Propafenone receptors in Kir2.x channels.

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INTRODUCTION

The cardiac inwardly rectifying K^+ current (I_{K1}) is characterized by a high conductance in the hyperpolarizing direction and a low conductance in the depolarizing direction as a consequence of the voltage-dependent block induced by intracellular Mg^{2+} and polyamines (1-3). I_{K1} plays a critical role in modulating cardiac excitability by setting the diastolic resting membrane potential and shaping the initial depolarization and final repolarization phases of the action potential (AP), in both atria and ventricles (1-3). Furthermore, the importance of I_{K1} in the establishment of a fast and stable reentry of spiral waves (rotors) and ventricular fibrillation dynamics has been demonstrated (4). Our group has demonstrated that flecainide, a class IC antiarrhythmic drug, increases Kir2.1 and ventricular I_{K1} currents by binding to Cys311 located within the βH-βI region of the cytoplasmic domain of the channel, without modifying Kir2.2, Kir2.3, and human atrial I_{K1} currents (5). These results led us to propose that in human heart, I_{K1} is mainly carried by Kir2.1 channels in ventricular cells, whereas relative contribution of Kir2.2 and Kir2.3 seems to be greater in atrial cells. Propafenone is a class IC antiarrhythmic drug widely used for the conversion of recent onset atrial fibrillation to sinus rhythm (6). However, it exerts proarrhythmic effects at the ventricular level. Indeed, propafenone increases mortality rate in patients with myocardial infarction, left ventricular dysfunction or heart failure, as demonstrated by the prospective, randomized Cardiac Arrest Study Hamburg trial (CASH, 7). However, the underlying mechanisms of the effects of this drug at the atrial and ventricular level are scarcely explored. Considering that the molecular architecture of the I_{K1} differs between atria and ventricles, we have analyzed whether

MATERIAL and METHODS

- Kir2.1, Kir2.2, and Kir2.3 (WT and mutants) currents ($I_{Kir2.x}$) were recorded in CHO cells, transiently transfected with the cDNA encoding the expression of these channels (1.6 µg). Native I_{K1} was recorded in human atrial myocytes enzymatically isolated from right atrial appendages obtained from patients that underwent cardiac surgery at the Hospital Gregorio Marañón in Madrid (5,8-12).
- Macroscopic and single channel currents were recorded at room temperature using the whole-cell and the cell-attached configurations of the patch-clamp technique, respectively (8-13).
- The voltage in the current-voltage (I-V) curves is adjusted according to the calculated liquid junction potentials: −13.2 and -12.1 mV at extracellular K^+ concentrations ($[K^+]_o$) of 4 and 20 mM, respectively. Chord conductance (Gc) was calculated as the ratio of the actual current and current predicted by assuming a linear unblocked current and fitted by a Boltzmann equation.
- In some experiments, currents were recorded at room temperature from excised inside-out macropatches from HEK-293 cells (5) by using a fluoride, vanadate, and pyrophosphate (FVPP)-potassium solution on both sides of the patch to prevent rundown.
- Molecular modeling was performed to obtain the lowest energy-minimized blind docking for propafenone with a full-length Kir2.2 channel (PDB ID code 3JYC) by using Autodock Vina. According to Vina best scored poses, the most stable complex configurations were considered.
- **Whole-cell solutions:**
  - ► External-CHO (mM): NaCl 136, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 10, glucose 10 (pH=7.4 NaOH). To obtain 1 and 100 mM $[K^+]_o$ solutions, equimolar substitution between KCl and NaCl was used.
  - ► External-native I_{K1} (mM): NaCl 120, KCl 20, $CaCl_2$ 1, $MgCl_2$ 1, HEPES 10, 4-aminopyridine 2, glucose 10, nifedipine (1 µM) and glibenclamide (10 µM) (pH 7.4 NaOH). To record I_{K1} in atrial myocytes, atropine (1 µM) was also added.
  - ► Internal (mM): K-aspartate 80, KCl 42, KH_2PO_4 10, Mg-ATP 5, phosphocreatine 3, HEPES 5, EGTA 5 (pH=7.2 KOH).
- **Cell-attached solutions:**

