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Contribution of BK_{Ca}-Channel Activity in Human Cardiac Fibroblasts to Electrical Coupling of Cardiomyocytes-Fibroblasts

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Abstract. Cardiac fibroblasts are involved in the maintenance of myocardial tissue structure. However, little is known about ion currents in human cardiac fibroblasts. It has been recently reported that cardiac fibroblasts can interact electrically with cardiomyocytes through gap junctions. Ca^{2+}-activated K^+ currents ($I_{K[Ca]}$) of cultured human cardiac fibroblasts were characterized in this study. In whole-cell configuration, depolarizing pulses evoked $I_{K(Ca)}$ in an outward rectification in these cells, the amplitude of which was suppressed by paxilline (1 μM) or iberiotoxin (200 nM). A large-conductance, Ca^{2+}-activated K^+ (BK_{Ca}) channel with single-channel conductance of 162 ± 8 pS was also observed in human cardiac fibroblasts. Western blot analysis revealed the presence of α-subunit of BK_{Ca} channels. The dynamic Luo-Rudy model was applied to predict cell behavior during direct electrical coupling of cardiomyocytes and cardiac fibroblasts. In the simulation, electrically coupled cardiac fibroblasts also exhibited action potential; however, they were electrically inert with no gap-junctional coupling. The simulation predicts that changes in gap junction coupling conductance can influence the configuration of cardiac action potential and cardiomyocyte excitability. $I_{k(Ca)}$ can be elicited by simulated action potential waveforms of cardiac fibroblasts when they are electrically coupled to cardiomyocytes. This study demonstrates that a BK_{Ca} channel is functionally expressed in human cardiac fibroblasts. The activity of these BK_{Ca} channels present in human cardiac fibroblasts may contribute to the functional activities of heart cells through transfer of electrical signals between these two cell types.

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Key words: Human cardiac fibroblast — Large-conductance, Ca^{2+}-activated K^+ channel — Simulation — Cardiac action potential — Electrical coupling

Introduction

The large-conductance, Ca^{2+}-activated (BK_{Ca}) channels, which are formed by α-subunit tetramers, are encoded by a nearly ubiquitous, alternatively spliced gene, *SLO* (*KCNMA1*) (Butler et al., 1993), and distinguished from most other K^+ channels in that their activation is under dual control, i.e., allosterically switched on either by membrane depolarization or by increased intracellular Ca^{2+}. Previous studies have demonstrated the existence of BK_{Ca} channels in vascular smooth myocytes, which are responsible for the regulation of vascular tone (Amberg et al., 2003; Wu et al., 2003). BK_{Ca} channels have been reported to be present in the cardiac inner mitochondrial membrane and to be responsible for the cardioprotective effect (Sato et al., 2005). The presence of a small-conductance, Ca^{2+}-activated K^+ channel in human and mouse cardiomyocytes has been described (Wang et al., 1999; Xu et al., 2003). A recent report demonstrated the presence of voltage-gated K^+ currents in ventricular fibroblasts (Chilton et al., 2005). However, it remains poorly understood whether BK_{Ca} channels are functionally expressed in human cardiac fibroblasts.

Heart cells consist of cardiac myocytes and nonmyocyte populations. Among nonmyocytes, cardiac fibroblasts constitute a significant fraction. Cardiac fibroblasts have been proposed to be important determinants of both structure and function of the myocardium (Kohl, Hunter & Noble, 1999; Gaudesius et al., 2003; Kizana et al., 2005). These cells can con-

tribute to structural, biochemical, mechanical and even electrical characteristics of cardiac function (Gaudesius et al., 2003; Camelliti, Borg & Kohl, 2005; Kamkin et al., 2005; Kohl et al., 2005). It has been recently reported that fibroblasts in the heart can interact electrically with cardiomyocytes through gap junctions (Camelliti et al., 2004; Rohr, 2004). The connexins that constitute the channel at gap junctions (C × 43 and C × 45) between cardiomyocytes and fibroblasts have been found at the point of contact of these two cells (Gaudesius et al., 2003; Camelliti et al., 2004; Rohr, 2004). The current may flow through these proteins that span the plasma membranes of cardiomyocytes and fibroblasts because connexin molecules in the plasma membrane of one cell can link up with those in an adjacent cell to form the channels. With the aid of a cardiac coculture system, a previous study demonstrated the role of gap junctional coupling for *in vitro* impulse conduction via nonmyocytes by showing that replacement of HeLa-C × 43 (i.e., HeLa cells transfected with C × 43) with wild-type HeLa cells lacking a direct electrotonic conduction afforded by C × 43 retarded the spread of excitation (Gaudesius et al., 2003). Therefore, based on experimental observations, it has been proposed that cardiac fibroblasts act as a substrate for electrical coupling. Cardiac fibroblasts could form a coupled network of cells, which is functionally interconnected to the cardiomyocytes (Camelliti et al., 2004, 2005). When genetically modified, cardiac fibroblasts were also demonstrated to become excitable and to be able to functionally link to cardiomyocytes (Kizana et al., 2005).

In this study, we identified functional expression of BK_{Ca} channels in cultured human cardiac fibroblasts but not in heart-derived H9c2 myoblasts. In addition, along with the theoretical simulations, we predict that alterations in the membrane potential can be observed in cardiomyocytes and cardiac fibroblasts when they are adjacently electrically coupled through gap junctions.

Materials and Methods

Cell Preparations

The human cardiac fibroblast, originally derived from normal human heart tissue, was obtained from Cell Applications (San Diego, CA). Cells were cryopreserved at first passage and could be cultured and propagated to at least eight population doublings. Cells were routinely cultured in human cardiac fibroblast growth medium (Cell Applications) in 50-ml plastic culture flasks in a humidified environment containing 5% CO_2/95% air. Cells were subcultured weekly after detachment using culture medium containing 0.25–0.5% trypsin and 0.02% ethylenediaminetetraacetic acid (EDTA). All cultures were grown to confluence before measurements were made. These cells were identified as fibroblasts because the expression of fibroblast surface protein has been verified by immunostaining with biotin-conjugated antibody (Peng et al., 2002). Electrophysiological studies were generally performed 5–7 days after cells were subcultured (60–80% confluence).

The H9c2 cell line, derived from embryonic rat ventricles, was obtained from American Type Culture Collection (CRL-1446; Manassas, VA). Cells were grown in monolayer culture in 50-ml plastic culture flasks in a humidified environment of 5% CO_2/95% air at 37°C. Cells were maintained at a density of 10^6/ml in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum and 2 mM glutamine (Lo et al., 2005).

Western Blot Analysis

To determine BK_{Ca} channel protein expression, a confluent dish of cells was solubilized with radioimmunoprecipitation assay buffer (50 mM Tris-HCl, 150 mM NaCl, 1% Nondet P-40, 0.5% sodium deoxycholate, 1% sodium dodecyl sulfate [SDS], at pH 7.5). Membrane proteins (20 µg) from human cardiac fibroblasts were separated on 10% SDS-polyacrylamide gels under reducing conditions and electrotransferred to polyvinylidene difluoride paper. Blots were blocked for 2 h at room temperature with TBST buffer (10 mM Tris-HCl, 150 mM NaCl, 0.2 % Tween-20, at pH 7.5) containing 5% nonfat dry milk. Blots were then incubated with 1:750 polyclonal anti-rabbit BK_{Ca} channel C-terminal peptide (Alomone Labs, Jerusalem, Israel) in TBST-5% nonfat dry milk overnight at 4°C, washed with TBST-5% nonfat dry milk five times for 10 min each time and then incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h. After being washed, blots were treated with enhanced chemiluminescent plus (Amersham, Arlington Heights, IL) and autoradiographed on X-ray film (Amersham). This anti-rat BK_{Ca} channel antibody can cross-react with the human BK_{Ca} channel. As shown below, human cardiac fibroblasts, unlike cardiac H9c2 cells, displayed a band with a molecular mass of ~120 kDa, as expected for the α-subunit of the BK_{Ca} channel (Tanaka et al., 1997; Wang et al., 2006).

