



# Modulation of Kir2.x channels by the antiarrhythmic drug propafenone

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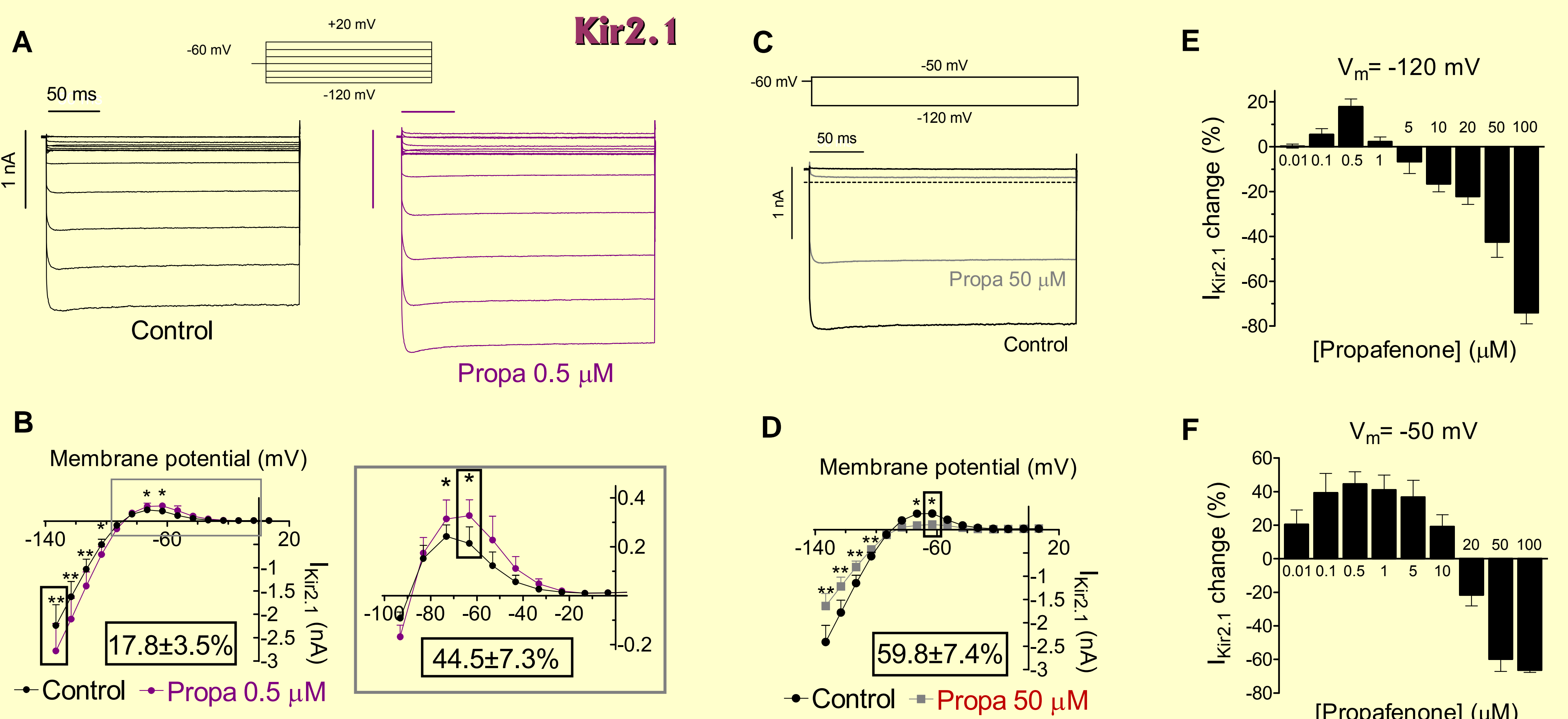
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## INTRODUCTION

