Nitric Oxide Increases Cardiac I_{K1} by Nitrosylation of Cysteine 76 of Kir2.1 Channels

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Rationale: The cardiac inwardly rectifying K^+ current (I_{K1}) plays a critical role in modulating excitability by setting the resting membrane potential and shaping phase 3 of the cardiac action potential.

Objective: This study aims to analyze the effects of nitric oxide (NO) on human atrial I_{K1} and on Kir2.1 channels, the major isoform of inwardly rectifying channels present in the human heart.

Methods and Results: Currents were recorded in enzymatically isolated myocytes and in transiently transfected CHO cells, respectively. NO at myocardial physiological concentrations (25 to 500 nmol/L) increased inward and outward I_{K1} and $I_{Kir2.1}$. These effects were accompanied by hyperpolarization of the resting membrane potential and a shortening of the duration of phase 3 of the human atrial action potential. The $I_{Kir2.1}$ increase was attributable to an increase in the open probability of the channel. Site-directed mutagenesis analysis demonstrated that NO effects were mediated by the selective *S*-nitrosylation of Kir2.1 Cys76 residue. Single ion monitoring experiments performed by liquid chromatography/tandem mass spectrometry suggested that the primary sequence that surrounds Cys76 determines its selective *S*-nitrosylation. Chronic atrial fibrillation, which produces a decrease in NO bioavailability, decreased the *S*-nitrosylation of Kir2.1 channels in human atrial samples as demonstrated by a biotin-switch assay, followed by Western blot.

Conclusions: The results demonstrated that, under physiological conditions, NO regulates human cardiac I_{K1} through a redox-related process. (*Circ Res*. 2009;105:383-392.)

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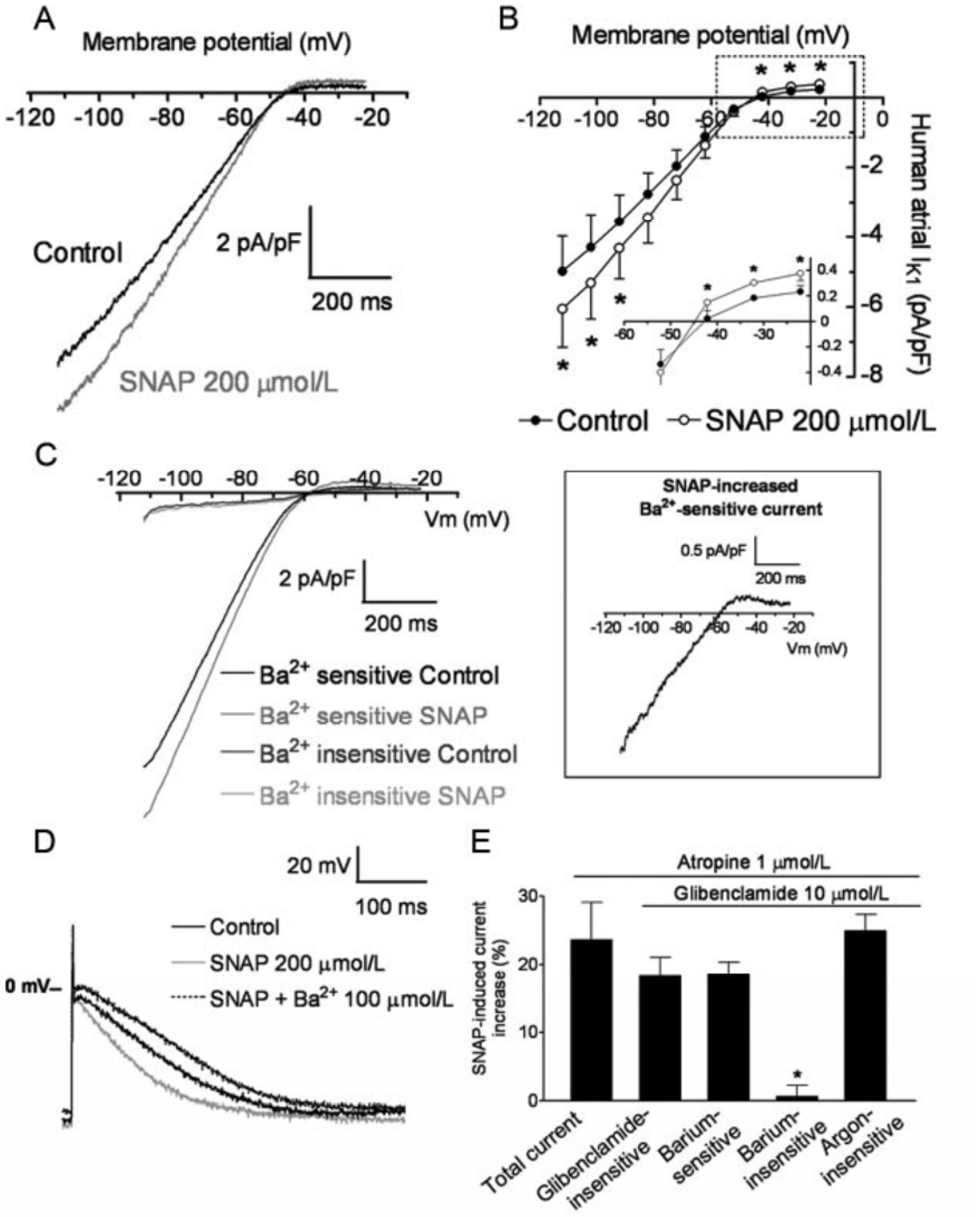
Why were we interested in analyzing the effects of NO?