Electrophysiological Measurements

Immediately before each experiment, cells were dissociated with 1% trypsin and an aliquot of cell suspension was placed in a recording chamber positioned on the stage of an inverted microscope (DM II; Leica, Wetzlar, Germany). Cells were bathed at room temperature (20–25°C) in normal Tyrode's solution containing 1.8 mM $CaCl_2$. Patch electrodes were made from Kimax-51 capillaries (Kimble Glass, Vineland, NJ) using a PP-830 microelectrode puller (Narishige, Tokyo, Japan). The pipette resistance was usually 3–5 MΩ, and seal resistance was at least 10 GΩ. Cell-membrane capacitance of 48 ± 5 pF (n = 22) was compensated. Series resistance, always in the range of 33–55 MΩ, was electrically compensated. Patch-clamp recordings were performed as described previously (Wu et al., 2003).

The signals were displayed on an HM-507 oscilloscope (Hameg, East Meadow, NY) and a PJ550-2 liquid-crystal-display projector (ViewSonic, Walnut, CA). Currents were low pass-filtered at 1 kHz. A Digidata 1322A interface (Axon Instruments, Union City, CA) was used for the analog-to-digital/digital-to-analog conversion. To minimize electrical noise, this interface device was connected to a Slimnote VX_3 computer (Lemel, Taipei, Taiwan) through a universal serial bus port and then controlled by pCLAMP 9.0 software (Axon Instruments).

The amplitudes of single BK_{Ca} channel currents observed in human cardiac fibroblasts were determined by fitting gaussian distributions to the amplitude histograms of the closed and open states. The channel open probability in a patch was expressed as $N \cdot P_o$, which can be estimated using the following equation: $N \cdot P_o = (A_1 + 2A_2 + 3A_3 + ... + nA_n)/(A_0 + A_1 + A_2 + A_3 + ... + A_n)$,

where N is the number of active channels in the patch, A_0 is the area under the curve of an all-points histogram corresponding to the closed state and $A_1...A_n$ represent the histogram areas reflecting the levels of distinct open state for one to n channels in the patch (Wu et al., 2003). Values are provided as means ± standard error of the mean (SEM) with sample sizes (n) indicating the number of cells examined. Paired or unpaired t-test and one-way ANOVA with the least significant difference method for multiple comparisons were used for statistical evaluation of differences among mean values, $P < 0.05$ or 0.01 was considered statistically significant.

Drugs and Solutions

Paxilline and ionomycin were purchased from Biomol Research Laboratories (Plymouth Meeting, PA) and angiotensin II was from Sigma (St. Louis, MO). Iberiotoxin and apamin were obtained from Alomone Labs. Squamocin was kindly provided by Dr. Yang-Chang Wu (Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung City, Taiwan) (Wu et al., 2003). The composition of normal Tyrode's solution was 136.5 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.53 mM $MgCl_2$, 5.5 mM glucose and 5.5 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)-NaOH buffer, pH 7.4. To record K^+ currents or membrane potential, the recording pipette was backfilled with a solution consisting of 140 mM KCl, 1 mM $MgCl_2$, 3 mM Na_2ATP, 0.1 mM Na_2GTP, 0.1 mM ethyleneglycoltetraacetic acid (EGTA) and 5 mM HEPES-KOH buffer, pH 7.2. For single-channel current recordings, the high-K^+ bathing solution contained 145 mM KCl, 0.53 $MgCl_2$ and 5 mM HEPES-KOH buffer, pH 7.4, and the pipette solution contained 145 mM KCl, 2 mM $MgCl_2$ and 5 mM HEPES-KOH buffer, pH 7.2. The value of free Ca^{2+} concentration was calculated assuming a dissociation constant for EGTA and Ca^{2+} (at pH 7.2) of 0.1 μM. To provide 0.1 μM free Ca^{2+} in bath solution, 0.5 mM $CaCl_2$ and 1 mM EGTA were added.

Mathematical Model

The simulations of cardiac action potential shown in this study were primarily conducted using the theoretical dynamic model of a mammalian ventricular action potential (Luo-Rudy model) with significant modifications (Luo & Rudy, 1994). In this model, the action potential is mathematically reconstructed from ionic processes that are formulated on the basis of experimental data obtained mostly from the guinea pig (Luo & Rudy, 1994). Because of the extent to which formulations of ion currents and model predictions have been validated, this has become one of the most extensively used cardiac ventricular cell models. The source program code in the C++ programming language for a single LRd model cell is available at http://www.cwru.edu/med/CBRTC/LrdOnline/content.htm. Single-channel Markovian models for the rapidly and slowly delayed rectifying K^+ currents (Clancy & Rudy, 2001; Silva & Rudy, 2005) were also reformulated and incorporated into the model. The pacing for each simulation was set at 1.0 Hz for 50 beats. The source code used in this study is available at http://senselab.med.yale.edu/senselab/modeldb.

The model of the BK_{Ca} channel was adopted from previous studies (Moczydlowski & Latorre, 1983; Keener & Keizer, 2002), given that it is consistently found in human cardiac fibroblasts. The source code for the modeled equations of BK_{Ca} channels adopted at our laboratory is available either at http://senselab.med.yale.edu/senselab/modeldb or at virtual cell environment (http://www.nrcam.urhc.edu). The parts of solutions to the different set of ordinary differential equations used in this study were always verified and approximated using the X-Win32 version of XPPAUT on a Dell Precision 670 workstation (Round Rock, TX) (Ermentrout, 2002). Although the running time for this software package is too slow to be employed for a long period of pacing (e.g., 50 beats), it can be a collection of differential equation solvers used for analyzing and understanding the behavior of physical systems that change over time. The information for XPP software is readily available at http://www.math.pitt.edu/~bard/XPP/XPP.html (Ermentrout, 2002).

As far as direct cell-to-cell coupling of cardiomyocte and fibroblast is concerned, the equations used for the simulations were as follows:

$$Cm\frac{dV_C}{dt} = -I_T + g_C \times (V_C - V_F)$$
$$Cm\frac{dV_F}{dt} = -I_{K(Ca)} + g_C \times (V_F - V_C),$$

where V_C and V_F are the membrane potential of cardiomyocyte and cardiac fibroblast, respectively; I_T and $I_{K(Ca)}$ are total ion currents in modeled cardiomycytes and Ca^{2+}-activated K^+ current in modeled cardiac fibroblasts, respectively; and g_C represents the net coupling conductance of all the gap junctions formed between the two cells. The left-hand terms of the equations represent a capacitive current, while the terms appearing at the right-hand side represent active ion currents that have been found to influence the membrane potential in cardiomyocytes and cardiac fibroblasts. For the sake of simplicity, we assumed that intercellular current is proportional to potential difference between cardiomyocytes and fibroblasts and that the net conductance of the gap junction is a constant, and that the capacitance between fibroblast and cardiomyocyte does not differ. K^+ outward currents in response to action potential waveform simulated from human cardiac fibroblasts were also examined, assuming that there is an electrical coupling of cardiomyocyte and fibroblast. Simulated action potentials of cardiac fibroblasts used as voltage templates were re-played to the cells though a digital-analogue converter (Lo et al., 2001).

