Effects of β-adrenoceptor stimulation in human atrial repolarizing currents

M. G. de la Fuente,[1] R. Caballero,[1,2] I. Amorós,[1,2] A. Barana,[1] P. Dolz-Gaitón,[1,2] R. Gómez,[1] S. Sacristán,[1,2]
F. Fernández-Avilés,[2] J. Tamargo,[1] and E. Delpón[1,2]

[1] Department of Pharmacology, School of Medicine, Universidad Complutense de Madrid. 28040 Madrid. SPAIN
[2] Instituto de Investigación Sanitaria Gregorio Marañón. 28007 Madrid. SPAIN

INTRODUCTION

Atrial fibrilation (AF) is the most prevalent arrhythmia and the main risk factor associated with myocardial-related cerebrovascular events (1). Nowadays, pharmacological treatment of AF is clearly suboptimal (2), mainly due to rapid changes (4 to 6 hours after the onset) in the electrical properties of the atria (electrical remodeling) induced by the arrhythmia itself (3). This electrical remodeling promotes the maintenance and recurrence of AF (4), and it is characterized by a marked shortening of the atrial action potential duration (APD) and refractoriness as a consequence of changes in Ca^{2+} and K^+ channel density (5). Our group has described that chronic AF (CAF) reduced the transient outward (I_{to1}) and the ultrarapid delayed rectifier (I_{Kur} or I_{sus}) K^+ currents differentially on each atria, whereas it increased the slow delayed rectifier (I_{Ks}) K^+ current in both (6). In fact, CAF-associated reduction of the I_{to1} amplitude was greater in the left atrium (LA), whereas the reduction of the I_{sus} was greater in the right atrium (RA). These effects increase the electrical heterogeneity between both atrium, promoting the AF recurrence. Moreover, the I_{Ks} augmentation, together with the increase of the inward rectifier currents (the I_{K1} and the agonist-independent component of the I_{KACh}), also produced by CAF (7), should critically contribute to the abbreviation of APD and refractoriness (6). It has been proposed that β-adrenergic stimulation has profound influence in the genesis and maintenance of AF. Indeed, CAF has been associated with an increased atrial sympathetic innervation (8), suggesting that autonomic remodeling may be part of atrial substrate for AF. Stimulation of β-adrenoceptors inhibited I_{to1} in dog Purkinje myocytes (9), but increased I_{sus} in human RA myocytes (10) and I_{Ks} in guinea-pig ventricular myocytes (11). Furthermore, it has been shown that the increase of the L-type Ca^{2+} current induced by β-adrenergic stimulation is potentiated by CAF (12). However, data on the effects of β-adrenoceptor stimulation on voltage-dependent K^+ repolarizing currents in patients with CAF are unavailable. Thus, in this study we analyzed the effects of isoproterenol, a β-adrenoceptor agonist, on I_{to1}, I_{Kur}, and I_{Ks} recorded in isolated myocytes obtained from RA and LA appendages (RAA and LAA, respectively) obtained from sinus rhythm (SR) and CAF patients.

MATERIAL & METHODS

- Human atrial myocytes were enzymatically isolated from RAA and LAA samples obtained from SR and CAF patients that underwent cardiac surgery at the Hospital Gregorio Marañón in Madrid (6,13-17).
- I_{sus}, I_{to1}, I_{Ks} and I_{CaL} were recorded using the whole-cell configuration of the patch-clamp technique (6,12-19). I_{to1} was measured as the difference between the peak current amplitude and the current amplitude at the end of the 250-ms depolarizing pulse, I_{sus} as the current amplitude at the end of the pulse, I_{Ks} as the difference between the current amplitudes at the beginning and the end of a 4-s depolarizing pulse, and I_{CaL} was measured as the difference between the peak current amplitude and the current amplitude at the end of the 500-ms pulse.
- For K^+ current recordings, external solution contained (in mM): NaCl 120, KCl 20, $CaCl_2$ 1, $MgCl_2$ 1, HEPES 10, glucose 10, nifedipine (1 μM), and atropine (1 μM) (pH=7.4, with NaOH). To record I_{to1} and I_{sus}, external solution was supplemented with TEA (10 mM), whereas to record I_{Ks}, 4-AP (2 mM) and dofetilide (1 μM) were added. Internal solution contained (in mM): K-aspartate 80, KCl 42, KH_2PO_4 10, Mg-ATP 5, phosphocreatine 3, HEPES 5, and EGTA 5 (pH=7.2, with KOH). To record I_{CaL} the external solution contained (in mM): TEA 137, $CaCl_2$ 1, $MgCl_2$ 0.5, HEPES 10, and glucose 10 (pH=7.4 with CsOH), while the internal solution contained (mM): CsCl 125, TEA 20, EGTA 10, MgATP 5, phosphocreatine 3.6, and HEPES 10 (pH=7.2 with CsOH).
- Action potentials were recorded from RAA myocytes under the current clamp configuration (14). The external solution contained (in mM): NaCl 150, KCl 4, MgCl 2, $CaCl_2$ 2, glucose 10, and HEPES 10 (pH 7.4, with NaOH), whereas internal solution contained K-aspartate 100, NaCl 8, KCl 40, Mg-ATP 5, EGTA 5, $CaCl_2$ 2, GTP 0.1, and HEPES 10 (pH 7.4, with KOH).
- To simulate the shapes of human atrial action potentials, a mathematical model previously validated and used for identical purposes was employed (20)
- mRNA was isolated from human atrial appendages and quantitative reverse transcription polymerase chain reaction (qPCR) analysis was performed (6).

