In silico model of SK channel gating, temperature dependence and calcium sensitivity

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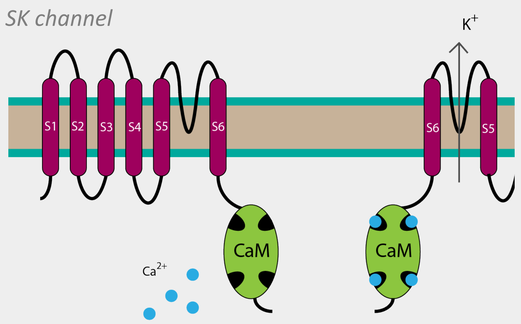
Introduction

The small conductance calcium activated potassium (SK) channel has emerged as an important atrial-specific target for atrial fibrillation (AF) treatment. Current pharmacological tools for AF treatment are moderately effective and carry significant risk. *In silico* modeling can provide frameworks for integrating and understanding disease mechanisms and compound (multi-target) drug actions, but current atrial myocyte models do not include SK channel formulations. A computational model of single SK2 channels was developed from experimental room temperature data by Hirschberg et al. [1998]. The temperature dependence of SK calcium-binding and gating is poorly described but may meaningfully alter the role of SK currents in the atrial action potential.



Objective

Our objective is to define a computational model of the SK current at physiological temperature, and use it to build predictive drug-binding models. These can be integrated in whole-cell models of healthy and diseased atrial myocytes.

Methods

Macropatch recording:

Inside-out patch clamp experiments of HEK293 cells with stable hSK2 and hSK3 expression were performed in symmetrical potassium solutions and a range (0.01-10 µM) of free intracellular Ca^{2+}. Patches were exposed to room or physiological temperature (37°C). Currents were recorded using Patchmaster software (HEKA Elektronik) with 200 ms voltage ramps ranging from -80 to +80 mV.

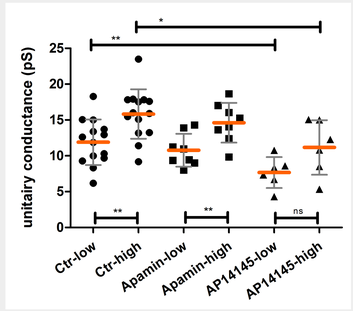
Single channel recording:

HEK293 cells were transfected with rSK2 and EGFP using 1:10 plasmid ratio and used after 48 hours. Quartz electrodes contained an isotonic K^+ solution with a free [Ca^{2+}] of 60 nM, while inside-out patches were excised into an isotonic K^+ solution containing 1 µM Ca^{2+}. Measurements were performed at room temperature with no drug (control), apamin (100 nM) or allosteric modulator AP14145 (10 µM) present in the electrode solution. Data was filtered at 1 kHz (8-pole Bessel) and acquired at 10 kHz using Pulse (Heka). Single channel transitions were analyzed with TAC (Bruxton) using the 50% threshold technique.

Results

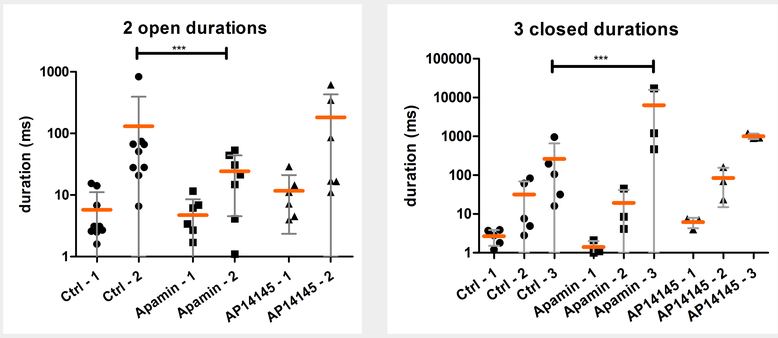
Two single channel conductances present in control and drug-modulated measurements

	n	low	high
Control (pS)	14	11.9 ± 3.2	15.8 ± 3.5
		75 ± 25 %	
Apamin (pS)	8	10.8 ± 2.3	14.6 ± 2.8
		42 ± 29 %	
AP14145 (pS)	6	7.67 ± 2.2	11.2 ± 3.8
		80 ± 33 %	



rSK2-mediated current show presence of two single channel conductances for control and drug groups, with only AP14145 significantly reduced channel conductance compared to control.