Results

Characterization of Whole-Cell Ca^{2+}-Activated K^+ Current ($I_{K[Ca]}$) in Human Cardiac Fibroblasts

In the first series of experiments, the whole-cell configuration of the patch-clamp technique was used to investigate ion currents in human cardiac fibroblasts. To examine K^+ currents, the cells were bathed in normal Tyrode's solution containing 1.8 mM $CaCl_2$ and the pipette solution contained a low concentration (0.1 mM) of EGTA and 3 mM of adenosine triphosphate (ATP). As shown in Figure 1, when the cell was held at −50 mV, voltage steps (from −60 to +60 mV, increment 10 mV) elicited a family of large, noisy outward currents with an outward rectification. The direction of this membrane current was reversed at -75 mV. When extracellular Ca^{2+} was removed, the amplitudes of these outward currents were greatly reduced throughout the voltage step. For example, at the level of +50 mV, removal of extracellular Ca^{2+} significantly reduced current amplitude from 1,306 ± 121 to 226 ± 28 pA ($P < 0.05$, $n = 8$). Figure 1B shows the averaged current-voltage (I-V)

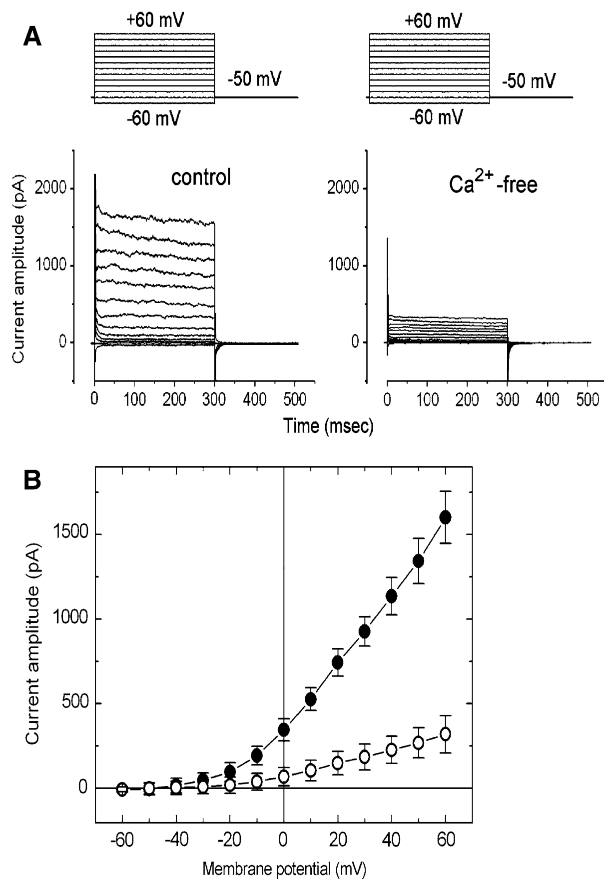

Fig. 1. Effect of removal of extracellular Ca^{2+} on $I_{K(Ca)}$ recorded from human cardiac fibroblasts. The cells were bathed in normal Tyrode's solution containing 1.8 mM $CaCl_2$. The cell was held at –50 mV, and voltage pulses ranging from −60 to +60 mV in 10-mV increments were applied with a duration of 300 ms. The patch solution contained 0.1 mM EGTA. (*A*) Superimposed current traces obtained in the control (*left*) and during exposure (*right*) to Ca^{2+}-free solution. The upper parts shown in each current record indicate the voltage protocol examined. (*B*) *I-V* relationships of I_K measured at the end of voltage pulses in the absence (○) and presence (●) of extracellular Ca^{2+} (1.8 mM). Each point represents the mean ± standard error (n = 7–10).

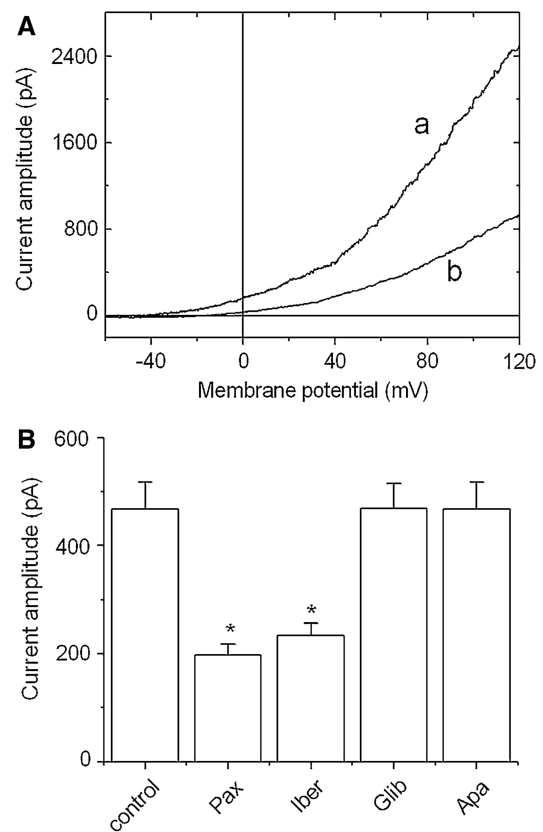

Fig. 2. Effects of paxilline, iberiotoxin, glibenclamide and apamin on $I_{K(Ca)}$ in human cardiac fibroblasts. The cells were bathed in normal Tyrode's solution containing 1.8 mM $CaCl_2$. Each cell was held at –50 mV, and ramp pulses from –60 to +120 mV were applied with a duration of 1 s. The recording pipette was filled with 0.1 mM EGTA. (*A*) Original current traces obtained in the absence (*a*) and presence (*b*) of 1 μM paxilline. (*B*) Effect of paxilline, iberiotoxin, glibenclamide and apamin on the amplitude of $I_{K(Ca)}$ in human cardiac fibroblasts. Current amplitude was measured at +40 mV. *Significantly different from control. Each point represents the mean ± SEM (n = 5–9). Pax, 1 μM paxilline; Iber, 200 nM iberiotoxin; Glib, 10 μM glibenclamide; Apa, 200 nM apamin.

relations for the amplitude of outward current measured at the end of voltage pulses in the presence and absence of extracellular Ca^{2+}. An increase in the intracellular EGTA concentration from 0.1 to 10 mM also almost abolished this current (*data not shown*). These outward currents are primarily considered to be Ca^{2+}-activated K^+ currents ($I_{K[Ca]}$), which were rather small when intracellular Ca^{2+} level was reduced. In addition, no presence of voltage-gated Na^+ or Ca^{2+} current was found in these cells.

To validate the nature of outward currents decreased by extracellular Ca^{2+}, another set of experiments was conducted in bath solution containing the different concentration of extracellular K^+. The current obtained in the absence of extracellular Ca^{2+} was subtracted from that in the presence of extracellular Ca^{2+} (1.8 mM). The reversal potential for this current in each cell was then measured. The data for these currents were pooled and plotted as a function of extracellular K^+ concentrations. The results revealed a slope of 58 mV per 10-fold increase in extracellular K^+.

