonswollen cells (data not shown). Thus, it appears that cell swelling blunts the ability of both dofetilide and La$^{3+}$ to block $I_{Kr}$.

**Effect of Swelling on $I_{Kr}$ in the Absence of Dofetilide**

In a further series of experiments, $I_{Kr}$ was evoked by stepping the voltage from −40 to +60 mV for 5 s under Ca$^{2+}$-free conditions, in the presence of dofetilide (0.2 μM). Under control conditions this produced a current of 0.81 ± 0.14 nA measured at 4.9 s ($n = 6$) and cell swelling caused an increase of 146.0 ± 42.5% to 1.78 ± 0.22 nA ($n = 6$, $p < 0.05$). $I_{Kr}$ was also increased by 136.6 ± 32.2% from 0.26 ± 0.05
Effect of Cell Swelling on $I_{\text{K}}$ and $I_{\text{V}}$

Figure 7. (A) The effect of switching from isoosmotic solution to an identical solution containing La$^{3+}$ (30 nM) was studied in five cells. La$^{3+}$ decreased $I_{\text{K}}$ under baseline conditions and swelling cells subsequently reduced $I_{\text{K}}$ further. *p < 0.05 level vs isoosmotic group (t test for paired data). (B) Effect of cell swelling, in the presence and absence of La$^{3+}$, on $I_{\text{K}}$ activated by a voltage step from -40 to -10 mV for 500 ms. Switching to hypoosmotic solution caused a significant reduction in $I_{\text{K}}$ and introducing La$^{3+}$ (30 nM; six cells per group) caused a slight, but not significant reduction in $I_{\text{K}}$. Thus, swelling seems to decrease the ability of La$^{3+}$ to block $I_{\text{K}}$. *p < 0.05 level vs control (t test for paired data).

nA under iso-osmotic conditions to 0.54 ± 0.06 nA on swelling (n = 6, p < 0.05). Cell swelling still increased $I_{\text{K}}$ in the presence of 30 nM tetrodotoxin (control current of 0.65 ± 0.23 nA increased to 1.15 ± 0.29 nA on swelling; n = 3, p < 0.05).

Finally, application of 10 nM UK66,914 decreased $I_{\text{K}}$ measured at the end of the 5 s pulse from 0.34 ± 0.04 nA to 0.16 ± 0.05 nA (53.0 ± 14.7% reduction; n = 3, p < 0.05). Similarly $I_{\text{K,cell}}$ was reduced from 138 ± 16 pA to 16 ± 27 pA (92.3 ± 19.0% decrease; n = 3, p < 0.05). Clearly, UK66,914 blocks both $I_{\text{K}}$ and $I_{\text{K,cell}}$ under our experimental conditions.

Perforated Patch Experiments

It could be argued that since the recording pipette represents a large reservoir of isoosmotic solution, it is unclear how this affects the steady state with respect to the osmotic balance between the pipette, the cell and the external solution. To address the question of whether this might affect the response of $I_{\text{K}}$ to swelling, we performed a small series of experiments using the perforated patch recording technique (see Methods).
Under these conditions, the mean value of $I_K$ in isoosmotic solution (measured at 0.95 s during a depolarizing pulse to +40 mV from a holding potential of -40 mV) was $0.40 \pm 0.03$ nA ($n = 3$). Application of hypoosmotic solution caused an increase in $I_K$ to a mean value of $0.95 \pm 0.10$ nA ($p < 0.05$ compared to control) which represents a $135.3 \pm 8.1\%$ increase. In two of these cells, tail currents were significantly increased from $159 \pm 12$ pA to $361 \pm 49$ pA (a $126.0 \pm 14.0\%$ increase, $p < 0.05$), while in the third cell a $61.4$ pA control tail current increased by $669\%$ to $472$ pA during cell swelling. In Ca$^{2+}$-free solutions, $I_{Ks}$ evoked by 5-s pulses to +60 mV from a holding potential of -40 mV (see above) increased by $51.3 \pm 2.9\%$ ($n = 5$) during cell swelling (control current of $0.97 \pm 0.14$ nA increased to $1.45 \pm 0.19$ nA; $p < 0.05$). Tail currents were also increased by $120 \pm 18\%$ from $0.22 \pm 0.05$ nA under isoosmotic conditions to $0.45 \pm 0.06$ nA on swelling ($n = 5$; $p < 0.05$). When $I_{Ks}$ was measured using the perforated patch technique by depolarizing cells to $-10$ mV for 500 ms from a holding potential of -40 mV in Ca$^{2+}$-free solutions, swelling caused a significant decrease in $I_{Ks}$, from $42.5 \pm 7$ pA to $22.1 \pm 7$ pA ($n = 5$; $p < 0.05$) representing a reduction of $52 \pm 9\%$. $I_{Ks}$ tail currents were also decreased by $82.7 \pm 7.8\%$, from $23.8 \pm 6.6$ pA under isoosmotic conditions to $-4.0 \pm 7.5$ pA under hypoosmotic conditions ($n = 5$; $p < 0.05$).