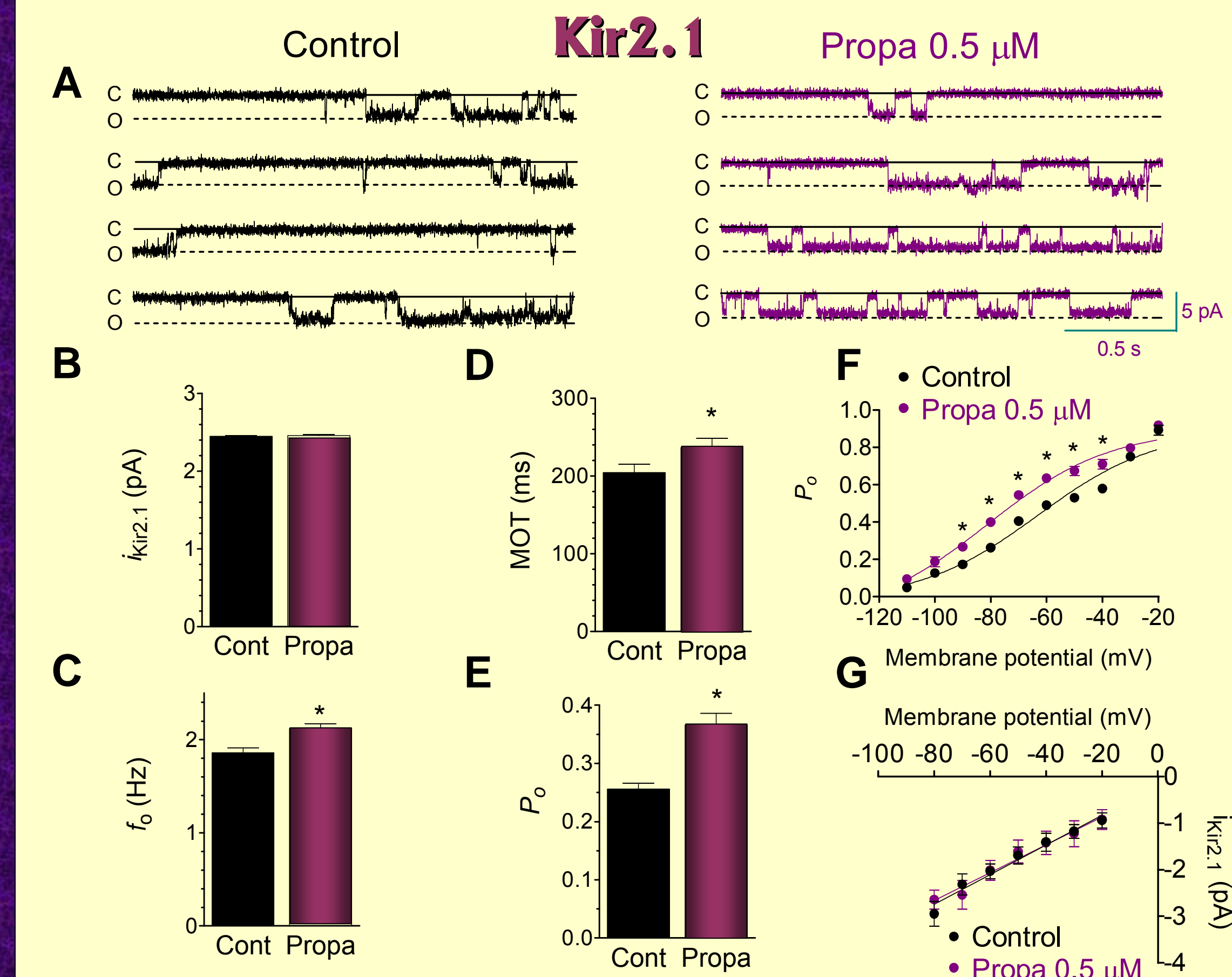
The cardiac inwardly rectifying K<sup>+</sup> current (I<sub>K1</sub>) is characterized by a high conductance in the hyperpolarizing direction and a low conductance in the depolarizing direction as a consequence of the voltage-dependent block induced by intracellular Mg<sup>2+</sup> and polyamines (1-3). I<sub>K1</sub> 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<sub>K1</sub> 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<sub>K1</sub> 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 atrial I<sub>K1</sub> currents (5). These results led us to propose that in human heart, I<sub>K1</sub> 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<sub>K1</sub> differs between atria and ventricles, we have analyzed whether putative effects of propafenone on I<sub>K1</sub> could account for its ventricular proarrhythmic effects. Thus, the aims of this study were to analyze: a) the effects of propafenone on human cardiac Kir2.1, Kir2.2, and Kir2.3 channels and b) the molecular determinants of these effects.