Effects of Various K^+ Channel Blockers on the Amplitude of $I_{K(Ca)}$ in Human Cardiac Fibroblasts

The effects of various K^+ channel blockers, including glibenclamide, paxilline and apamin, on $I_{K(Ca)}$ in these cells were further examined and compared. In these experiments, cells were bathed in normal Tyrode's solution containing 1.8 mM $CaCl_2$ and ramp pulses from −60 to +120 mV with a duration of 1 s were applied. The results demonstrated that neither

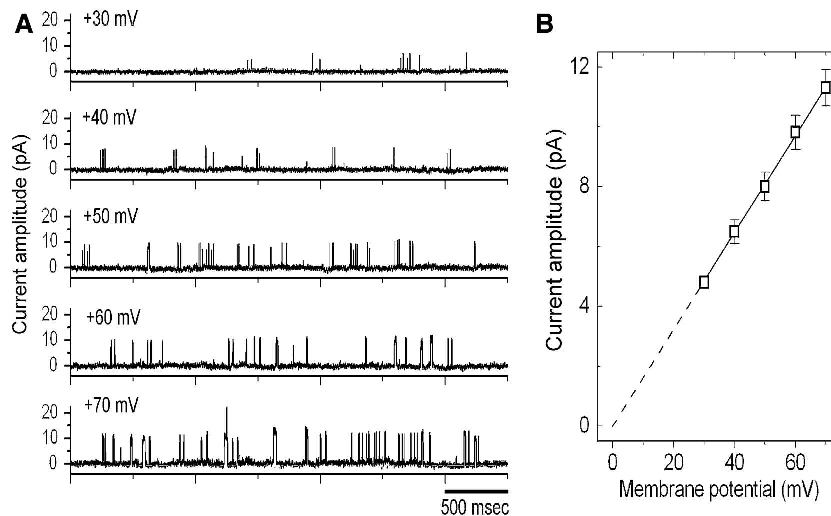

Fig. 3. Activity of BK_{Ca} channels recorded from human cardiac fibroblasts. The experiments were conducted in symmetrical K^+ concentration (145 mM), and a cell-attached configuration was made. Cells were bathed in high-K^+ solution containing 1.8 mM $CaCl_2$. Channel activity was measured at various membrane potentials ranging from +30 to +70 m*V*. Channel openings are shown as an upward deflection. (*A*) Original current traces obtained at different levels of holding potential. (*B*) *I-V* relationship of BK_{Ca} channels in human cardiac fibroblasts. Each point represents the mean ± SEM (n = 7–9).

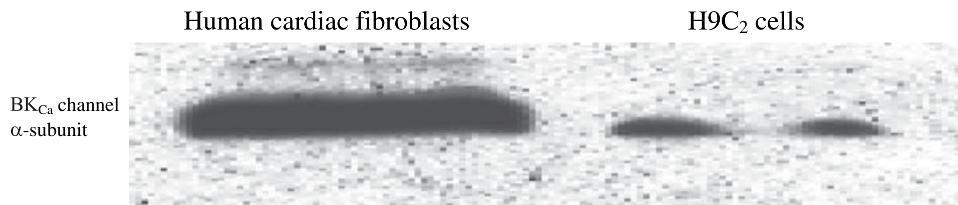

Fig. 4. Quantitative immunoblot of BK_{Ca} channels in human cardiac fibroblasts and cardiac H9c2 cells. *Left lane* shows the presence of the α-subunit of BK_{Ca} channels in cultured human cardiac fibroblasts, while *right lane* shows negligible presence of this subunit.

apamin (200 nM) nor glibenclamide (10 μM) was found to have any significant effect on the amplitude of $I_{K(Ca)}$ in these cells. However, paxilline (1 μM) or iberiotoxin (200 nM) was effective in suppressing $I_{K(Ca)}$ (Fig. 2). For example, at the level of +40 m*V*, paxilline (1 μM) significantly decreased current density form 9.4 ± 1.0 to 4.0 ± 0.4 pA/pF Thus, it is likely that outward K^+ currents in human cardiac fibroblasts are sensitive to block by paxilline or iberiotoxin but not by glibenclamide or apamin.

Properties of BK_{Ca} Channels in Human Cardiac Fibroblasts

In order to characterize the properties of $I_{K(Ca)}$ in these cells, single-channel experiments were performed. The plot of current amplitude as a function of holding potential was constructed. Figure 3 illustrates the *I-V* relationship of BK_{Ca} channel in these cells, which were bathed in symmetrical K^+ concentration (145 mM). In cell-attached configuration, the single-channel conductance of BK_{Ca} channels calculated from a linear *I-V* relationship was 162 ± 8 pS (n = 7) with a reversal potential of 0 ± 3 m*V* (n = 7). The activity of these channels recorded during cell-attached recordings was also sensitive to activation by application of ionomycin (10 μM) or squamocin (10 μM). The activity of BK_{Ca} channels in human cardiac fibroblasts could primarily contribute to the generation of whole-cell $I_{K(Ca)}$.

Immunodetection of BK_{Ca} Channels in Human Cardiac Fibroblasts

To verify the channel proteins present in these cells, we also performed immunoblots using antibodies generated against BK_{Ca} channel α - subunit. In this analysis, a polyclonal antibody raised against the C-terminal peptide of the BK_{Ca} channel $\alpha_{(1184–1200)}$, which can cross-react with the human entity, was used. The results revealed the presence of one protein band in human cardiac fibroblasts but not in cardiac H9c2 cells. This major band was detected at a relative molecular weight of ~120 kDa, as expected for the α-subunit of the BK_{Ca} channel (Fig. 4) (Tanaka et al., 1997; Wang et al., 2006). Unlike cardiac H9c2 myoblasts, these cells were found to display a band with a molecular mass of ~120 kDa, as expected for the α-subunit of the BK_{Ca} channel. In previous results at our laboratory, positive controls, prepared from the samples of pituitary GH_3 cells, also revealed this band (*data not shown*).

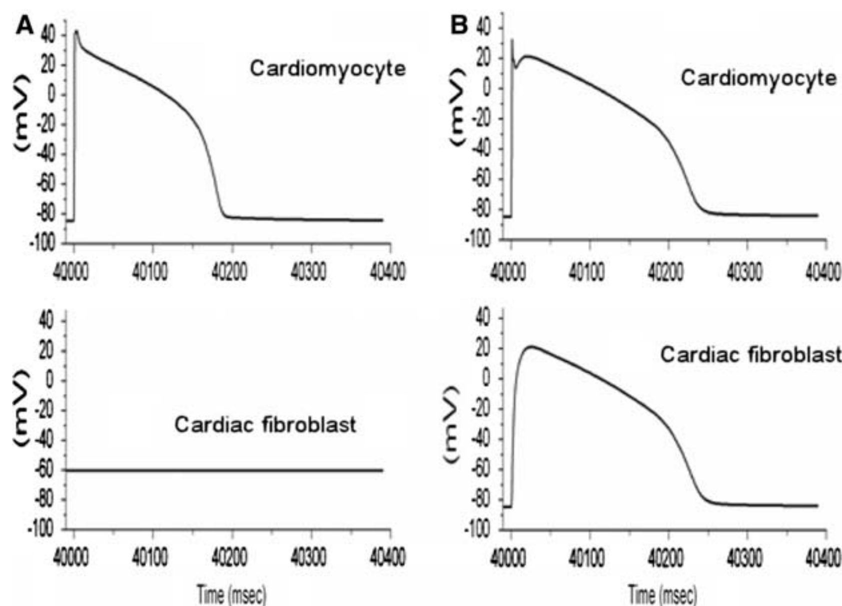

Fig. 5. Change in membrane potential of cardiomyocytes and cardiac fibroblasts in the absence (*A*) and presence (*B*) of electrical coupling. The modified model originally developed by Luo and Rudy was used to account for electrical coupling of cardiomyocytes and cardiac fibroblasts. The simulation was performed with a constant cycle length of 1 s for 50 beats. Changes in the membrane potential in both cardiomyocytes and fibroblasts are well aligned and illustrated here after 40 driving beats. Of note, when these two cells are not electrically coupled, fibroblast remains nonexcitable.