These results demonstrate that when using the perforated patch technique, cell swelling produces differential effects on $I_{Ks}$ and $I_{Ko}$ which are qualitatively similar to those recorded with the conventional whole-cell voltage-clamp method. Whether there are systematic quantitative differences in the responses to cell swelling measured by these two experimental approaches remains to be determined.

**DISCUSSION**

An increase in cell volume has been shown to affect various ion transport mechanisms in the heart. In rabbit ventricular myocytes, two cotransporters have been shown to be involved in the swelling response, the sodium-potassium-chloride (Na-K-2Cl) and NaCl cotransporters (Drewnowska and Baumgarten, 1991). Cl$^-$ current has also been shown to be activated by cell swelling or stretch (Hagiwara, Masuda, Shoda and Irisawa, 1992; Sorota, 1992; Tseng, 1992; Vandenberg et al., 1994). In addition, stretching/swelling single ventricular cells has been shown to modulate intracellular Ca$^{2+}$ (Gannier, White, Lacampagne, Garnier, and Le Guennec, 1994) and to decrease Na$^+$-Ca$^{2+}$ exchange (Wright, Rees, Vandenberg, Twist, and Powell, 1994, 1995). As stated earlier, changes in cell volume have been shown to modulate K$^+$ efflux, thus it was the aim of this study to investigate the effect of cell swelling on $I_K$ and $I_{Ko}$, both of which appear to be activated at voltages relevant to the cardiac action potential.

The results presented here have shown that $I_K$ is affected by cell swelling, consistent with the work of Sasaki et al. (1992, 1994) and have clarified the effect of swelling on the two components of $I_K$. The pharmacological and electrophysiological evidence indicates that $I_{Ks}$ is potentiated by cell swelling whereas $I_{Ko}$ is inhibited. When ventricular myocytes were swollen and then exposed to one of two $I_K$ blockers, we found that UK66,914, which we found blocked both $I_K$ subtypes, was much more effective in blocking swelling-activated $I_K$ than dofetilide, which is highly se-
lective for $I_{Kr}$. To further discriminate between effects on the two $I_K$ subtypes we then used the envelope of tails protocol and found that current amplitude at shorter pulses was decreased by swelling, whereas the amplitude at longer pulses was increased, confirming that swelling had differential effects on the two components of $I_K$. Swelling also had differential effects on the deactivation time constant ($\tau$) depending on the pulse duration; swelling increased $\tau$ at short durations and decreased it at longer durations. The time course of deactivation of the tail current was best fitted by a mono-exponential curve. This is in agreement with the results of Walsh, Begenisich, and Kass (1989), Sanguinetti and Jurkiewicz (1990a) and Balser, Bennett, Hondeghem, and Roden (1991) also using guinea pig ventricular myocytes. However, others using the same cell type have found that $I_K$ deactivation is best fitted by a biexponential curve (Kiyosue, Arita, Muramatsu, Spindler, and Noble, 1993; Daleau and Turgeon, 1994). This dichotomy may be explained in part by the results of Kiyosue et al. (1993): their $I_K$ tail deactivation curves were best fit by two exponentials over a range of temperatures, but at temperatures above $33^\circ C$ the faster component contributed more than $80\%$ of the total tail current amplitude. Thus, at temperatures such as those used in this study ($35^\circ C$) the fast time constant would dominate, masking the slow component. Also Matsuura, Ehara, and Imoto (1987) found that the time course of tail currents could be mono- or biexponential depending on the voltage to which the cell was repolarized.