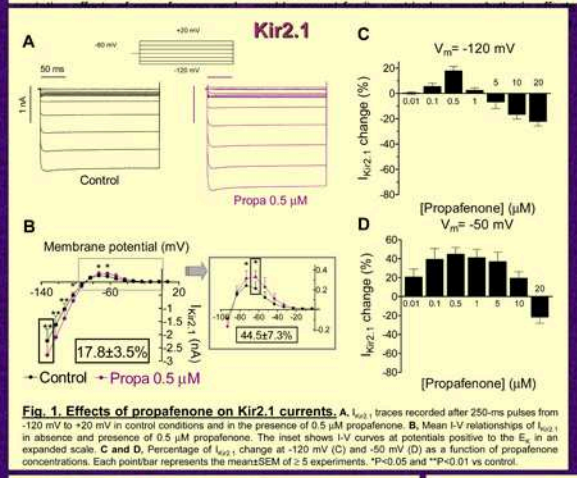


Fig. 1. Effects of propafenone on Kir2.1 currents. **A**, $I_{Kir2.1}$ traces recorded after 250-ms pulses from -120 mV to +20 mV in control conditions and in the presence of 0.5 µM propafenone. **B**, Mean I-V relationships of $I_{Kir2.1}$ in absence and presence of 0.5 µM propafenone. The inset shows I-V curves at potentials positive to the E_K in an expanded scale. **C and D**, Percentage of $I_{Kir2.1}$ change at -120 mV (C) and -50 mV (D) as a function of propafenone concentrations. Each point/bar represents the mean±SEM of ≥ 5 experiments. *P<0.05 and **P<0.01 vs control.

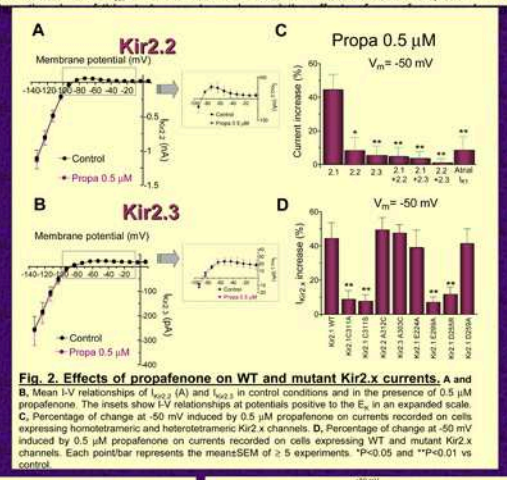


Fig. 2. Effects of propafenone on WT and mutant Kir2.x currents. **A and B**, Mean I-V relationships of $I_{Kir2.2}$ (A) and $I_{Kir2.3}$ in control conditions and in the presence of 0.5 µM propafenone. The insets show I-V relationships at potentials positive to the E_K in an expanded scale. **C**, Percentage of change at -50 mV induced by 0.5 µM propafenone on currents recorded on cells expressing homotetrameric and heterotetrameric Kir2.x channels. **D**, Percentage of change at -50 mV induced by 0.5 µM propafenone on currents recorded on cells expressing WT and mutant Kir2.x channels. Each point/bar represents the mean±SEM of ≥ 5 experiments. *P<0.05 and **P<0.01 vs control.

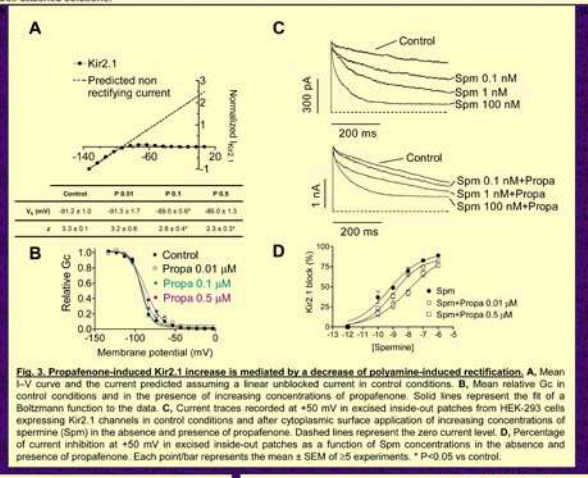


Fig. 3. Propafenone-induced Kir2.1 increase is mediated by a decrease of polyamine-induced rectification. **A**, Mean I-V curve and the current predicted assuming a linear unblocked current in control conditions. **B**, Mean relative Gc in control conditions and in the presence of increasing concentrations of propafenone. Solid lines represent the fit of a Boltzmann function to the data. **C**, Current traces recorded at +50 mV in excised inside-out patches from HEK-293 cells expressing Kir2.1 channels in control conditions and after cytoplasmic surface application of increasing concentrations of spermine (Spm) in the absence and presence of propafenone. Dashed lines represent the zero current level. **D**, Percentage of current inhibition at +50 mV in excised inside-out patches as a function of Spm concentrations in the absence and presence of propafenone. Each point/bar represents the mean ± SEM of ≥5 experiments. * P<0.05 vs control.