Simulation Studies to Determine how Electrical Coupling of Cardiac Fibroblast to Cardiomyocyte Contributes to the Membrane Potential

It is important to note that intercellular transfer of electrical signals occurs between fibroblasts and cardiomyocytes via gap junctions. The activity of ion channels in cardiac fibroblasts tends to influence the membrane potential of cardiac action potential, thereby exacerbating the propensity of cardiac arrhythmias (Gaudesius et al., 2003). Therefore, using a modified model developed by Luo and Rudy (1994), we further investigated how changes in gap junction coupling conductance influenced the configuration of cardiac action potential. A model for BK_{Ca} channels was also used to account for the presence of BK_{Ca} channels in human cardiac fibroblasts (Moczydlowski & Latorre, 1983; Keener & Keizer, 2002). As shown in Figure 5, when cardiomyocytes and cardiac fibroblasts are electrically uncoupled, no action potential in cardiac fibroblasts can be demonstrated. However, interestingly, when gap junction conductance of 3 μS/μF was arbitrarily added to the simulated cells, there was a cardiac action potential in cardiac fibroblasts, along with a slow upstroke velocity (i.e., about 4.6 mV/ms). In addition, the configuration of cardiac action potential was altered after an electrical interaction between cardiomyocytes and fibroblasts. The duration of action potential at 90% in electrically coupled cardiomyocytes was prolonged from 192 to 238 ms. Based on these simulations, it would thus be tempting to postulate that changes in electrical coupling between cardiomyocytes and fibroblasts can be effective in prolonging the duration of cardiac action potential. The results

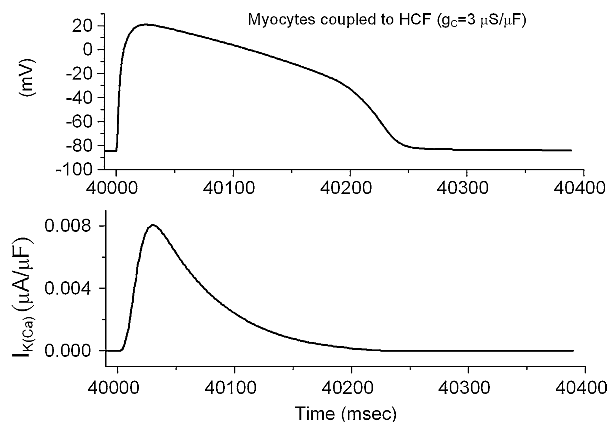

Fig. 6. Effect of electrical coupling on membrane potential (*upper*) and $I_{K(Ca)}$ (*lower*) in modeled human cardiac fibroblasts (HCF). In this simulation, gap junction conductance of 3 μS/μF was incorporated to the modeled cells. Changes in the membrane potential and $I_{K(Ca)}$ in adjacent electrically coupled fibroblasts are well aligned and illustrated here after 40 driving beats. Of note, in electrically coupled cardiac fibroblasts, an action potential with a slowing of upstroke velocity was observed and the amplitude of $I_{K(Ca)}$ corresponding to the appearance of action potential was greatly increased during membrane depolarization.

imply that such coupling increases the propensity of arrhythmias in myocardium.

Figure 6 illustrates the time course of changes in membrane potential and the amplitude of $I_{K(Ca)}$ in cardiac fibroblasts when electrically coupled to cardiomyocytes with a gap junction conductance of 3 pS/pF. The $I_{K(Ca)}$ shown in modeled fibroblasts was activated, with a delay of several milliseconds after the onset of action potential. The results clearly

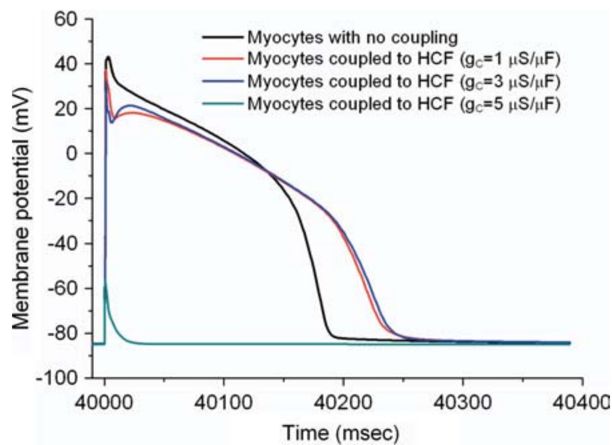

Fig. 7. Effect of gap junction conductance on cardiac action potential in a modeled cardiomyocyte which is coupled or not with a modeled human cardiac fibroblast (HCF). In this simulation, different levels of gap junction conductance were applied to the cells and current stimuli were set at 80 μA. Changes in membrane potential are illustrated after 40 driving beats. Of note, the increased conductance in gap junction tends to prolong action potential duration. At a gap junction conductance of 5 μS/μF, excitability of the cardiomyocyte is greatly reduced and no generation of action potential occurs.

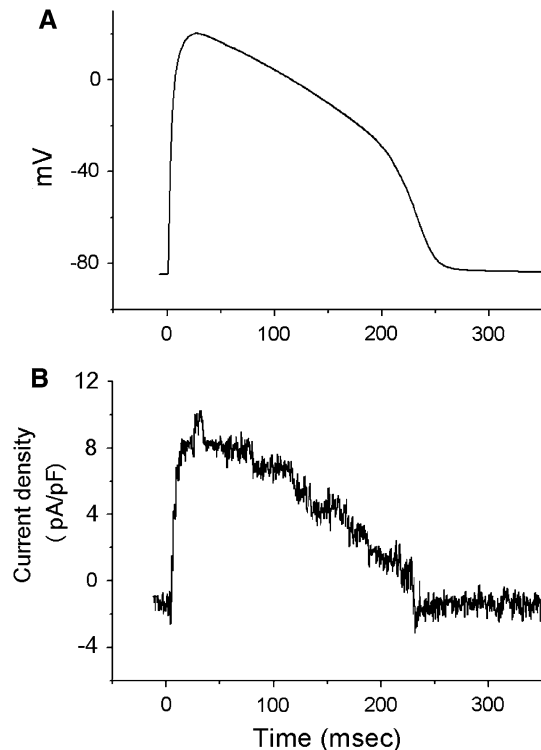

Fig. 8. K^+ outward current elicited by an action potential waveform of human cardiac fibroblast when it is electrically coupled to cardiomyocyte. In these experiments, cells were bathed in normal Tyrode's solution containing 1.8 mM $CaCl_2$. The upper part shows simulated waveform of action potentials generated from a electrically-coupled human cardiac fibroblast.

predict that when electrical coupling between these two cells occurs, the amplitude of $I_{K(Ca)}$ in cardiac fibroblasts will be greatly increased because membrane depolarization caused the generation of cardiac action potential. However, the initial rise of action potential in cardiac fibroblasts is relatively slow compared to that in cardiac action potential. In addition, the peak current density amplitude of $I_{K(Ca)}$ is relatively small as compared to that elicited by rectangular pulses as shown in Fig. 1.