In a series of experiments aimed at characterising further the effects of swelling on the two $I_K$ subtypes, cells were swollen under Ca$^{2+}$-free conditions. In the absence of Ca$^{2+}$ the activation of $I_{Ks}$ is shifted to more negative potentials and the activation of $I_{Kr}$ is shifted to more positive potentials, thus aiding analysis of the two subtypes individually. Swelling caused a large increase of $I_{Ks}$ when activated by a 5-s pulse from $-40$ to $+60$ mV in Ca$^{2+}$-free solutions containing dofetilide to block any contaminating $I_{Kr}$. In contrast cell swelling caused a significant decrease in $I_{Kr}$ when it was activated by a 500-ms pulse from $-40$ to $-10$ mV in Ca$^{2+}$-free solutions. Thus, these data were again consistent with the contention that swelling increases $I_{Ks}$ in the absence of $I_{Kr}$ and decreases $I_{Kr}$ in the absence of $I_{Ks}$.

An interesting observation with regard to the effect of swelling on $I_{Kr}$, was that when an alternative protocol was used to minimize $I_{Ks}$ activation (stepping from $-40$ to $-10$ mV for 250 ms) we noted that swelling cells caused a decrease in $I_{Kr}$ that introducing dofetilide (0.2 or 1.0 $\mu$M) produced a further decrease in current, but did not completely abolish current. More detailed experiments then showed clearly that dofetilide is a potent blocker of $I_{Ks}$ when used over the concentration range 10 nM to 10 $\mu$M and our finding that in nonswollen cells the drug blocks $\sim88\%$ of $I_{Kr}$ at 0.2 $\mu$M is in good agreement with the work of Jurkiewicz and Sanguinetti (1993). Surprisingly, while this drug concentration prevents the subsequent response to swelling, this inhibitory effect is approximately halved when dofetilide is applied during cell swelling. Under our experimental conditions, 10 $\mu$M dofetilide was required to block $I_{Ks}$ in swollen cells to the same degree as 0.2 $\mu$M under isoosmotic conditions. The mechanism(s) underlying the attenuation of the ability of dofetilide to block $I_{Kr}$ during swelling are as yet unknown, but cannot be unique to this drug, since swelling also blunts the blocking effects of La$^{3+}$ on $I_{Kr}$.
The mechanisms underlying the effects of swelling on both $I_{K}$ and $I_{K_{s}}$ also remain unknown. One hypothesis is that since cell swelling increases intracellular free Ca$^{2+}$ and since this has been shown to increase $I_{K}$ (Tohse et al., 1987; Tohse, 1990) and more specifically $I_{K_{s}}$ (Busch and Maylie, 1994) the two effects may be linked. In the present study intracellular Ca$^{2+}$ was buffered by 10 mM EGTA, however if swelling were to release high concentrations of Ca$^{2+}$ locally, then the local buffering capacity of EGTA may be overwhelmed. Thus, we cannot rule out swelling-induced alterations in intracellular Ca$^{2+}$ as a mechanism for increasing $I_{K}$. Also, $I_{K}$ has been shown to be modulated by extracellular Ca$^{2+}$ (Armstrong and Lopez-Barneo, 1987). However, all of these proposals will have to be reconciled with the results of our present experiments, which show clearly that when extracellular Ca$^{2+}$ was removed a significant response to hypoosmotic stress was still observed.

Another possible explanation for the swelling-induced alteration in $I_{K}$ could be that an increase in cell volume causes dilution of intracellular ions such as K$^{+}$. If this is the case in our experiments, then such changes are not accompanied by a dramatic change in the reversal potential for $I_{K}$ measured from the whole-cell I-V relationship (see Fig. 1B). It is possible that any dilution of K$^{+}$ that may occur as a result of swelling is compensated for by pipette dialysis. Dilution of intracellular Mg$^{2+}$ as a result of an increase in cell volume may be another reason for the increase in $I_{K_{s}}$ since it has been shown that Mg$^{2+}$ blocks $I_{K}$ in frog atrial cells (Tarr, Trank, and Goertz, 1989; Duchatelle-Gourdon, Lagrutta, and Hartzell, 1990), thus, any decrease in intracellular Mg$^{2+}$ may relieve this block of $I_{K}$.

In the present study we have shown using a range of experimental protocols that swelling ventricular myocytes has differential effects on the two components of $I_{K}$, causing a decrease in $I_{K_{s}}$ and an increase in $I_{K_{s}}$. Future experiments will aim to investigate the mechanism of action of swelling in producing these effects and the possible contribution such changes in membrane conductance might make to ischaemia- and reperfusion-induced arrhythmogenesis.

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