## MATERIAL and METHODS

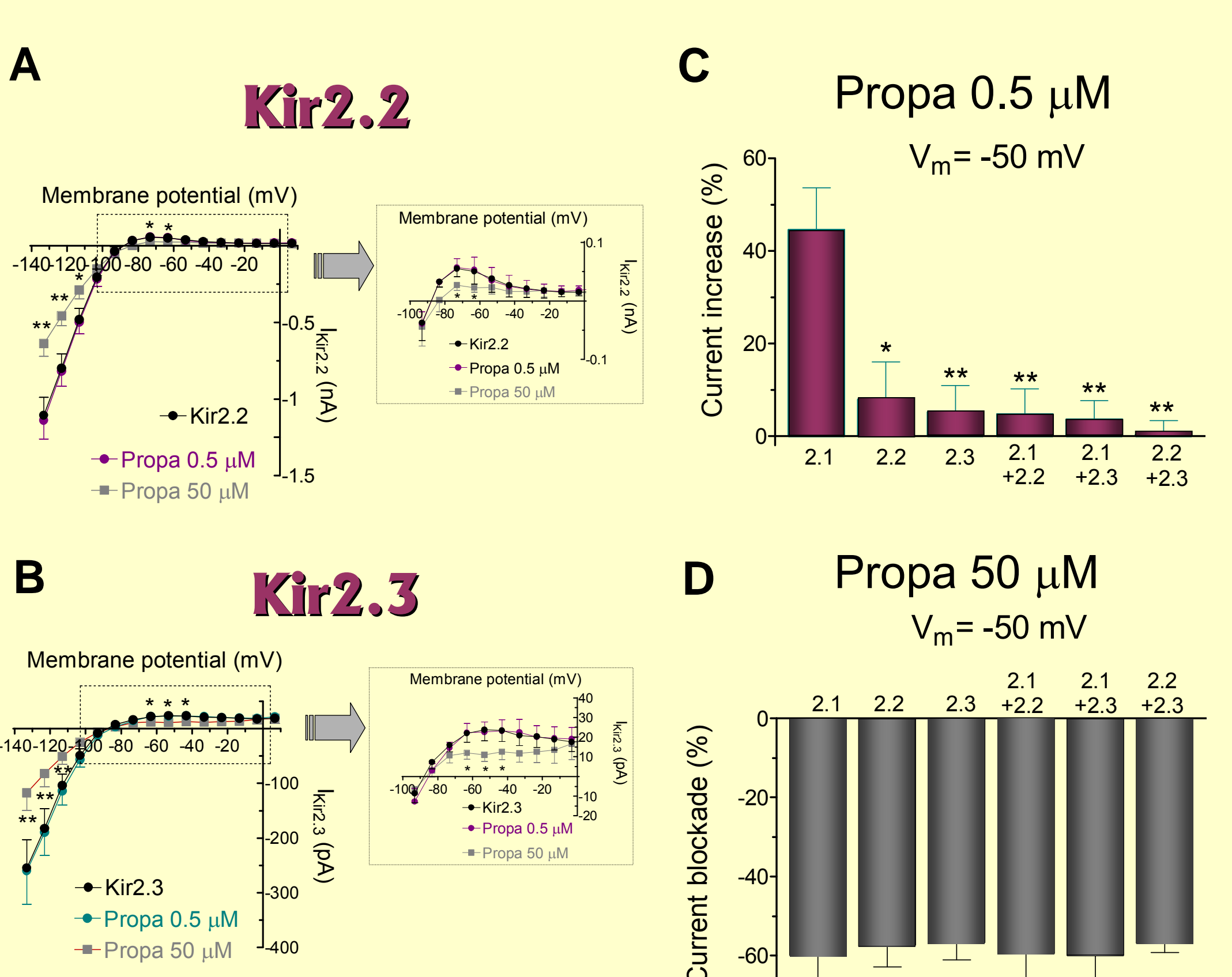
- Kir2.1 (WT and mutants), Kir2.2, and Kir2.3 currents (I<sub>Kir2.x</sub>) were recorded in CHO cells, transiently transfected with the cDNA encoding the expression of these channels (1.6 μg). Native I<sub>K1</sub> was recorded in human atrial myocytes enzymatically isolated from right atrial appendages 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<sup>+</sup> concentrations ([K<sup>+</sup>]<sub>o</sub>) of 4 and 20 mM, respectively. Chord conductance (G<sub>c</sub>) was calculated as the ratio of the actual current and current predicted by assuming a linear unblocked current and fitted by a Boltzmann equation  $y = [1/(1 + \exp(-\lambda(V - V_h)))]$ , where V is the membrane potential, V<sub>h</sub> represents the midpoint of the curve, and  $\lambda = zF/RT$ , where z stands for the effective valency or steepness of rectification, F is Faraday's constant, R is the gas constant, and T is the absolute temperature.
- Whole-cell solutions:
  - ▶ External-CHO (mM): NaCl 136, KCl 4, CaCl<sub>2</sub> 1.8, MgCl<sub>2</sub> 1, HEPES 10, glucose 10 (pH=7.4 NaOH). To obtain 1 and 100 mM [K<sup>+</sup>]<sub>o</sub> solutions, equimolar substitution between KCl and NaCl was used.
  - ▶ External-native I<sub>K1</sub> (mM): NaCl 120, KCl 20, CaCl<sub>2</sub> 1, MgCl<sub>2</sub> 1, HEPES 10, 4-aminopyridine 2, glucose 10, nifedipine (1 μM) and glibenclamide (10 μM) (pH 7.4 NaOH). To record I<sub>K1</sub> in atrial myocytes, atropine (1 μM) was also added.
  - ▶ Internal (mM): K-aspartate 80, KCl 42, KH<sub>2</sub>PO<sub>4</sub> 10, Mg-ATP 5, phosphocreatine 3, HEPES 5, EGTA 5 (pH=7.2 KOH).
- Cell-attached solutions:
  - ▶ External (mM): KCl 140, CaCl<sub>2</sub> 1.8, MgCl<sub>2</sub> 1, HEPES 10, glucose 10 (pH=7.4 KOH).
  - ▶ Internal (mM): KCl 140, CaCl<sub>2</sub> 1, HEPES 10 (pH=7.2 KOH).
- Drugs: Propafenone was initially dissolved in methanol to obtain 10 mM stock solutions and further dilutions were carried out in external solution to obtain the desired final concentration.