Effect of Changes in Gap Junction Conductance on Cardiac Action Potential

Previous studies have demonstrated that expression of the gap junction proteins C × 45 and C × 43 in the sinus node region could be altered upon regional ischemia (Camelliti et al., 2004). Electrical coupling in the heart can be regulated by adrenergic activation (Salameh et al., 2006). In our simulation, we also arbitrarily increased gap junction conductance stepwise to examine the configuration of cardiac action potential. During pacing at a frequency of 1.0 Hz, prolongation of cardiac action potential became clearly discernible (Fig. 7). More importantly, when gap junction conductance was elevated to 5 μS/μF, cell excitability was so depressed that action potential was no longer generated with a stimulus of 80 μA. The simulation results predict that cardiomyocytes, when tightly electrically coupled to cardiac fibroblasts, are susceptible to alterations in action potential configuration and membrane excitability.

Properties of K^+ Outward Currents in Response to Action Potentials Simulated from Electrically-coupled Fibroblasts

Because the size and time course of ion currents in response to chang in waveforms of action potentials was different from those during a rectangular voltage-clamp pulse (Lo et al. 2001), the outward currents during simulated action potentials generated from human cardiac fibroblasts, when it is electrically coupled to cardiomyocyte, were further characterized. In these experiments, simulated waveforms of action potentials from an electrically-coupled fibroblast were digitally generated as voltage templates and re-played to evoke ion currents in GH3 cells (Lo et al., 2001). As shown in Fig. 8, the K+ outward current in human cardiac fibroblasts could be generated in response to simulated action potential waveforms. Similar results were obtained in seven different cells. Because the currents in response to simulated action potential waveforms were sensitive to block by 1 μM paxilline, the results suggest that $I_{K(Ca)}$ may actually be K+ flux through the BK_{Ca} channel. The current trajectory in response to action potential waveform of simulated electrically-coupled fibroblasts closely resembles simulated density of

$I_{K(Ca)}$ shown in Fig. 6. Thus, it is possible that $I_{K(Ca)}$ can be evoked by action potential waveforms of cardiac fibroblasts, if fibroblasts are electrically coupled to cardiomyocytes.

Discussion

In this study, we clearly identified the presence of Ca^{2+}-activated K^+ currents in human cardiac fibroblasts. The electrophysiological and pharmacological properties of BK_{Ca} channels have been also characterized in these cells but not in heart-derived H9c2 myoblasts. The results demonstrate that the pharmacological and electrophysiological properties of these channels are similar to those in other types of cells described previously (Lippiat, Standen & Davies, 2000; Wu, 2003; Lin et al., 2004). These BK_{Ca} channels were expressed in cultured human cardiac fibroblasts but not in cardiomyocytes. However, the physiological significance of BK_{Ca} channels expressed in cultured human cardiac fibroblasts remains poorly understood. Based on our simulation study, it is anticipated that upon electrical coupling between cardiac fibroblasts and cardiomyocytes, the amplitude of $I_{K(Ca)}$ in cardiac fibroblasts will be greatly increased as membrane depolarization caused the generation of cardiac action potential. Indeed the amplitude of $I_{K(Ca)}$ in cardiac fibroblast can also be enhanced in response to simulated action potential wave forms of electrically coupled fibroblast. The activity of ion channels in cardiac fibroblasts tends to alter cardiac action potential, thereby exacerbating the propensity of cardiac arrhythmias.

The macroscopic outward currents in response to membrane depolarizations seen in human cardiac fibroblasts were found to reveal a slope of 58 mV per 10-fold increase in extracellular K^+. This can be interpreted to mean that, unlike the properties of proton currents recently described in human cardiac fibroblasts (El Chemaly et al., 2006), the decrease of outward current caused by removal of extracellular Ca_{2+} in human cardiac fibroblasts can follow the Nernstian behavior of a K^+-selective channel.

Consistent with recent observations (Chilton et al., 2005), this study clearly demonstrated the presence of voltage-gated K^+ currents in human cardiac fibroblasts. The ability of paxilline or iberitoxin to block this type of outward K^+ current also suggests the presence of $I_{K(Ca)}$ in these cells. Paxilline, an indole alkaloid isolated from Penicillium paxilli, has been reported to be a selective inhibitor of BK_{Ca} channels (Wu, 2003). In single-channel recordings, the single-channel conductance of BK_{Ca} channels in human cardiac fibroblasts, was 162 $\pm$ 8 pS (n = 7), a value that is similar to that of prototypical BK_{Ca} channels present in various excitable and non-excitable cells (Wu, 2003; Wu et al., 2003). However, this value is apparently greater than that of small-conductance Ca^{2+}-activated K^+ channels (Wang et al., 1999; Xu et al., 2003) and of G proteins-coupled inwardly rectifying K^+ (GIRK) channels described previously in atrial myocytes (Wu, Liu & Hwang, 1998). BK_{Ca}-channel activity recorded in cell-attached configuration was increased 2 min after exposure to ionomycin (10μM) or squamocin (10μM). These compounds were reported to the ionophores for Ca^{2+} ions (Wu et al., 2002). Immunoblot studies shown here have also revealed the presense of BK_{Ca} channel-α subunit as verified in cultured human cortical neurons and in pituitary GH_3 cells (Wang et al., 2006). Taken together, it is anticipated that the activity of BK_{Ca} channel's primarily constitutes the generation of whole-cell $I_{K(Ca)}$ in human cardiac fibroblasts, not in cardiac H9c2 cell. In fact, the BK_{Ca} channel has been shown to have an important bearing on the role of the firing in neurons and endocrine cells, and of the contractile force in smooth muscle cells (Amberg et al., 2003; Wu et al., 2003; Lin et al., 2004). Its activation causes membrane hyperpolarization, which inhibits cell firing and limits the firing frequency of action potentials.

The BK_{Ca} channels are activated by membrane depolarization and/or by increased intracellular Ca^{2+}. The activity of BK_{Ca} channels in fibroblast can thus be influenced by action potential from cardiomyocyte, if these two types of cells are functionally linked. As a result, as predicted from the present simulations, the configuration of cardiac action potential will be altered by electrical activity of fibroblast adjacently coupled to cardiomyocyte, in pathophysiological conditions where fibroblasts are formed and electrically coupled to cardiomyocytes. Heterocellular coupling likely occurs under physiological conditions as well as pathophysiological ones. The K^+ outward current to which BK_{Ca}-channel activity seen in cardiac fibroblast contibute tend to have an impact on electrical activity of cardiomyocyte. However, as shown Fig. 1 and 2A, a substantial outward current remained after Ca^{2+}-activated K^+ current was blocked, it is also likely that other types of voltage-gated K^+ channels contribute to the generation of membrane potential in cardiac fibroblasts as described previously (Chilton et al., 2005).