Consistent structure of SK channel gating scheme

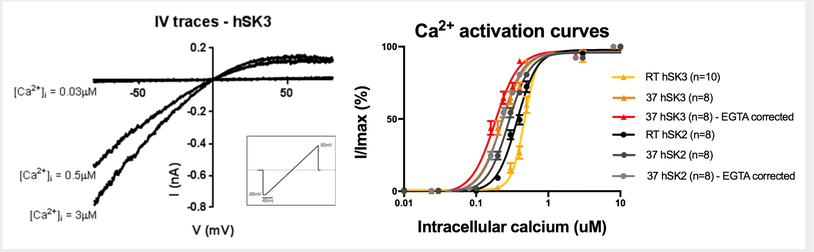


Consistent number of transitions over all groups show similar Markov model structure with varying transition rates. Apamin significantly prolonged longest open and closed durations.

Conclusions

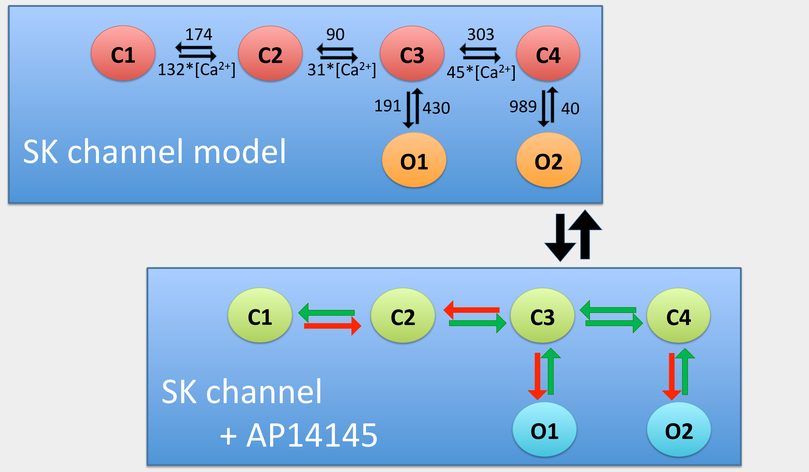
- Single channel measurements support a Markov structure consisting of 2 open and 4 closed states
- SK channel inhibitors act via different dynamics
- Physiological temperature increased maximum SK conductance and calcium sensitivy at macropatch level

Temperature increased calcium sensitivity and maximum current of hSK2 and hSK3 macropatches



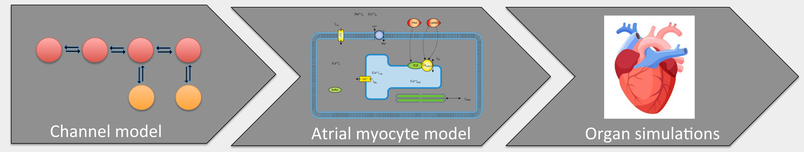
Increase from room temperature to 37°C decreased the (EGTA buffering corrected) EC50 for hSK2 (0.38±0.02 µM to 0.22±0.01 µM; P<0.0001, n=8), while shifting the EC50 from 0.53±0.07 µM to 0.18±0.02 µM for hSK3-mediated current (P=0.0006, n=10;n=8). The calcium sensitivity increased with factor 1.44 for hSK2 and 2.05 for hSK3 for every 10C increase. The maximum current Q10 is 1.13 and 1.24 for hSK2 and hSK3 respectively.

Parameterized Markov model for SK channel gating



Presence of inhibitor AP14145 up- and down (green and red arrows, respectively) regulated transition rates, affecting channel gating dynamics.

Future perspectives



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