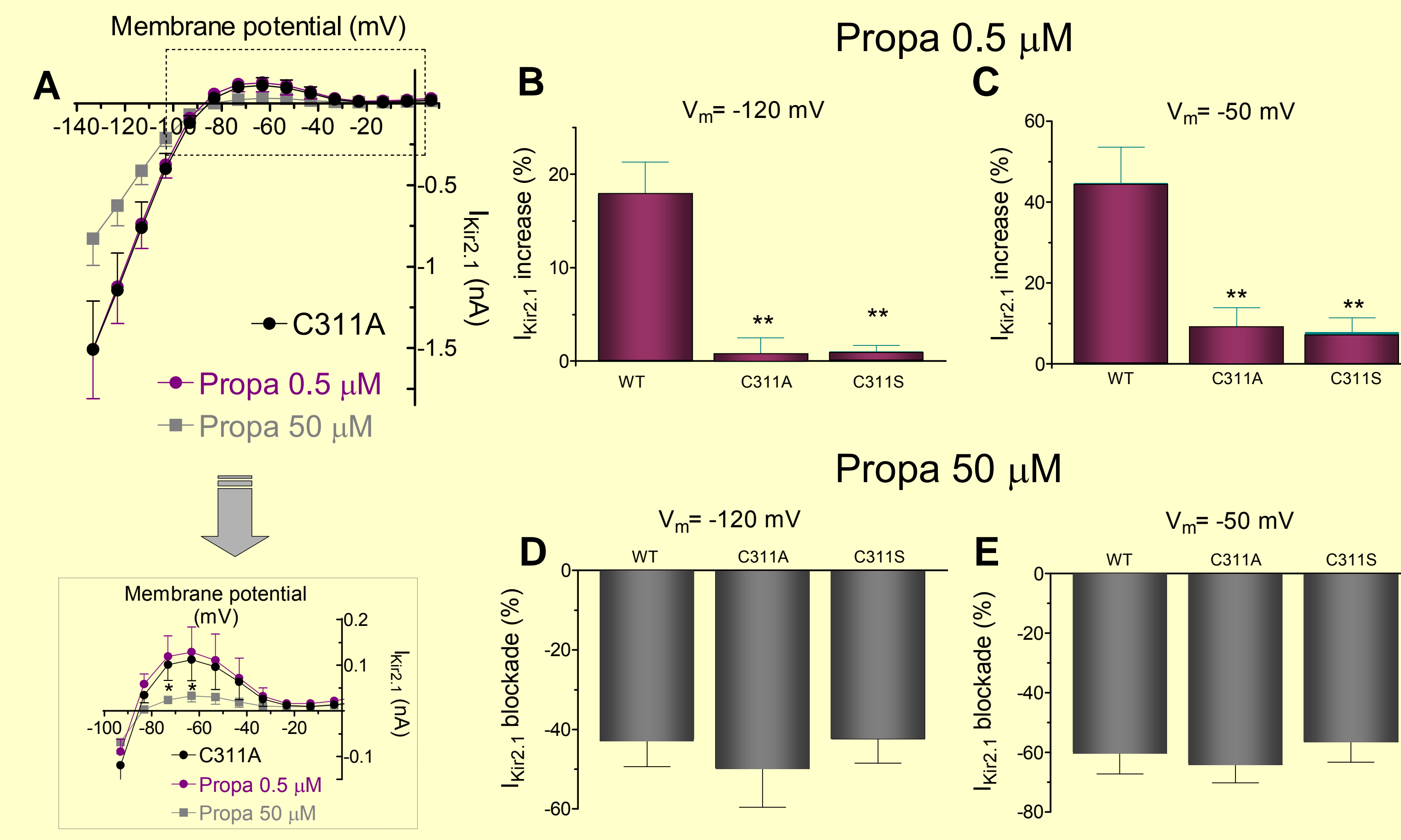
**Fig. 1. Effects of propafenone on Kir2.1 currents.** A, Kir<sub>2.1</sub> traces recorded after 250-ms pulses from -120 to +20 mV in control conditions and in the presence of 0.5 μM propafenone. B, Mean I-V relationships of I<sub>Kir2.1</sub> in absence and presence of 0.5 μM propafenone. The inset shows I-V curves at potentials positive to the E<sub>K</sub> in an expanded scale. C and D, Kir<sub>2.1</sub> traces recorded after 250-ms pulses to -120 and -50 mV (C) and mean I-V relationships (D) of I<sub>Kir2.1</sub> in the absence and presence of 50 μM propafenone. E and F, Percentage of I<sub>Kir2.1</sub> change at -120 mV (E) and -50 mV (F) 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.