At present, there are few experimental observations clearly showing fibroblast-cardiomyocyte coupling in native cardiac tissue. Electrophysiological studies have been hampered by the fact that cardiac fibroblasts have a high membrane resistance (in the GΩ range), although this may be advantageous for direct electrical coupling if fibroblasts are functionally linked to cardiomyocytes. In this study, we employed a modified dynamic Luo-Rudy model of cardiomyocytes (Luo & Rudy, 1994) in an attempt to delineate the potential effect of electrically coupled cardiac fibroblasts on cardiac action potential. Based on our sim-

ulation studies, when cardiac fibroblasts are tightly coupled to cardiomyocytes, they can readily alter the membrane potential dynamics of cardiomyocytes. The activity of BK_{Ca} channels functionally expressed in cardiac fibroblasts can also be activated. In terms of the emergence of action potential in cardiac fibroblasts, the results of our simulation study were very much in line with experimental observations by Kamkin et al. (2005). More interestingly, when the coupling strength was increased with a g_C value of 4 pS/pF, cardiomyocyte excitability was greatly depressed because $I_{K(Ca)}$ present in cardiac fibroblasts contributed to a change in membrane potential of cardiomyocytes. Therefore, using a mathematical model, we propose that electrical properties of cardiomyocytes are altered by an increase in electrical coupling to cardiac fibroblasts though gap junctions.

Cardiac fibroblasts, which are electrically nonexcitable, were found to contain background conductances for K^+, Na^+ and Cl^- which can contribute to the level of resting potential of these cells (Kohl et al., 1999). In this study, the BK_{Ca} channel activity was the only one employed for the simulation of membrane potential in cardiac fibroblasts for the sake of simplicity. Therefore, in our stimulation data, the resting potential of electrically coupled cardiac fibroblasts is relatively more negative compared with the experimental observations (Kamkin et al., 2005). However, zero current potential recorded by Chilton et al. (2005) was close to the Nernst potential for K^+ and compatible with the value provided by our simultations. The present findings showing the intial rise of action potential in cardiac fibroblasts is relatively slow as compared to that in cardiomycyte also suggest that activation kinetics of BK_{Ca} channels in response to membrane depolarization is comparatively slower than that in voltage-gated Na^+ channels. Nevertheless, the simulation results are shown to correlate well with the experimental observations described previously (Kamkin et al., 2005). Our experimental studies also showed that $I_{K(Ca)}$ can be generated in response to simulated action potential waveforms of cardiac fibroblast.

Dissociated chondrocytes have been reported to be de-differentiated under culture conditions. Properties of ion channels may be altered in cell cultured chondrocytes (Wilson et al., 2004). However, none of reports regarding changes in the activity of ion channels under culture conditions have been described in fibroblasts. It still remains to be clarified whether the BK_{Ca} channel characterized in this study is an artifact of the cells being in culture.

Based on more complicated gating of the channel, a wide variety of simulations for the BK_{Ca} channel have been described (Horrigan, Cui & Aldrich, 1999; Rothberg & Magleby, 1999). We also incorporated an allosteric model (Horrigan et al., 1999) for the BK_{Ca} channel to Luo-Rudy phase 1 model. The simulation results were similar to those shown in this study. However, when cardiac fibroblasts were exposed to various stimuli (e.g., angiotensin II) or when cardiomyocytes exhibited variable changes in membrane potential (e.g., early or delayed afterdepolarizations), the kinetic gating for BK_{Ca} channels would be significantly altered. Therefore, more complex mathematical models incorporated with the cardiomyocyte model need to be developed to clearly describe how the activity of BK_{Ca} channels in cardiac fibroblasts modulates cardiac action potential through an interaction with cardiomyocytes via electrical coupling.

The present model is a minimal one, i.e., a model of minimal complexity but containing the essential components to produce electrical interaction between cardiomyocytes and fibroblasts. In this simulation, we simply considered intercellular coupling between cardiomyocytes and fibroblasts to be instantaneous and bidirectional. In fact, because there were variable input resistances of the cells, suggesting that monodirectional current flow between these two cells should occur. In addition, the variety of connexins may provide a rich repertoire for diverse modulatory possibilities not simply by voltage but also by a variety of factors, including intracellular pH, intracellular Ca^{2+}/calmodulin or second messengers (Peracchia, 2004; Salameh et al., 2006). Therefore, more detailed simulations need to be further considered by introducing more realistic assumptions in asymmetrical and heterogeneous coupling of cardiomyocytes and fibroblasts. In addition, as the guinea-pig action potential mathematically used in this study is different from that of any region of the human ventricle, another model derived from ten Tusscher et al. (2004) was also linked to cardiac fibroblast, the simulation results were similar (data not shown).

It also needs to be noted that this cell line used in this study could be representative of a myofibroblast and not a fibroblast, although the expression of fibroblast surface protein was identified. The important difference between myofibroblast and fibroblast remains to be further explored on the basis of structural, immunocytochemical and functional data.

In summary, this study provides evidence that BK_{Ca} channels are functionally expressed in human cardiac fibroblasts. The simulations predict that the activity of these channels in cardiac fibroblasts may contribute to alteration in cardiac action potential when there is a tight electrical coupling between cardiomyocytes and cardiac fibroblasts. As a result, cardiac fibroblasts may act as a current sink and prevent impulse propagation.

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References

Amberg, G.C., Bonev, A.D., Rossow, C.F., Nelson, M.T., Santana, L.F. 2003. Modulation of the molecular composition of large conductance, Ca^{2+} activated K^+ channels in vascular smooth muscle during hypertension. *J. Clin. Invest*. **112**:717–724

Butler, A., Tsunoda, S., McCobb, D.P., Wei, A., Salkoff, L. 1993. *mSlo*, a complex mouse gene encoding "maxi" calcium-activated potassium channels. *Science* **261**:221–224

Camelliti, P., Borg, T., Kohl, P. 2005. Structural and functional characterization of cardiac fibroblasts. *Cardiovasc. Res*. **65**:40–51

Camelliti, P., Green, C.R., LeGrice, I., Kohl, P. 2004. Fibroblast network in rabbit sinoatrial node: Structural and functional identification of homogeneous and heterogeneous cell coupling. *Circ. Res*. **94**:828–835

Chilton, L., Ohya, S., Freed, D., George, E., Drobic, V., Shibukawa, Y., Maccannell, K.A., Imaizumi, Y., Clark, R.B., Dixon, I.M., Giles, W.R. 2005. K^+ currents regulate the resting membrane potential, proliferation, and contractile responses in ventricular fibroblasts and myofibroblasts. *Am. J. Physiol*. **288**:H2931–H2939

Clancy, C.E., Rudy, Y. 2001. Cellular consequences of HERG mutations in the long QT syndrome: Precursors to sudden cardiac death. *Cardiovasc. Res*. **50**:301–313

El Chemaly A, Guinamard R, Demion M, Fares N, Jebara V, Faivre JF, Bois P. 2006. A voltage-activated proton current in human cardiac fibroblasts. *Biochem Biophys Res Common* **340:** 512–516

Ermentrout, G.B. 2002. Simulating, Analyzing, and Animating Dynamical System. A Guide to XPPAUT for Researchers and Students. Society for Industrial and Applied Mathematics (SIAM), Philadelphia

Gaudesius, G., Miragoli, M., Thomas, S.P., Rohr, S. 2003. Coupling of cardiac electrical activity over extended distances by fibroblasts of cardiac origin. *Circ. Res*. **93**:421–429

Guinamard, R., Chatelier, A., Demion, M., Potreau, D., Patri, S., Rahmati, M., Bois, P. 2004. Functional characterization of a Ca^{2+}-activated non-selective cation channel in human atrial cardiomyocytes. *J. Physiol*. **558**:75–83