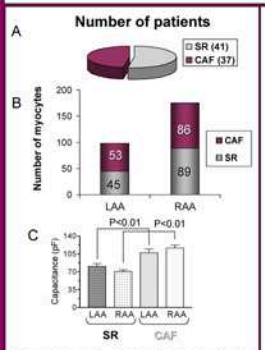

Figure 1. A-C, Distribution of patients (A), number (B), and mean capacitance values (C) of LAA and RAA myocytes obtained from SR and CAF patients.

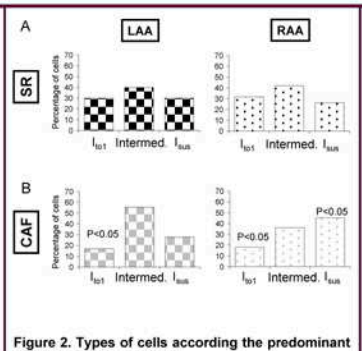

Figure 2. Types of cells according the predominant K^+ current during plateau.
A and B, Bar graphs showing the percentage of cells that exhibited I_{to1}-predominant, I_{sus}-predominant, and intermediate patterns in SR (A) and CAF (B) myocytes.

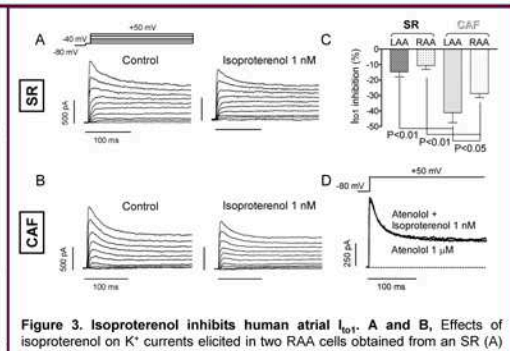

Figure 3. Isoproterenol inhibits human atrial I_{to1}. A and B, Effects of isoproterenol on K^+ currents elicited in two RAA cells obtained from an SR (A) and a CAF (B) patient. **C,** Percentage of isoproterenol-induced I_{to1} inhibition at +30 mV in LAA and RAA myocytes from SR and CAF patients. Each bar represents the mean±SEM of n>8. **D,** Effects of isoproterenol in the presence of atenolol on K^+ currents recorded in an RAA myocyte from a CAF patient.

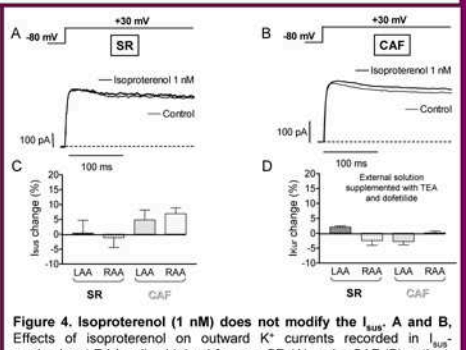

Figure 4. Isoproterenol (1 nM) does not modify the I_{sus}. A and B, Effects of isoproterenol on outward K^+ currents recorded in I_{sus}-predominant RAA cells obtained from an SR (A) and a CAF (B) patient. **C and D,** Percentage of I_{sus} (C) and I_{Kur} (D) change at +30 mV induced by isoproterenol on LAA and RAA cells from SR and CAF patients. Each point represents the mean±SEM of n>10.