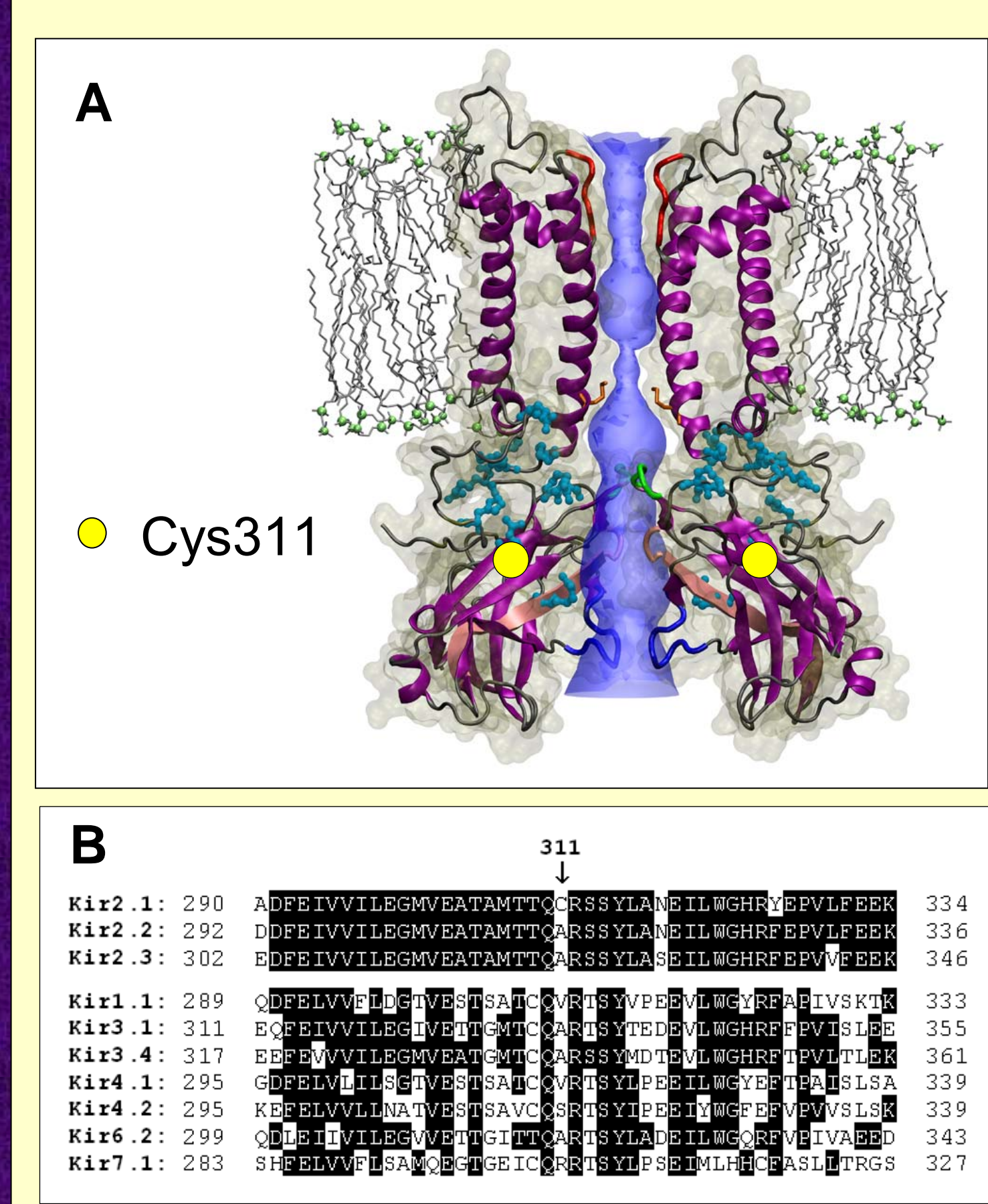
**Fig. 2. Effects of 0.5 μM propafenone on unitary Kir2.1 currents.** A, Kir<sub>2.1</sub> single channel recordings obtained by applying 8-s pulses from a holding potential of 0 mV to -80 mV in control conditions and in the presence of propafenone. Continuous and discontinuous lines represent the closed (C) and open (O) channel levels, respectively. B-E, Unitary current amplitude (B), opening frequency (f<sub>o</sub>, C), mean open time (MOT, D), and open probability (P<sub>o</sub>, E) in control conditions and in the presence of propafenone. F and G, P<sub>o</sub> (F) and single-channel current-voltage (G) relationships in the absence and presence of propafenone. Each bar/point represents the mean±SEM of 6 experiments. \*P<0.05 vs control.