Horrigan, F.T., Cui, J., Aldrich, R.W. 1999. Allosteric voltage gating of potassium channels I. Mslo ionic currents in the absence of Ca^{2+}. *J. Gen. Physiol*. **114**:277–304

Kamkin, A., Kiselva, I., Lozinsky, I., Scholz, H. 2005. Electrical interaction of mechanosensitive fibroblasts and myocytes in the heart. *Basic Res. Cardiol*. **100**:337–345

Keener J.P., Keizer J.E. 2002. Fast and slow time scales. In: C.P., Fall, E.S., Marland, J.M., Wagner, J.J., Tyson, editors. Computational Cell Biology. Springer-Verlag, New York pp. 77–100

Kizana, E., Ginn, S.L., Allen, D.G., Ross, D.L., Alexander, I.E. 2005. Fibroblasts can be genetically modified to produce excitable cells capable of electrical coupling. *Circulation* **111**:394–398

Kohl, P., Camelliti, P., Burton, F.L., Smith, G.L. 2005. Electrical coupling of fibroblasts and myocytes: Relevance for cardiac propagation. *J. Electrocardiol*. **38**(Suppl. 4):45–50

Kohl, P., Hunter, P., Noble, D. 1999. Stretch-induced changes in heart rate and rhythm: Clinical observations, experiments and mathematical models. *Prog. Biophys. Mol. Biol*. **71**:91–139

Lin, M.W., Yang, S.R., Huang, M.H., Wu, S.N. 2004. Stimulatory actions of caffeic acid phenethyl ester, a known inhibitor of NF-κB activation, on Ca^{2+}-activated K^+ current in pituitary GH_3 cells. *J. Biol. Chem*. **279**:26885–26892

Lippiat, J.D., Standen, N.B., Davies, N.W. 2000. A residue in the intracellular vestibule of the pore is critical for gating and permeation in Ca^{2+}-activated K^+ (BK_{Ca}) channels. *J. Physiol*. **529**:131–138

Lo, Y.C., Yang, S.R., Huang, M.H., Liu, Y.C., Wu, S.N. 2005. Characterization of chromanol 293B-induced block of the delayed rectifier K^+ current in heart-derived H9c2 cells. *Life Sci*. **76**:2275–2286

Lo YK, Wu SN, Lee CT, Li HF, Chiang HT 2001. Characterization of action potential waveform-evoked L-type calcium currents in pituitary GH3 cells. *Pflügers Arch*. **442:** 547–557

Luo, C.H., Rudy, Y. 1994. A dynamic model of the cardiac ventricular action potential. I. Simulation of ionic currents and concentration changes. *Circ. Res*. **74**:1071–1096

Moczydlowski, E., Latorre, R. 1983. Gating kinetics of Ca^{2+}-activated K^+ channels from rat muscle incorporated into planar lipid bilayers. Evidence for two voltage-dependent Ca^{2+} binding reactions. *J. Gen. Physiol*. **82**:511–542

Peng, J., Gurantz, D., Tran, V., Cowling, R.T., Greenberg, B.H. 2002. Tumor necrosis factor-alpha-induced AT1 receptor upregulation enhances angiotensin II-mediated cardiac fibroblast responses that favor fibrosis. *Circ. Res*. **91**:1119–1126

Peracchia, C. 2004. Chemical gating of gap junction channels: Roles of calcium, pH and calmodulin. *Biochem. Biophys. Acta* **1662**:61–80

Rohr, S. 2004. Role of gap junctions in the propagation of the cardiac action potential. *Cardiovasc. Res*. **62**:309–322

Rothberg, B.S., Magleby, K.L. 1999. Gating kinetics of single large-conductance Ca^{2+}-activated K^+ channels in high Ca^{2+} suggest a two-tiered allosteric gating mechanism. *J. Gen. Physiol*. **114**:93–124

Salameh, A., Frenzel, C., Boldt, A., Rassler, B., Glawe, I., Schulte, J., Muhlberg, K., Zimmer, H.G., Pfeiffer, D., Dhein, S. 2006. Subchronic alpha- and beta-adrenergic regulation of cardiac gap junction protein expression. *FASEB J*. **20**:365–367

Sato, T., Saito, T., Saegusa, N., Nakaya, H. 2005. Mitochondrial Ca^{2+}-activated K^+ channels in cardiac myocytes: A mechanism of the cardioprotective effect and modulation by protein kinase A. *Circulation* **111**:198–203

Silva, J., Rudy, Y. 2005. Subunit interaction determines I_{Ks} participation in cardiac repolarization and repolarization reserve. *Circulation* **112**:1384–1391

Tanaka, Y., Meera, P., Song, M., Knaus, H.G., Toro, L. 1997. Molecular constituents of maxi K_{Ca} channels in human coronary smooth muscle: Predominant $\alpha + \beta$ subunit complexes. *J. Physiol*. **502**:545–557

ten Tusscher KH, Noble D, Noble PJ, Panfilov AV. 2004. A model for human ventricular tissue. *Am J Physiol Heart Circ Physiol*. **286:** H1573–1589.

Wang, W., Watanabe, M., Nakamura, T., Kudo, Y., Ochi, R. 1999. Properties and expression of Ca^{2+}-activated K^+ channels in H9c2 cells derived from rat ventricle. *Am. J. Physiol*. **276**:H1559–H1566

Wang, Y.J., Liu, Y.C., Chang, H.D., Wu, S.N. 2006. Diosgenin, a plant-derived sapogenin, stimulates Ca^{2+}-activated K^+ current in human cortical HCN-1A neuronal cells. *Planta Med*. **72**:430–436

Wilson JR, Duncan NA, Giles WR, Clark RB. 2004. A voltage-dependent K^+ current contributes to membrane potential of acutely isolated canine articular chondrocytes. *J Physiol (London)*. **557:**93–104.

Wu, S.N. 2003. Large-conductance Ca^{2+}-activated K^+ channels: Physiological role and pharmacology. *Curr. Med. Chem*. **10**:1241–1253

Wu, S.N., Chiang, H.T., Chang, F.R., Liaw, C.C., Wu, Y.C. 2002. Stimulatory effects of squamocin, an annonaceous acetogenin, Ca^{2+}-acitvated K^+ current in cultured smooth muscle cells of human coronary artery. *Chem. Res. Toxicol*. **16**:15–22

Wu, S.N., Lin, P.H., Hsieh, K.S., Liu, Y.C., Chiang, H.T. 2003. Behavior of nonselective cation channels and large-conductance Ca^{2+}-activated K^+ channels induced by dynamic changes in membrane stretch in cultured smooth muscle cells of human coronary artery. *J. Cardiovasc. Electrophysiol.* **14**:44–51

Wu, S.N., Liu, S.I., Hwang, T.L. 1998. Activation of muscarinic K^+ channels by extracellular ATP and UTP in rat atrial myocytes. *J. Cardiovasc. Pharmacol.* **31**:203–211

Xu, Y., Tuteja, D., Zhang, Z., Xu, D., Zhang, Y., Rodriguez, J., Nie, L., Tuxson, H.R., Young, J.N., Glatter, K.A., Vázquez, A.E., Yamoah, E.N., Chiamvimonvat, N.C. 2003. Molecular identification and functional roles of a Ca^{2+}-activated K^+ channel in human and mouse hearts. *J. Biol. Chem.* **278**:49085–49094