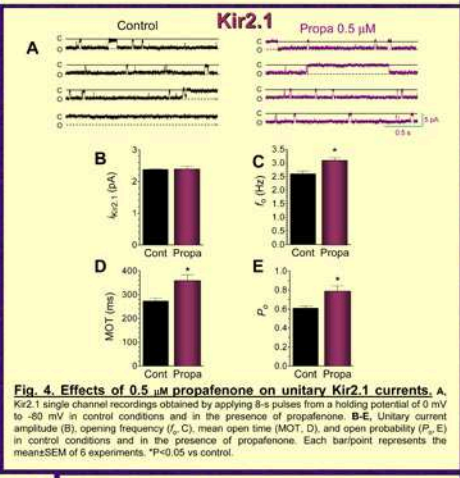
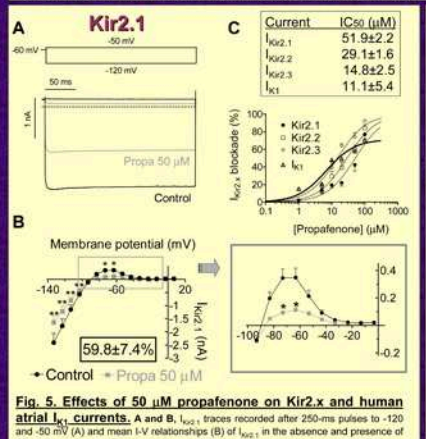


Fig. 4. Effects of 0.5 µM propafenone on unitary Kir2.1 currents. **A**, Kir2.1 single channel recordings obtained by applying 8-s pulses from a holding potential of 0 mV to -80 mV in control conditions and in the presence of propafenone. **B-E**, Unitary current amplitude (B), opening frequency (f_o, C), mean open time (MOT, D), and open probability (P_o, E) in control conditions and in the presence of propafenone. Each bar/point represents the mean±SEM of 6 experiments. *P<0.05 vs control.



Current	IC50 (µM)
$I_{Kir2.1}$	51.9±2.2
$I_{Kir2.2}$	29.1±1.6
$I_{Kir2.3}$	14.8±2.5
I_{K1}	11.1±5.4

Fig. 5. Effects of 50 µM propafenone on Kir2.x and human atrial I_{K1} currents. **A and B**, $I_{Kir2.1}$ traces recorded after 250-ms pulses to -120 and -50 mV (A) and mean I-V relationships (B) of $I_{Kir2.1}$ in the absence and presence of 50 µM propafenone. The inset shows I-V curves at potentials positive to the E_K in an expanded scale. **C**, Percentage of $I_{Kir2.x}$ and human atrial I_{K1} change at -120 mV as a function of propafenone concentrations. Each point/bar represents the mean±SEM of ≥ 5 experiments. *P<0.05 and ** P<0.01 vs control.

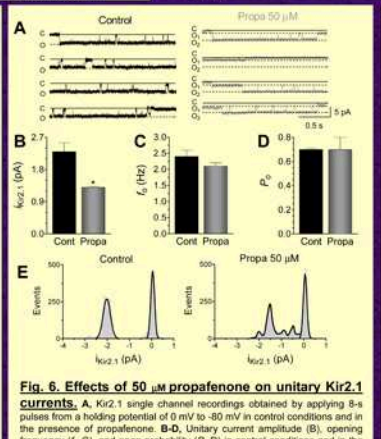


Fig. 6. Effects of 50 µM propafenone on unitary Kir2.1 currents. **A**, Kir2.1 single channel recordings obtained by applying 8-s pulses from a holding potential of 0 mV to -80 mV in control conditions and in the presence of propafenone. **B-D**, Unitary current amplitude (B), opening frequency (f_o, C), and open probability (P_o,D) in control conditions and in the presence of propafenone. **E**, Amplitude histograms at -80 mV in the absence and presence of 50 µM propafenone constructed by pooling amplitude data from 6 experiments. Each bar/point represents the mean±SEM of 6 experiments. *P<0.05 vs control.