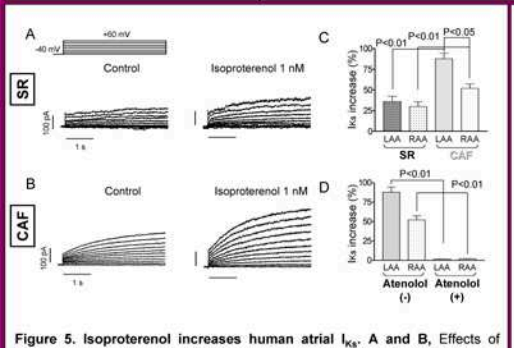

Figure 5. Isoproterenol increases human atrial I_{Ks}. A and B, Effects of isoproterenol on 2 mM 4-AP-resistant K^+ currents elicited in two RAA cells obtained from an SR (A) and a CAF (B) patient. **C,** Percentage of isoproterenol-induced I_{Ks} increase at +30 mV in LAA and RAA myocytes from SR and CAF patients. **D,** Percentage of isoproterenol-induced I_{Ks} increase at +30 mV in LAA and RAA myocytes from CAF patients in the absence and presence of atenolol. Each bar represents the mean±SEM of n>8.

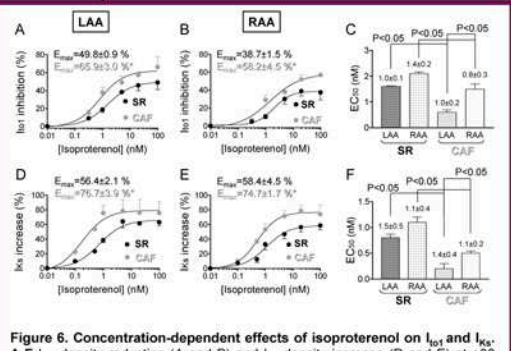

Figure 6. Concentration-dependent effects of isoproterenol on I_{to1} and I_{Ks}. A-F I_{to1} density reduction (A and B) and I_{Ks} density increase (D and E) at +30 mV as a function of isoproterenol concentration in LAA (A and D) and RAA (B and E) myocytes from SR and CAF patients. Continuous lines represent the fit of a Hill equation to the data. *P<0.05 *vs* SR. EC_{50} values for the isoproterenol-induced I_{to1} inhibition (C) and I_{Ks} increase (F). In C and F, Hill coefficients appear over the data bar. Each point/bar represents the mean±SEM of n>8.

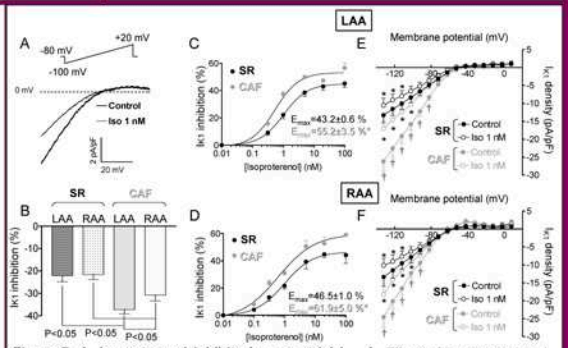

Figure 7. A, Isoproterenol inhibits human atrial I_{K1}. A, Effects of isoproterenol on I_{K1} recorded by applying a voltage-ramp (800 ms) in a LAA myocyte from a CAF patient. **B,** Isoproterenol-induced I_{K1} inhibition at -100 mV in LAA and RAA myocytes from SR and CAF patients. **C and D,** Concentration-dependent I_{K1} inhibition produced by isoproterenol at -100 mV in LAA (C) and RAA (D) myocytes from SR and CAF patients. **E and F,** Effects of isoproterenol on I_{K1} density-voltage curves obtained in LAA (**E**) and RAA (F) myocytes from SR and CAF patients. Each point/bar represents the mean±SEM of n>8. *P<0.05 *vs.* control. †P<0.05 *vs* SR.

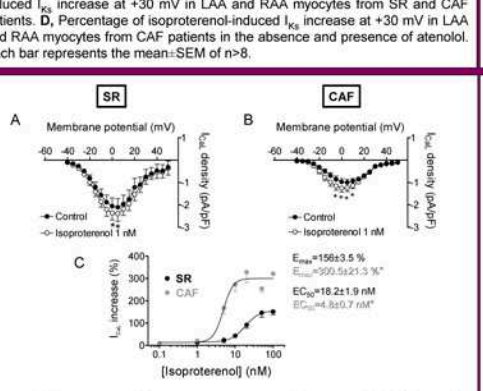

Figure 8. Isoproterenol increases human atrial I_{CaL}. A and B, Effects of 1 nM isoproterenol on the current density-voltage relationships for I_{CaL} recorded in RAA myocytes from SR (A) and CAF (B) patients. *P<0.05 *vs* control. The protocol to obtain current-voltage relationships of I_{CaL} consisted of 500-ms pulses that were imposed in 5 mV increments between -40 and +50 mV. **C,** Percentage of isoproterenol-induced I_{CaL} increase at +5 mV in RAA myocytes from SR and CAF patients. Continuous lines represent the fit of a Hill equation to the data. *P<0.05 *vs* SR.