1. Ionic channels in HUMAN atrial myocytes under normal and pathological conditions: Atrial Fibrillation
2. Atrial fibrillation increases the amplitude of the inward rectifier current (IK1)
3. The increase of atrial IK1 stabilizes the spiral reentry waves (rotors) that underlie atrial fibrillation:

- **An increase in IK1 promotes Atrial fibrillation**

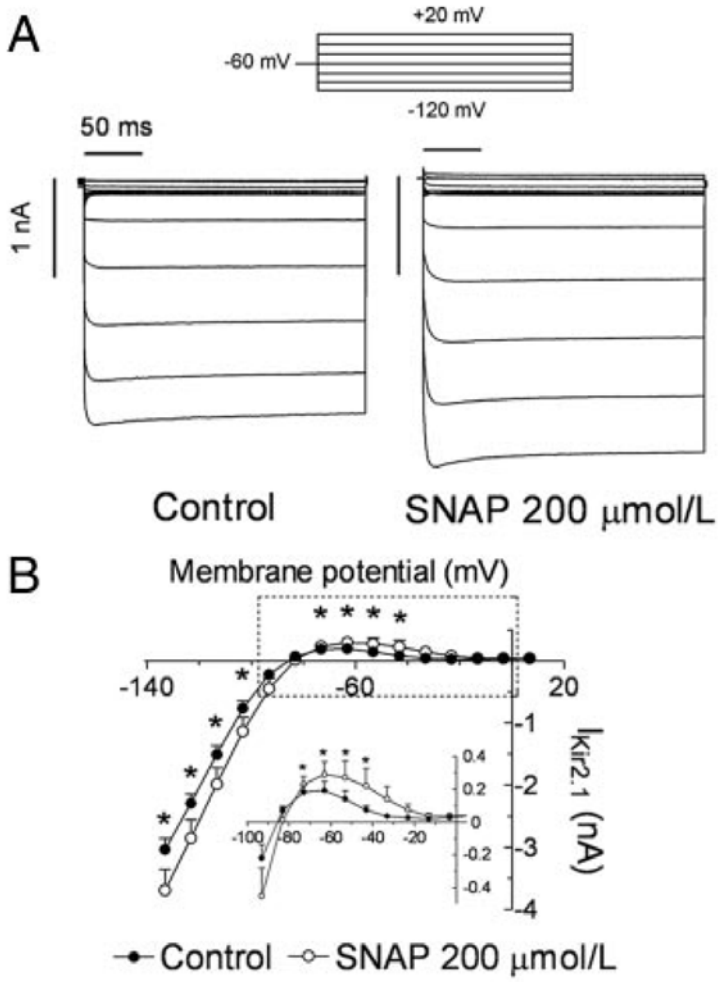
5. We are interested in the endogenous and pharmacological modulation of IK1
6. It has been described that AF markedly reduces the NO bioavailability in the atria
7. We decided to analyze whether NO regulates IK1

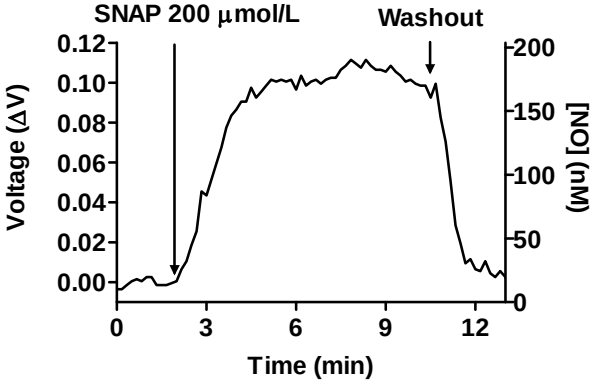
1. NO increases human atrial IK1
2. This effect produces:
- A shorthening of the duration of the action potential
- Hyperpolarizes the resting membrane potential