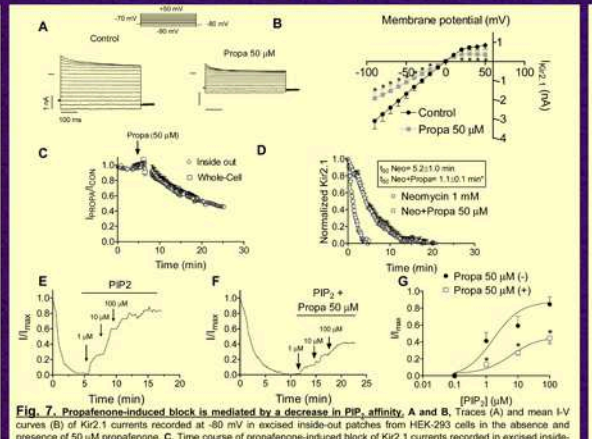


Fig. 7. Propafenone-induced block is mediated by a decrease in PIP_2 affinity. **A and B**, Traces (A) and mean I-V curves (B) of Kir2.1 currents recorded at -80 mV in excised inside-out patches from HEK-293 cells in the absence and presence of 50 µM propafenone. **C**, Time course of propafenone-induced block of Kir2.1 currents recorded in excised inside-out patches or under the whole-cell configuration. **D**, Time course of neomycin-induced block of Kir2.1 currents recorded in excised inside-out patches in the absence and presence of propafenone. **E and F**, Normalized Kir2.1 current amplitude as a function of time in the presence of neomycin and increasing concentrations of PIP_2 in the absence (E) or presence (F) of propafenone. **G**, Percentage of current increase at -80 mV in excised inside-out patches as a function of PIP_2 concentrations

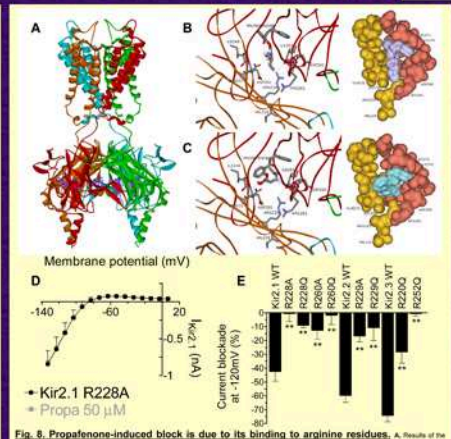


Fig. 8. Propafenone-induced block is due to its binding to arginine residues. **A**, Results of the blind-docking study of propafenone within Kir2.2 channels (PDB ID code 3JYC). **B and C**, Predicted bonds between propafenone and some amino acids located within the cytoplasmic domain. **D**, Mean I-V curves for currents recorded in CHO cells expressing R228A Kir2.1 channels in the absence and presence of 50 µM propafenone. **E**, Propafenone-induced block on the current recorded at -120 mV in cells expressing Kir2.x WT and mutant channels. ** P<0.01 vs control. Each point/bar represents the mean ± SEM of ≥5 experiments.

CONCLUSIONS

1. **At therapeutical concentrations (≈1 µM) propafenone increases inward and outward $I_{Kir2.1}$ generated by homotetrameric Kir2.1 channels by increasing the mean open time, the opening frequency, and thus, the P_o of the channels. However, it does not modify Kir2.2, Kir2.3, heterotetrameric Kir2.x, and human atrial I_{K1} currents.**
2. **Increasing effects depend on the presence of a cysteine at position 311, which is only present in Kir2.1 channels, and are abolished by some mutations that decrease polyamine-induced block.**
3. **The Kir2.1 current induced-increase could be involved in the proarrhythmogenic effects of propafenone at the ventricular level.**
4. **At concentrations ≥20 µM, propafenone inhibits inward and outward Kir2.1-3 and human atrial I_{K1} currents. The order of potency for the propafenone-induced block is Kir2.3 ≈ human atrial I_{K1} > Kir2.2 > Kir2.1.**
5. **Propafenone inhibits Kir2.x currents by allosterically decreasing channel affinity for PIP_2, which, in turn, decreases channel conductance by favouring the appearance of subconductance states.**
6. **Blocking effects of propafenone are due to its binding to a low-affinity binding site present in all Kir2.x channels, which comprises residues R220 and R252 of the cytoplasmic domain.**

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