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Novel Negative Allosteric Modulator Inhibits Small Conductance Ca²⁺-Activated K⁺-Channels and Selectively Prolongs Atrial Refractoriness in Conscious Pigs

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Background: Small conductance Ca²⁺-activated K⁺-channels (SK channels) represent a promising atrial-selective target for treatment of atrial fibrillation (AF). Establishing the molecular mechanism for SK channel inhibition by small molecules is important for generating new drugs.

Objective: To establish the mechanism of SK inhibition by AP14145 and to confirm a prolongation of the atrial effective refractory period (AERP) in pigs without comparable effects in the ventricles.

Methods: Using site directed mutagenesis the putative drug binding site of AP14145 was established AP14145 subtype selectivity and mechanism of action were investigated by inside-out and whole cell patch clamp recordings of expressed SK channels. A selectivity profile of AP14145 was performed on heterologous expressed channels. Effects of AP14145 (i.v. infusion of 5 mg/kg over 30 min) on the AERP were recorded in three conscious healthy pigs using an implanted pacemaker.

Results: AP14145 was found to be a selective negative allosteric modulator of SK2 and SK3 channels. The presence of AP14145 10 μM increased the EC₅₀ of Ca²⁺ from 0.37 ± 0.02 μM (n=9) to 1.2 ± 0.1 μM (n=7). This inhibitory effect depended on two amino acids, S508 and A533 found in the inner pore helix of S5 and S6, as earlier found for another negative SK channel modulator NS8593 indicating a common binding site AP14145 did not display subtype selectivity (IC₅₀ = 1.1 ± 0.3 μM SK2 vs IC₅₀ = 1.1 ± 0.3 μM SK3, n=7). In the three conscious pigs, AP14145 increased the AERP by 40 ms (22%), 32 ms (22%) and 20 ms (18%) without prolonging the QTcB, -7 ms (-2%), -16ms (-4%) and +38 ms (+8%) , respectively.

Conclusions: AP14145 is a selective negative allosteric modulator of SK2 and SK3 that shifts the calcium dependence of channel activation, an effect dependent on two identified amino acids. Moreover, SK current inhibition prolongs atrial refractoriness in conscious healthy pigs without affecting the QT interval, thereby highlighting SK channels as an atrial-selective target. The understanding of how SK inhibition is accomplished at the molecular level should help future development when targeting SK channels for the treatment of AF.