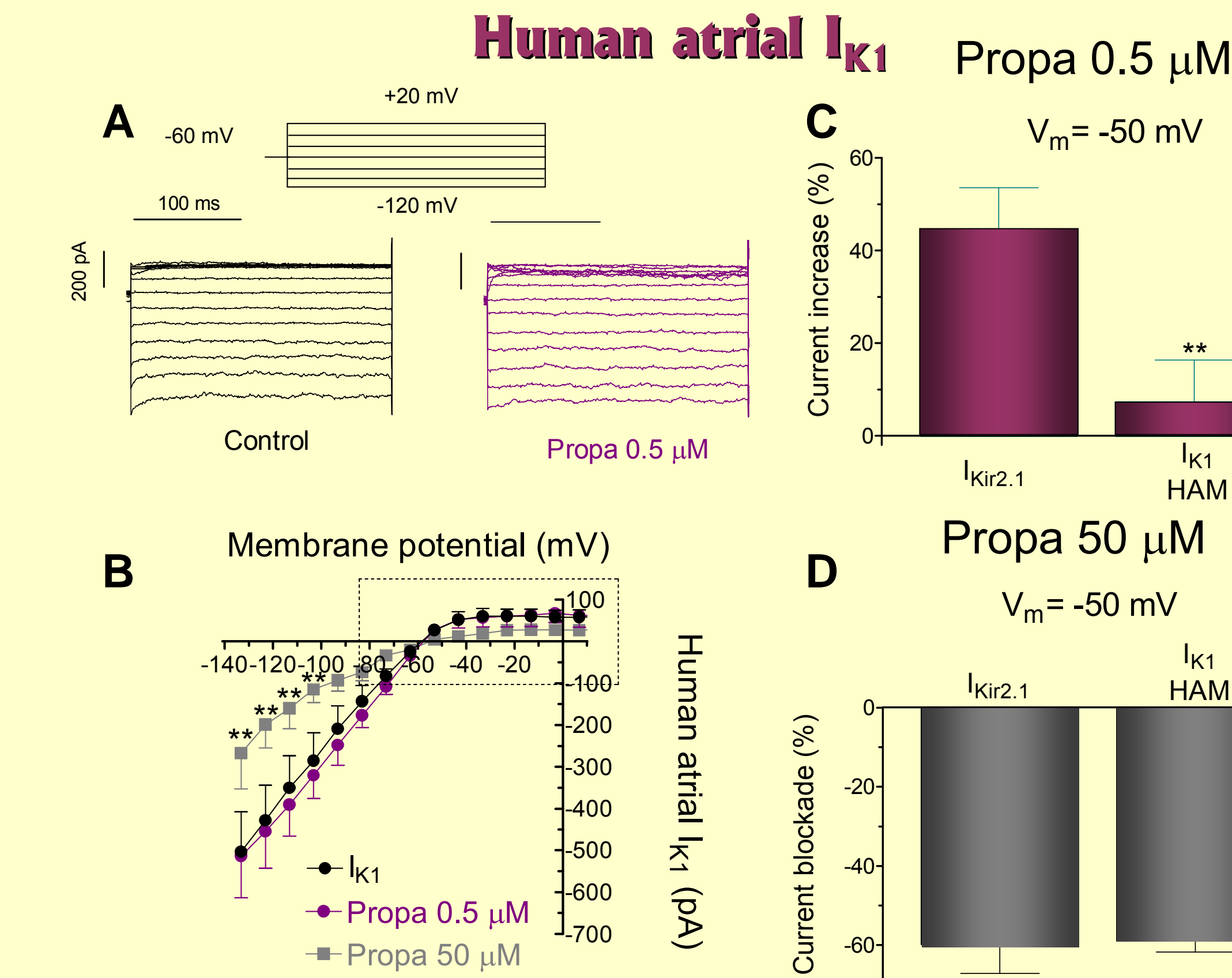
**Fig. 3. Effects of propafenone on Kir2.2 and Kir2.3 currents.** A and B, Mean I-V relationships of I<sub>Kir2.2</sub> (A) and I<sub>Kir2.3</sub> in control conditions and in the presence of 0.5 μM and 50 μM propafenone. The insets show I-V relationships at potentials positive to the E<sub>K</sub> in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