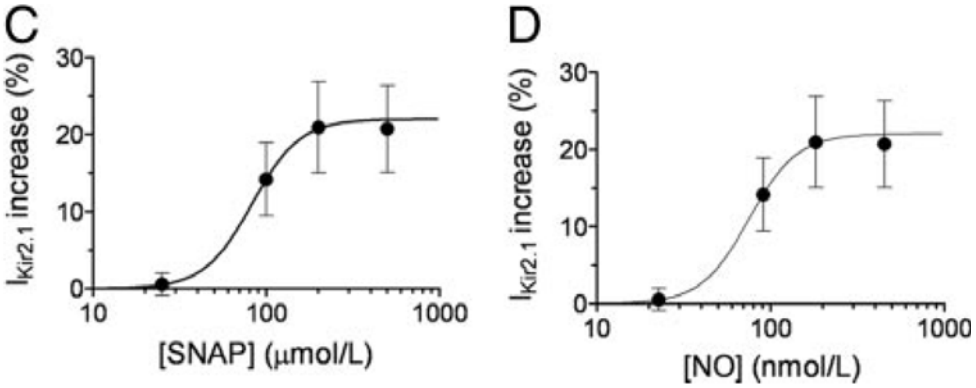


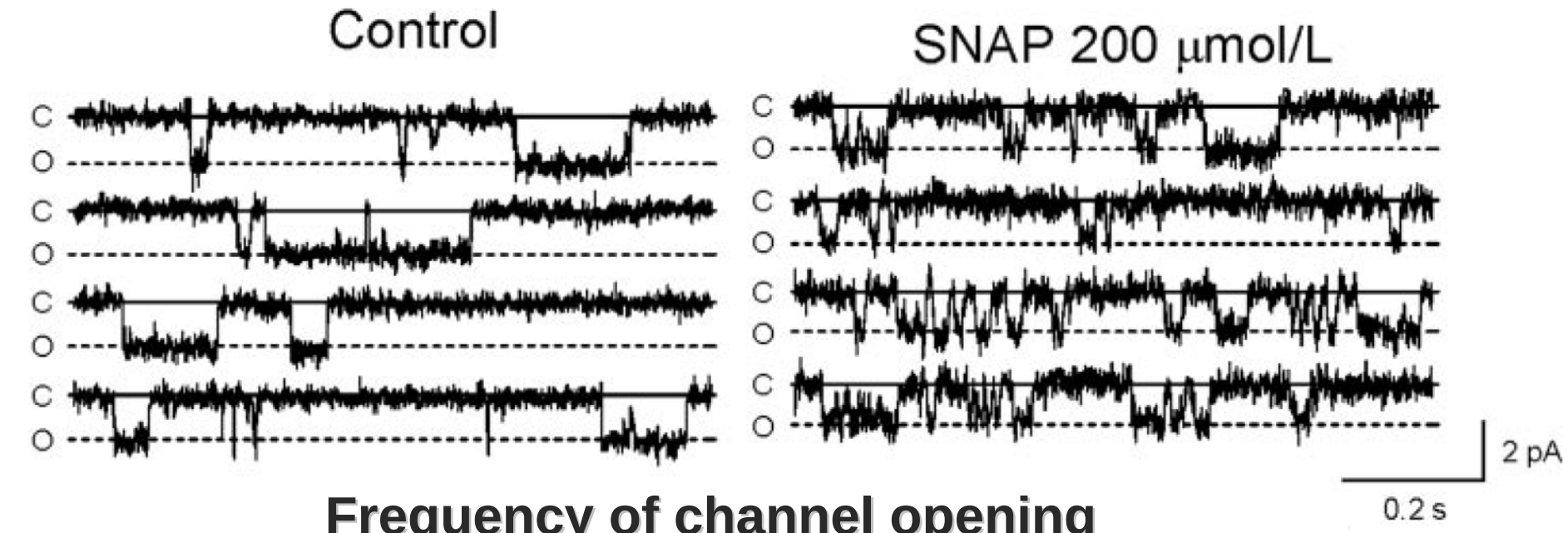
NO increases in a concentration-dependent manner currents carried by Kir2.1, Kir2.2, and Kir2.3 human channels

Considering the physiological cardiac concentrations of NO (200-900 μM) and the IC_{50} for the increasing effect (80 nM), it can be concluded that Kir2.x human cardiac channels are extremely sensitive to NO

