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Dual effects of propafenone on Kir2.x channels

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Increase of the inward rectifying K^+ current (I_{K1}) plays a key role in the establishment of fast and stable reentries of spiral electrical waves (rotors) and fibrillation dynamics. Therefore, cardiac inward rectifier Kir2.x channels underlying I_{K1} are putative targets for the control of fibrillatory arrhythmias. However, data on the pharmacological properties of Kir2.x channels are scarce. In the present study we have analyzed the effects of propafenone, a class IC antiarrhythmic drug, on human Kir2.1, 2.2, and 2.3 channels transiently expressed in CHO cells and on human I_{K1} recorded in myocytes isolated from right atrial appendages. Currents were recorded using the patch-clamp technique. Results are the mean±s.e.m. of ≥8 experiments. Statistical analysis was done by Student *t* test or one-way ANOVA followed by Newman Keuls test. To compare concentration-response curves, an F-test was used. A $P<0.05$ was considered significant.

Propafenone produced dual effects (increasing and decreasing) on Kir2.1 channels whereas only blocking effects were produced on Kir2.2 and 2.3 channels ($P<0.05$). At low concentrations (10 nM-1 μM) propafenone significantly increased the mean open time and the open probability of Kir2.1 channels by decreasing the affinity of the channel for intracellular polyamines ($P<0.05$). Indeed, 0.5 μM propafenone increased the IC_{50} for spermine induced-block from 0.5±0.1 to 11.1±0.1 nM ($P<0.05$), as demonstrated in inside-out experiments. The propafenone-increasing effects critically depended on its interaction with Cys311 located at the HI-loop of the cytoplasmic domain of the channel. At the equivalent position Kir2.2 and Kir2.3 channels exhibit an alanine which explains the Kir2.1-specificity of the propafenone-increasing effects. At concentrations above the therapeutic range (5-100 μM) propafenone inhibited Kir2.x currents, being 3.5 fold more potent for blocking Kir2.3 (IC_{50}=15.0±2.5 μM) than Kir2.1 (IC_{50}=52.0±2.2 μM) channels ($P<0.05$). Propafenone significantly decreased the Kir2.x unitary current amplitude ($P<0.05$), an effect which was abolished by the mutation of two arginines conserved in the three Kir2.x channels (Arg228 and 260 in Kir2.1). Propafenone binding to these residues allosterically decreased the Kir2.x channel affinity for phosphatidylinositol 4,5-bisphosphate (PIP_2), an essential modulator of Kir2.x channel activity, an effect which ultimately decreases the current. In fact, 50 μM propafenone significantly reduced the PIP_2-induced increase of Kir2.1 currents recorded in inside-out macropatches ($P<0.05$). On human atrial I_{K1} only the propafenone-inhibitory effects ($P<0.05$) were apparent.

In conclusion Kir2.1 channels exhibit two propafenone binding sites, the high affinity site being responsible for the propafenone-increasing effects. The low affinity site, also located in the cytoplasmic domain, is present in all Kir2.x channels and is allosterically coupled to the PIP_2 binding. Thus, propafenone blocks Kir2.x channels by decreasing the channel affinity for PIP_2 which represents a novel specific blocking mechanism.