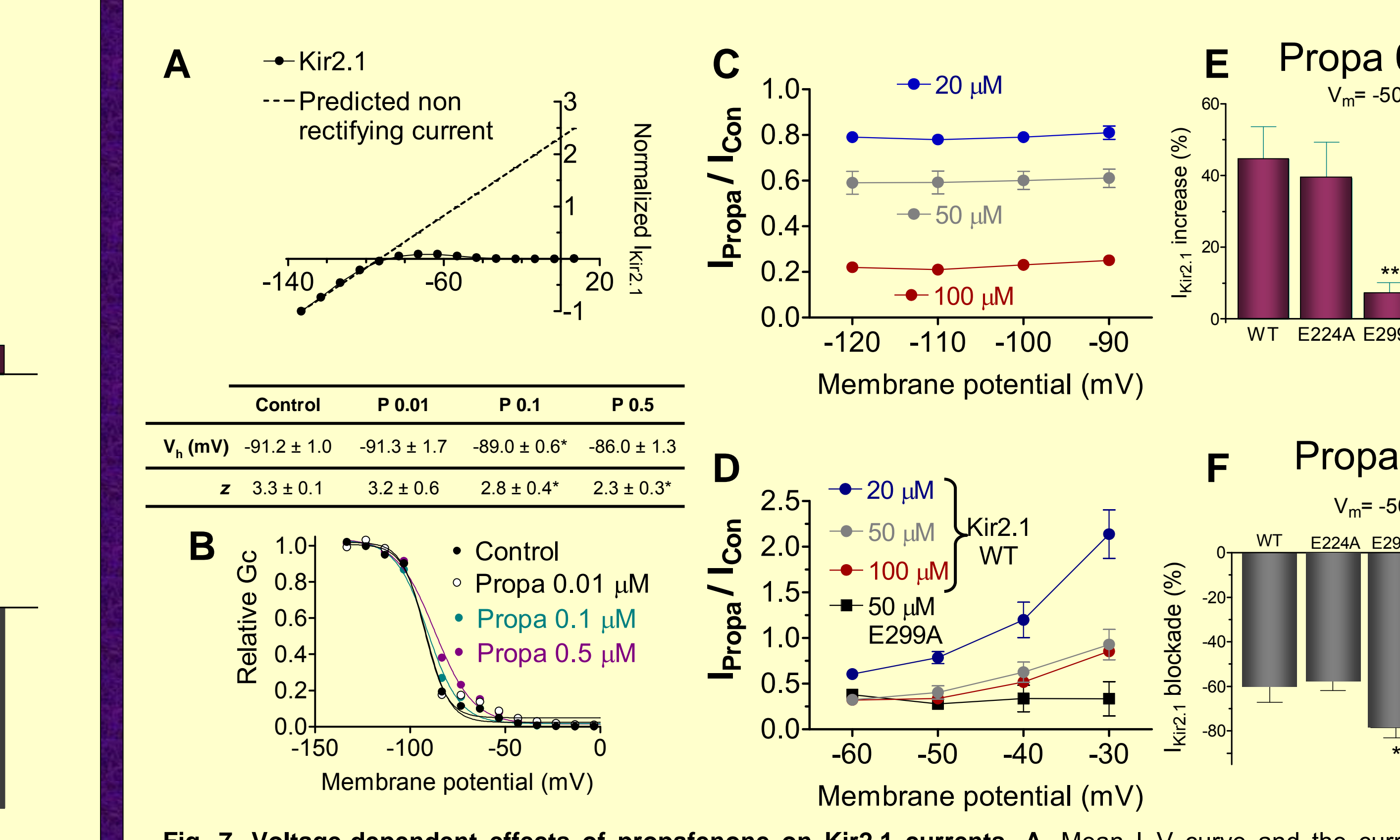
**Fig. 4. Propafenone-induced increase is determined by Cys311.** A, Mean I-V curves for currents recorded in CHO cells expressing C311A Kir2.1 channels in the absence and presence of 0.5 and 50 μM propafenone. The inset shows I-V relationships at potentials positive to the E<sub>K</sub> in an expanded scale. B and C, Percentage of Kir<sub>2.1</sub> increase on currents recorded at -120 mV (B) and -50 mV (C) in CHO cells expressing WT or C311A and C311S Kir2.1 channels at 0.5 μM (B and C) and 50 μM (D and E). \*\*P<0.01 vs WT. Each point/bar represents the mean±SEM of ≥5 experiments.



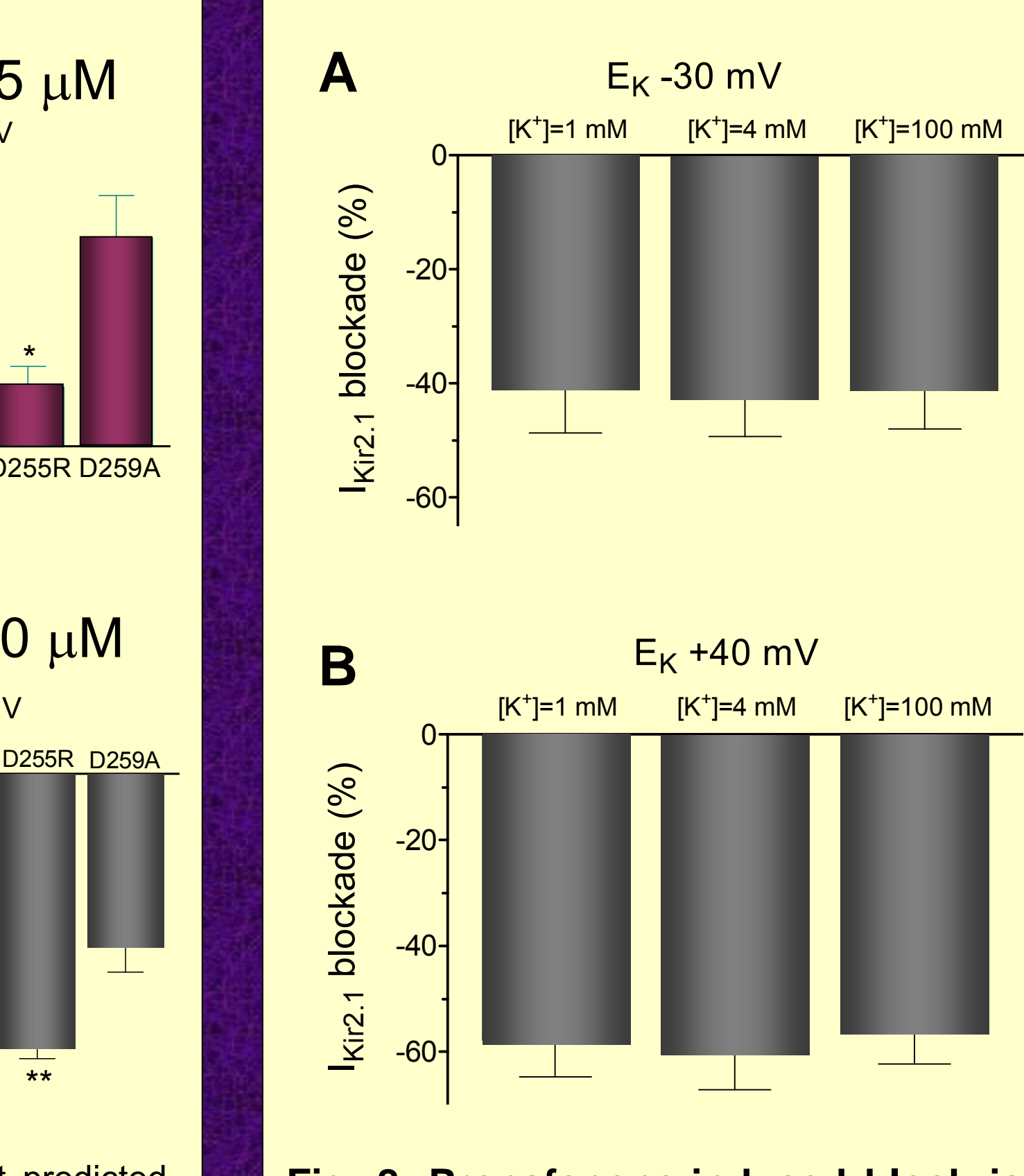
**Fig. 5. Kir2.1 channel structure.** A, Model of Kir<sub>2.1</sub> channel architecture (obtained from <http://www.physiology.vcu.edu>). B, Amino acid sequence alignments of representative members of the human Kir superfamily showing sequence similarities in the Cys311-flanking regions of the conserved C-terminus.