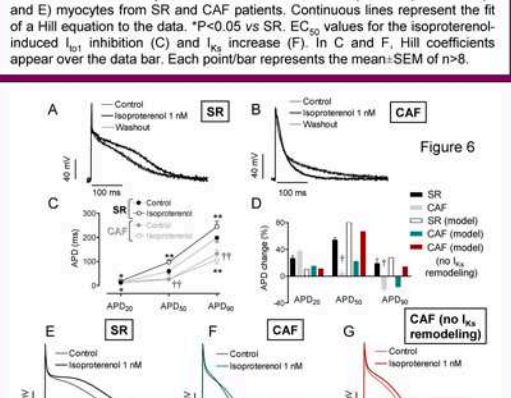

Figure 9. CAF modifies the effects of isoproterenol on human atrial action potentials (APs). A and B, Effects of isoproterenol on APs recorded in RAA myocytes obtained from an SR (A) and a CAF (B) patient. **C,** Effects of isoproterenol on APD measured at 20%, 50%, and 90% of repolarization. *P<0.05 *vs* control. **P<0.01 *vs* control. ††P<0.01 *vs* SR. **D,** Percentage of change in the APD produced by isoproterenol in SR and CAF myocytes. †P<0.05 *vs* SR. In C and D, each point/bar represents the mean±SEM of n>8. **E and F,** Mathematically modeled steady-state APs obtained in an SR (E), a CAF (F), and a CAF myocyte without considering the CAF-induced increase on I_{Ks} (G) in absence and presence of isoproterenol at a frequency of 1 Hz.

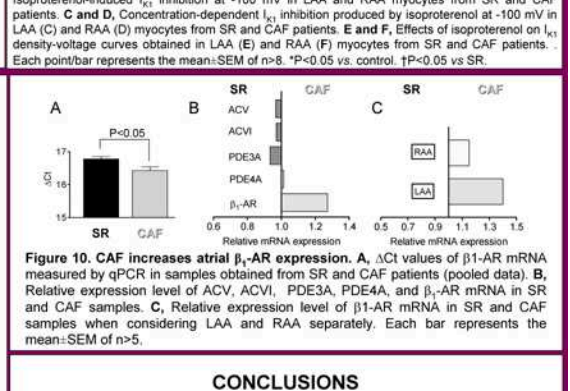

Figure 10. CAF increases atrial β_1-AR expression. A, ΔCt values of β1-AR mRNA measured by qPCR in samples obtained from SR and CAF patients (pooled data). **B,** Relative expression level of ACV, ACVI, PDE3A, PDE4A, and β_1-AR mRNA in SR and CAF samples. **C,** Relative expression level of β1-AR mRNA in SR and CAF samples when considering LAA and RAA separately. Each bar represents the mean±SEM of n>5.

CONCLUSIONS

- CAF potentiates the inhibition of the I_{to1} and the increase of the I_{Ks} produced by β-AR stimulation, this effect being greater in LAA than in RAA myocytes.
- CAF potentiates the β-adrenergic-induced increase of the I_{CaL}.
- β-adrenergic stimulation does not modify the I_{Kur} either in SR or in CAF myocytes and inhibits I_{K1} only at potentials negative to the equilibrium potential for K^+.
- The CAF-induced potentiation of the β-adrenergic effects on human atrial ion currents can be attributed to an increase in the β1-AR expression. Moreover, the mRNA expression of the β1-AR is higher in LAA than in RAA samples.
- The increase in β1-AR expression as well as the ion channel derangements produced by CAF, could account for the different effects produced by the β-AR stimulation on the APD in myocytes from SR (prolongation) and CAF patients (shortening).
- The CAF-induced increase on I_{Ks} is critical to account for the β1-AR-induced shortening of APD in CAF myocytes.
- The CAF-induced potentiation of the effects of β1-adrenoceptor stimulation on human atrial K^+ currents could contribute to the shortening of APD observed in CAF and, thus, to promote reentry.

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