by adjusting gel stiffness with different mixing ratios of PVA and crosslinker. **Results:** Myocyte contraction and calcium transients were measured in-gel and compared with load-free cells. Contracting cells in-gel showed a significantly lower fractional shortening (12.6 ± 0.9 in-gel vs. 18.2 ± 0.9 load-free, $p<0.001$), demonstrating a ''knock-down factor'' of 31% when the myocyte is pulling mechanical load. Contraction departure and return velocities were significantly slower in-gel than in the load-free state as expected. However, the systolic calcium transient was greater in-gel than load-free (Fura-2 fluorescence ratio peak height 1.47 ± 0.09 in-gel vs. 0.87 ± 0.04 load-free, $p<0.0001$), revealing the mechano-chemotransduction that translates external stress to intracellular Ca^{2+} increase. The calcium transient departure and return velocities were also significantly higher in-gel than load-free. **Conclusions:** Our newly-developed versatile Cell-in-Gel system provides a novel experimental method to control mechanical stress at the single cell level for investigating mechano-chemotransduction pathways in intact myocytes. The above experimental results are consistent with our modeling predictions, demonstrating the mechanical load effects on altering myocyte Ca^{2+} handling and contraction dynamics.

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Functional Interaction with Filamin a Enhances Atrial-Specific Small Conductance Ca2 Activated K^+ Channel (SK2) Surface Membrane Expression

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For ion channels to function properly, a precise number of channel proteins need to be trafficked to exact locations on the cell surface membrane. Small-conductance, Ca^{2+}-activated K^+ channels (SK) are predominantly expressed in the atria and their role has been implicated in atrial fibrillation (AF).

Using yeast two-hybrid screen against human heart library, we identify filamin A (FLNA) as a putative interacting protein with SK2 channel. Patch-clamp recordings and immunofuorescence studies in neonatal and adult cardiac myocytes as well as HEK293 cells suggest the interaction leads to an increase in SK2 membrane localization. SiRNA knockdown of FLNA in neonatal myocytes results in a decrease in the membrane localization of SK2 channels. Additionally, the calcium dependency of SK2 channel membrane expression was examined using Total Internal Reflection Microscopy (TIRF-M) and immunofluorescence microscopy. Importantly, intracellular Ca^{2+} (Ca^{2+}_i) plays a critical role in the membrane localization of SK2 channel when the channel is co-expressed with α-actinin2, another cytoskeletal protein which we have previously shown to interact with SK2 channel. In conclusion, FLNA is a regulator of SK2 channel expression. Moreover, SK2 membrane expression is critically dependent on Ca^{2+}_i. An increase in Ca^{2+}_i, for example, during AF, is predicted to result in an increase in SK2 channel expression leading to shortening of the action potentials.

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A-Actinin2 and Filamin a Cytoskeletal Interacting Proteins Facilitate SK2 Channels Recycling from Endosomes to the Surface Membrane

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The normal function of ion channels depends critically on the precise subcellular localization and the number of channel proteins on the cell surface membrane. Small-conductance, Ca^{2+}-activated K^+ channels (SK) are unique in that they are gated solely by changes in intracellular Ca^{2+}. The channels are expressed in atrial cardiomyocytes and responsible for shaping atrial action potentials. Understanding the mechanisms of SK channel trafficking may provide new insights into the regulation controlling the repolarization of atrial myocytes.

Surface membrane localization of SK2 channels were evaluated using Total Internal Reflection Fluorescence (TIRF) Microscopy. SK2 channels were tagged with Tomato fluorescent protein and expressed in HEK 293 cells. We have previously demonstrated that the C and N termini of SK2 channels interact with actin-binding proteins, α-actinin2 and filamin A, respectively. When SK2 channels were co-expressed with filamin A, the membrane fluorescence intensity of SK2 channels increased significantly. Similar findings were observed with the putative interacting cytoskeletal protein, α-actinin2. These observations supported our notion that filamin A and α-actinin2, facilitate the forward trafficking or decrease the retrograde trafficking. We next tested the effects of primaquine and dynasore. Primaquine has been shown to block the recycling pathway from endosomes while dynasore is known to be a specific inhibitor of dynamin which is responsible for endocytosis. Treatment with primaquine significantly reduced the membrane expression of SK2 channels. While treatment with dynasore failed to alter the surface membrane expression of SK2 channels. Further investigations using constitutively-active or dominant-negative forms of Rab GTPases provide additional insights into the distinct roles of the two putative cytoskeletal proteins on the recycling processes of SK2 channels from endosomes. α-actinin2 and filamin A facilitates SK2 channels recycling to the surface membrane through different endosomal pathways.

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Critical Roles of SK3 Calcium-Activated Potassium Channels in the Repolarization of Atrial Myocytes

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Small conductance Ca^{2+}-activated K^+ channels (SK channels) have been first identified in central nervous system, where they aid in integrating changes in intracellular Ca^{2+} with the intrinsic excitability of neurons and affect the synaptic transmission and plasticity. We have demonstrated that three members of SK channel family (SK1, SK2 and SK3) are present in human and mouse cardiac myocytes and contribute significantly to the repolarization process in both mouse and human atria. Moreover, the three members can heteromultimerize to form functional channels. In this study, we directly tested the contribution of SK3 channels to the overall repolarization of atrial action potentials. We used a mouse model with site-specific insertion of a tetracycline-based genetic switch in the 5' untranslated region of the *KCNN3* (SK3 channel) gene so that SK3 expression could be decreased by dietary doxycycline administration without interfering with the normal profile of SK3 expression. Whole-cell patch-clamp recording showed a significant shortening of the action potential duration mainly at 90% repolarization (APD_{90}) in atrial myocytes from the homozygous $SK3^{T/T}$ animals. Conversely, treatment with dietary doxycycline results in a significant prolongation of APD_{90} in atrial myocytes from $SK3^{T/T}$ animals. We further demonstrated that the shortening of action potential durations in SK3 over-expression mice predisposes the animals to inducible atrial arrhythmias. In conclusion, SK3 contributes toward atrial action potential repolarization, suggesting the pivotal role of the SK channels in atrial myocytes.

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Targeted Deletion of KCNE4 Impairs Ventricular Repolarization in Mice

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With 1000 people succumbing to sudden cardiac death each day in the United States alone, it is imperative to continue to elucidate the molecular basis for cardiac ion channel function and dysfunction. KCNE4 (MiRP3) is a 1TM K^+ channel β subunit, inherited variants in which are associated with atrial fibrillation (AF) and Long QT Syndrome, a disorder linked to defective ventricular myocyte repolarization. The mechanisms of pathology are unclear. Here, we deleted the *Kcne4* gene in mice and assessed the effects on cardiac function. Transcriptomic analysis suggested no global gene remodeling and hemodynamic parameters were normal. Electrocardiographic measurements revealed normal cardiovascular electrical function at 5 months, but genotype-specific QTc prolongation at 18 months of age. Consistent with this finding, using patch clamp analysis of cardiac myocyte currents we discovered that older $Kcne4^{-/-}$ mice exhibit diminished K^+ current in septal ventricular myocytes compared to that of age- and sex-matched $Kcne4^{+/+}$ littermates. Kinetic and pharmacological analyses pinpointed the affected current to be the 50 μM 4-aminopyridine (4-AP)-sensitive component of the $I_{K,slow}$ current, which is conducted in mouse ventricles by the Kv1.5 delayed rectifier α subunit. We also found that in CHO cells, KCNE4 co-expression increases the sensitivity of $K_V1.5$