**Fig. 6. Effects of propafenone on human atrial I<sub>K1</sub>.** A, Human atrial I<sub>K1</sub> traces recorded in an enzymatically isolated myocyte by applying 250-ms pulses from -120 to +20 mV in control conditions and in the presence of 0.5 μM propafenone. B, Mean I-V relationships for human atrial I<sub>K1</sub> recorded in the absence and presence of 0.5 μM and 50 μM propafenone. C and D, Percentage of change induced by 0.5 μM (C) and 50 μM (D) propafenone on human atrial I<sub>K1</sub>. Each point represents the mean±SEM of ≥ 5 experiments.



**Fig. 7. Voltage-dependent effects of propafenone on Kir2.1 currents.** A, Mean I-V curve and the current predicted assuming a linear unblocked current in control conditions. B, Mean relative G<sub>c</sub> 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 and D, Fractional I<sub>Kir2.1</sub> block (I<sub>Prop</sub>/I<sub>Con</sub>) induced by 20, 50, and 100 μM propafenone at membrane potentials for voltages negative (C) and positive (D) to E<sub>K</sub>. In D, block induced by propafenone 50 μM in E299A Kir2.1 channels is included. E and F, Propafenone-induced change on the current recorded at -50 mV in CHO cells expressing WT, E224A, E299A, D255R, and D259A Kir2.1 channels at 0.5 μM (E) and 50 μM (F). \* P<0.05 and \*\* P<0.01 vs. control. Each point/bar represents the mean ± SEM of ≥5 experiments.



**Fig. 8. Propafenone-induced block is not dependent on [K<sup>+</sup>]<sub>o</sub>.** A and B, Block produced by 50 μM propafenone on the I<sub>Kir2.1</sub> recorded at 30 mV negative (A) and 40 mV positive (B) to E<sub>K</sub> in CHO cells perfused with 1, 4, and 100 mM [K<sup>+</sup>]<sub>o</sub>. Each bar represents the mean ± SEM of ≥5 experiments.

## CONCLUSIONS

1. At therapeutical concentrations (≈1 μM) propafenone increases inward and outward I<sub>Kir2.1</sub> generated by homotetrameric Kir2.1 channels by increasing the mean open time, the opening frequency, and thus, the P<sub>o</sub> of the channels. However, it does not modify Kir2.2, Kir2.3, heterotetrameric Kir2.x, and human atrial I<sub>K1</sub> 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 proarrhythmic effects of propafenone at the ventricular level.
4. At concentrations ≥20 μM, propafenone inhibits inward and outward Kir2.1-3 and native I<sub>K1</sub> currents. The propafenone-induced inhibition was not affected by either substitution of Cys311 or polyamine-binding site residues or by changes in the [K<sup>+</sup>]<sub>o</sub>.
5. Propafenone binds to a high-affinity binding site in Kir2.1 channels involving Cys311, which is responsible for its increasing effects. Moreover, it also binds to a low-affinity binding site present in all Kir2.x channels, which is responsible for the blocking effects and is not located within the cytoplasmic or transmembrane pore.

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