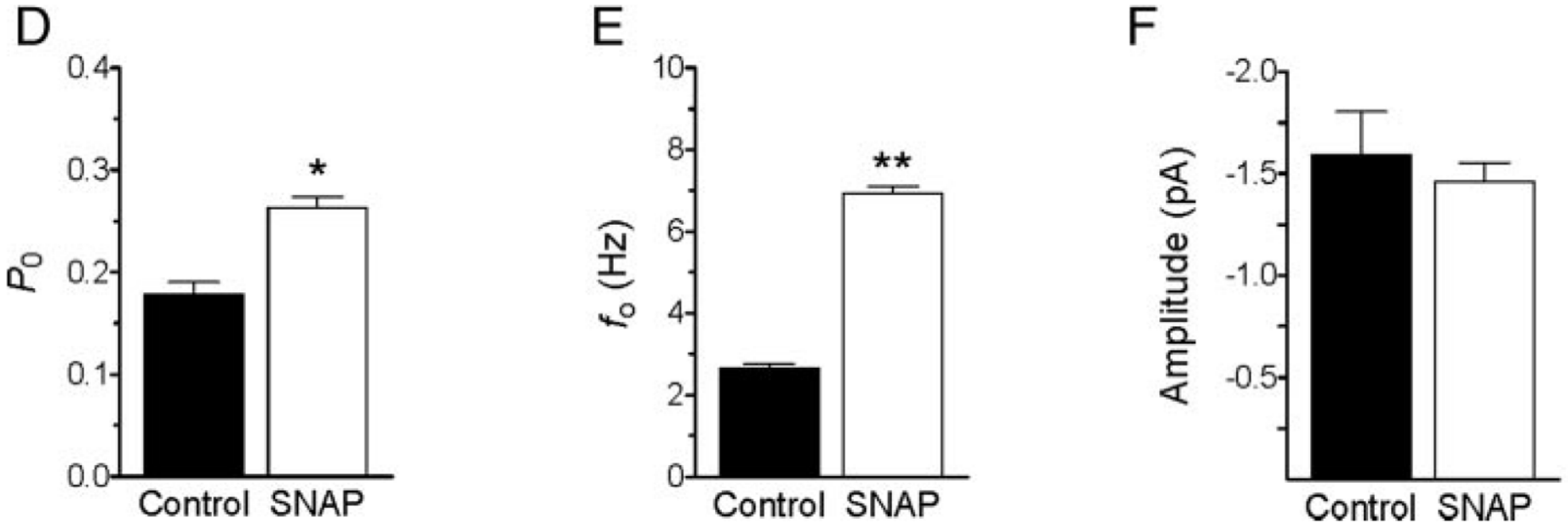






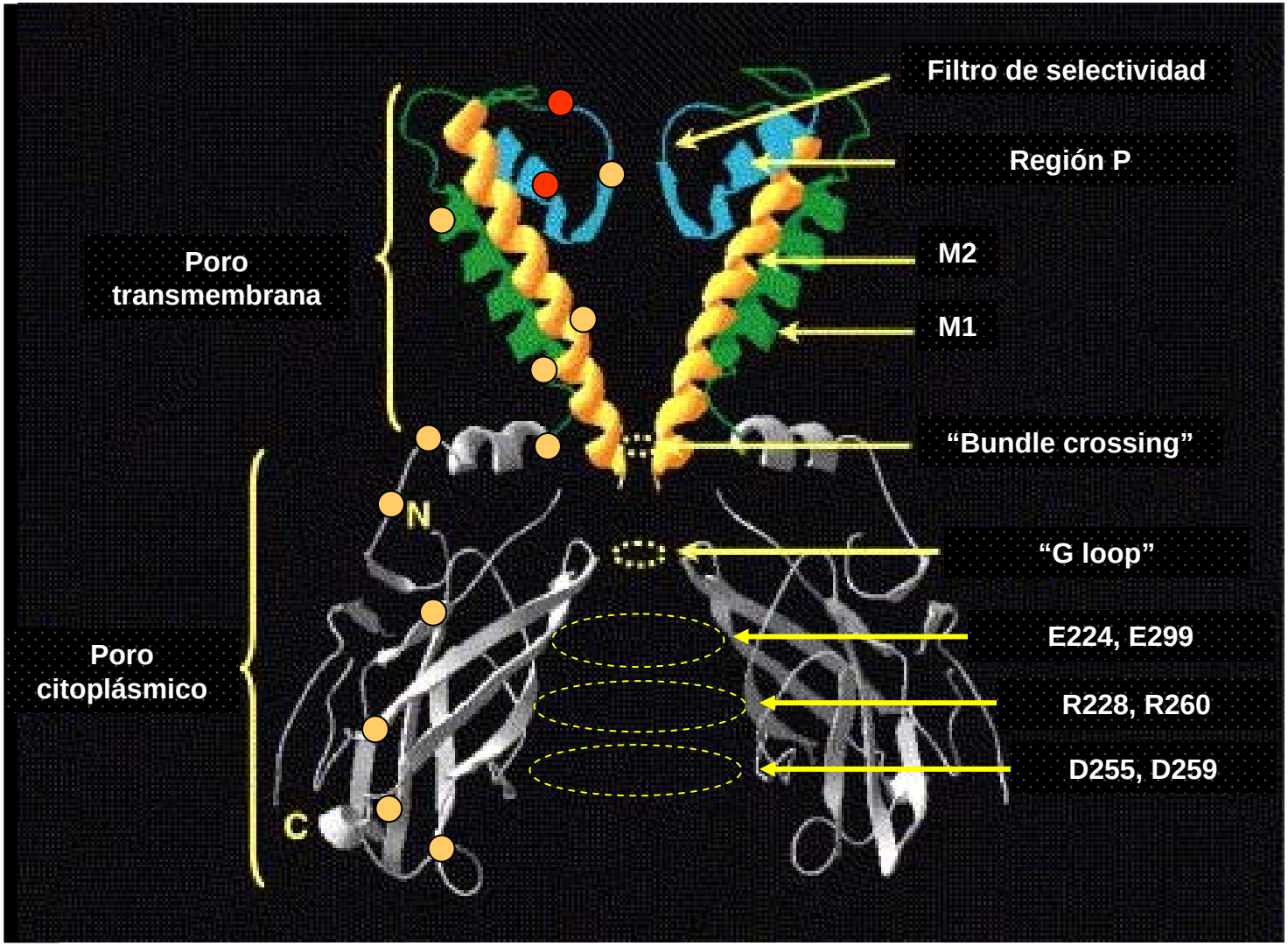


Frequency of channel opening

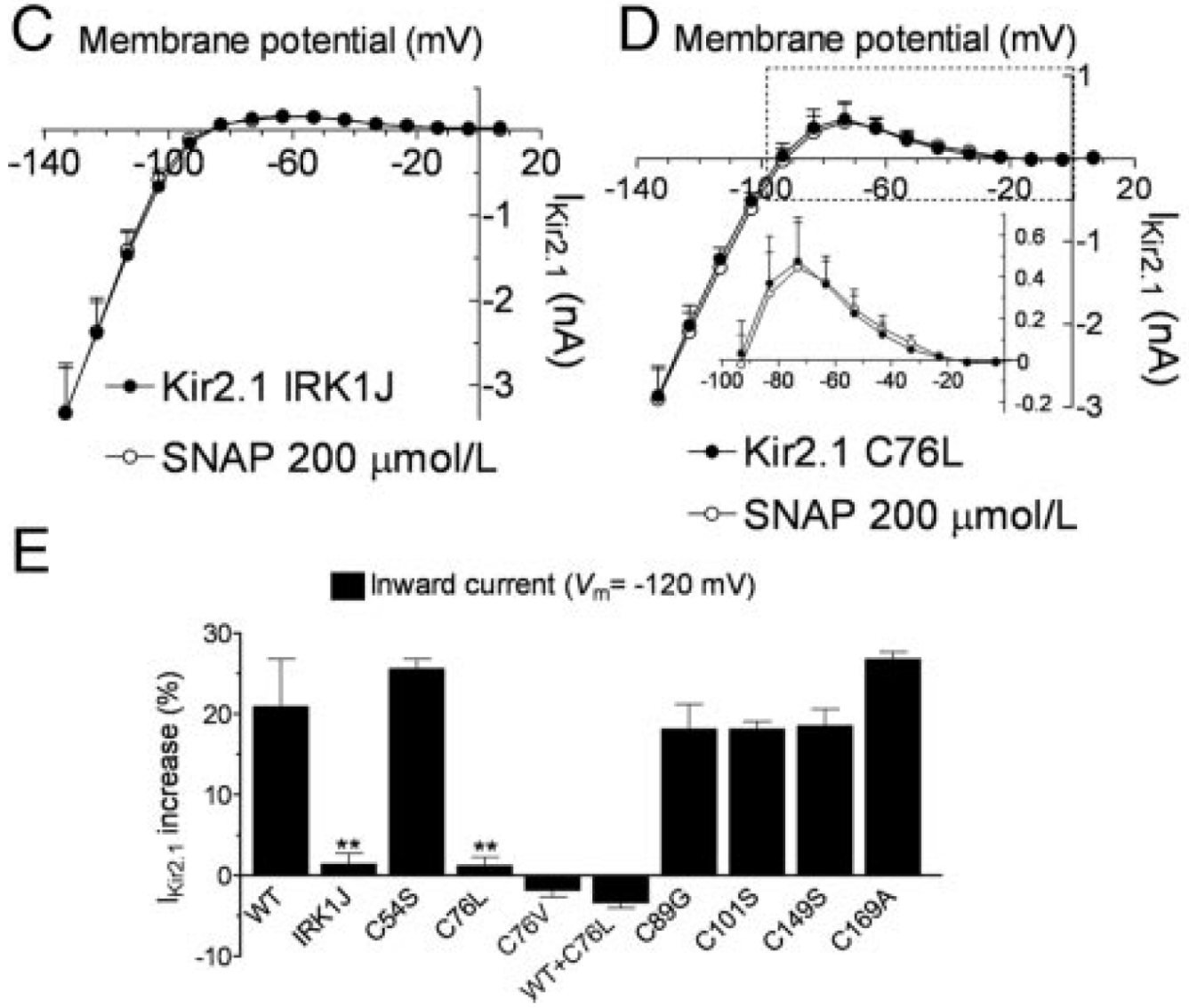


Kir2.1 channel structure

C43
C54
C76
C89
C101
C122
C149
C154
C169
C209
C311
C356
C375



NO increases IK1 by the selective S-nitrosylation of Cys76



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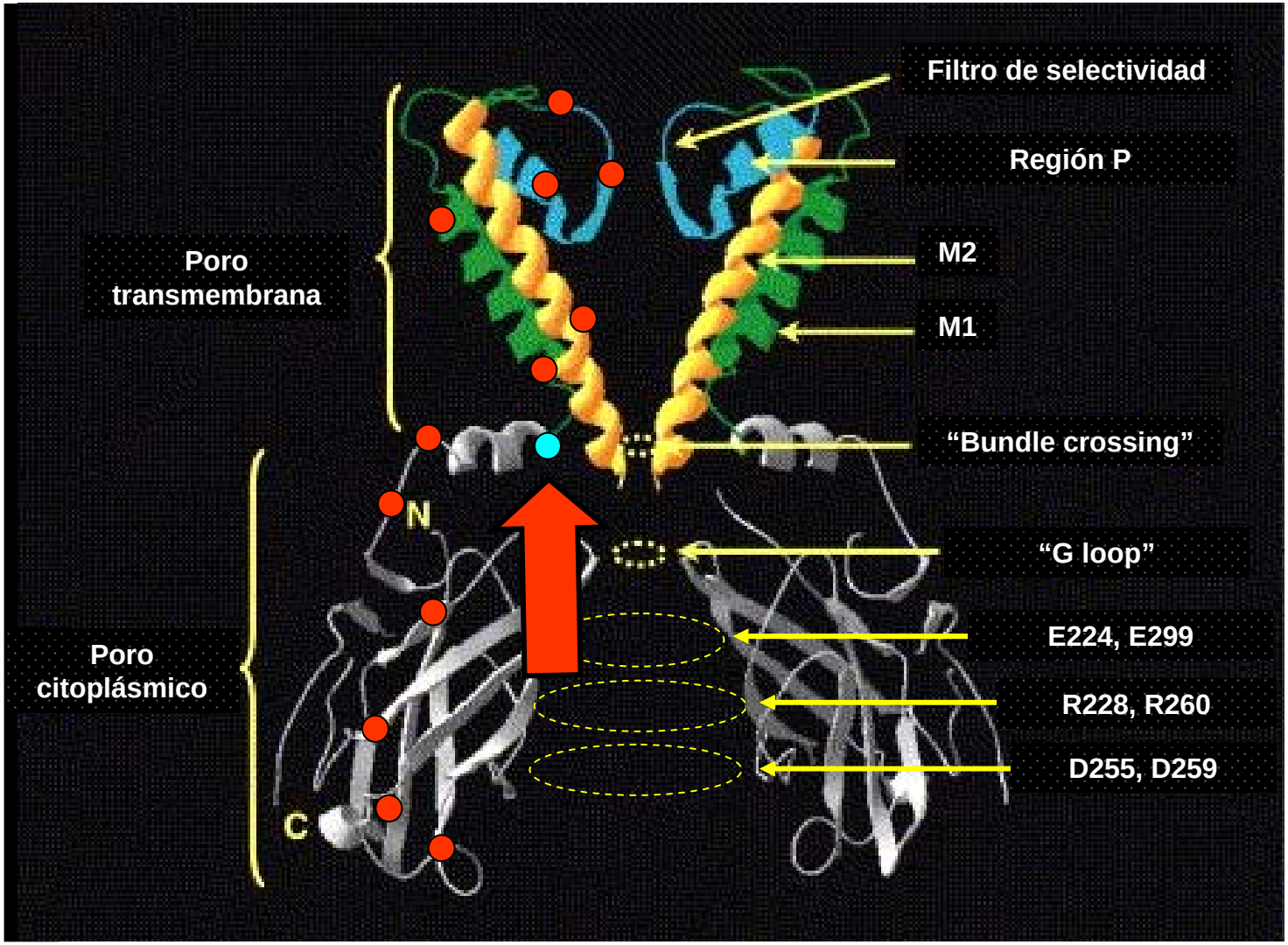
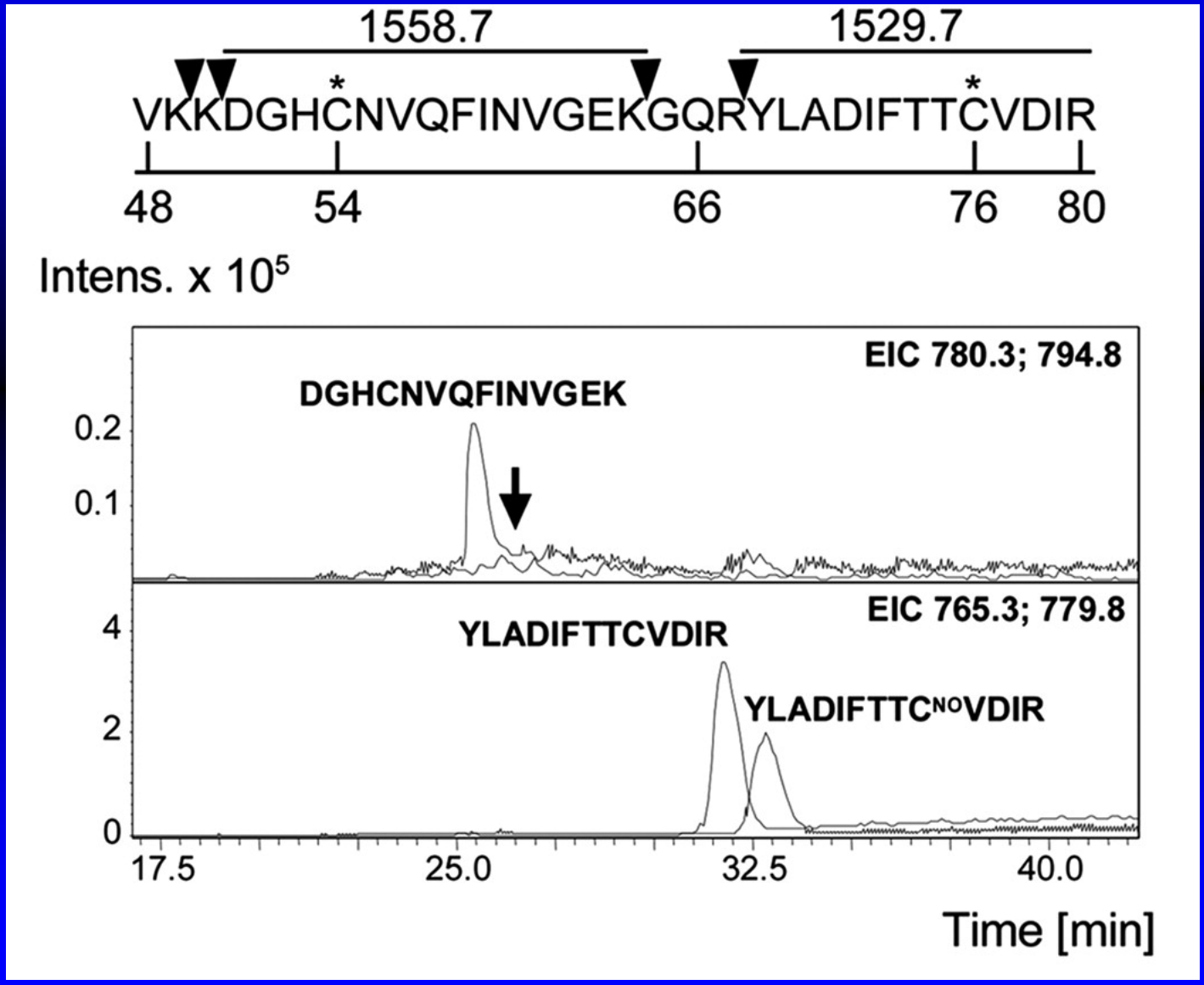
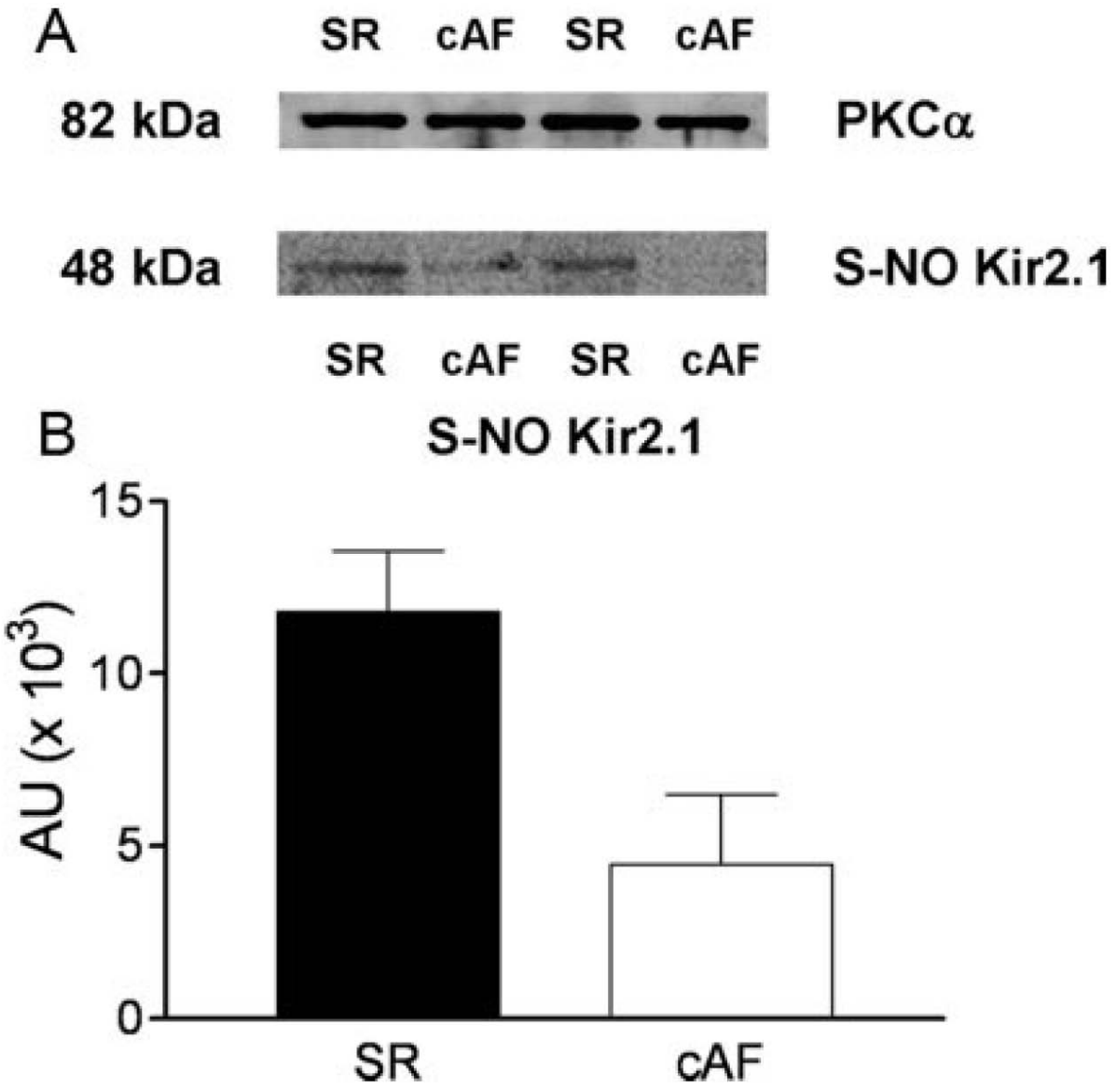


Kir2.1 Cysteine selectivity is determined by its environment within the primary sequence



Oxidative stress produced by AF significantly reduces the S-nitrosylation of the Kir2.1 channel



Conclusions

NO critically determines human atrial excitability and refractoriness by controlling the IK1 amplitude. This modulation is produced by means of the selective S-nitrosylation of Cys76 of Kir2.1 channels

Thank you very much!