Effects of β-adrenoceptor stimulation on human atrial voltage-dependent K^+ currents

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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}, and I_{Ks} were recorded using the whole-cell configuration of the patch-clamp technique (6,13-19). 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 depolarizing pulse, and I_{Ks} as the difference between the current amplitudes at the beginning and the end of a 4-s depolarizing pulse (6,19). For 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).
- Capacitance of the myocytes from CAF patients was greater than that of myocytes from SR patients (110±6.5 pF *vs* 68.4±5.3, n=120, *P*<0.0001).
- 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, CaCl2 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, CaCl2 2, GTP 0.1, and HEPES 10 (pH 7.4, with KOH).
- mRNA was isolated from human atrial appendages and semi- and quantitative reverse transcription polymerase chain reaction (qPCR) analysis (6) were performed.

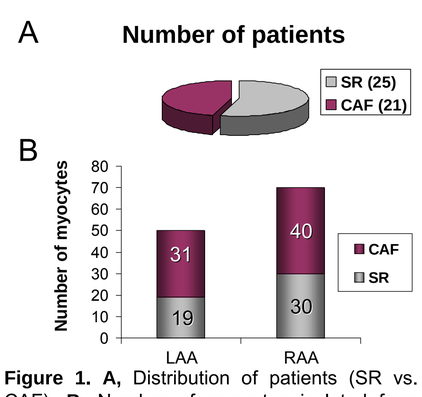

Figure 1. A, Distribution of patients (SR vs. CAF). **B,** Number of myocytes isolated from LAA and RAA samples studied.

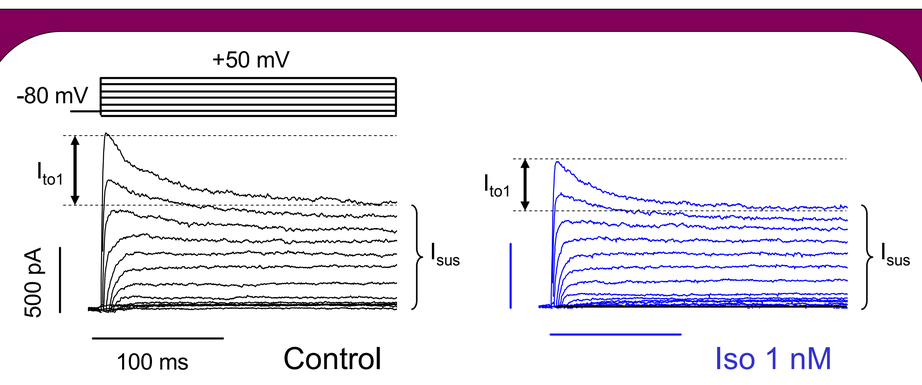

Figure 2. Outward K^+ current traces obtained by applying 250-ms pulses from -80 mV to potentials ranging between -90 and +50 mV in an enzymatically isolated LAA myocyte from a SR patient, in control conditions (left) and in the presence of isoproterenol (1 nM) (right).

Table 1. Effects of isoproterenol on the time course and voltage dependence of I_{to1} inactivation.

	CONTROL				ISOPROTERENOL 1 nM			
	SR		CAF		SR		CAF	
	LAA	RAA	LAA	RAA	LAA	RAA	LAA	RAA
τ_f inact (ms)	22.3 ± 3.9	20.4 ± 2.2	29.1 ± 3.6	26.7 ± 4.2	27.4 ± 4.2	24.0 ± 3.6	31.0 ± 1.8	25.3 ± 5.6
τ_s inact (ms)	137 ± 31	103 ± 12	135 ± 31	103 ± 19	174 ± 30	183 ± 38	154 ± 32	92 ± 13
V_h inact (mV)	-41.7 ± 1.1	-38.1 ± 2.9	-30.7 ± 1.6	-37.7 ± 5.1	-39.9 ± 1.9	-40.8 ± 3.1	-31.2 ± 2.3	-36.1 ± 4.8

I_{sus}

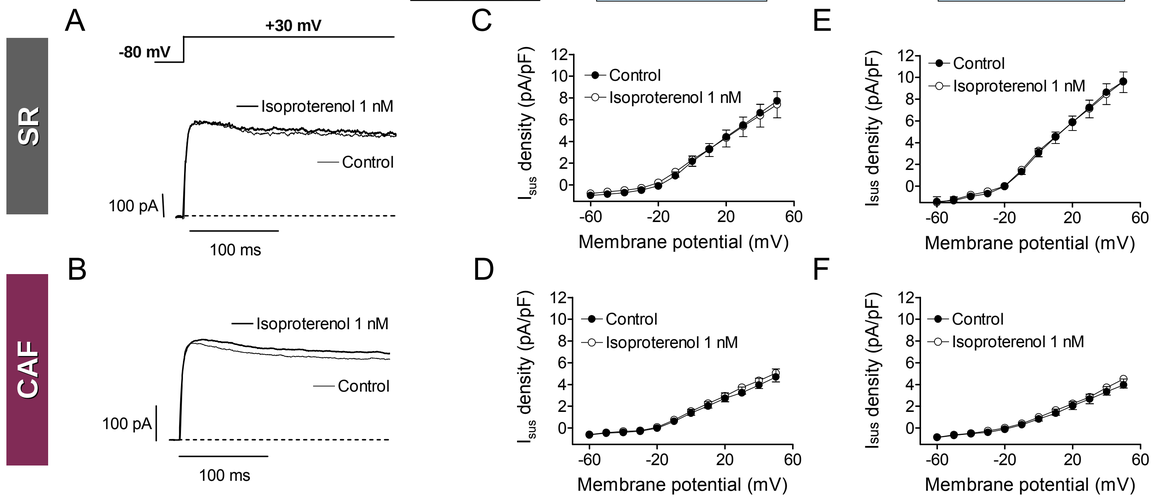

Figure 3. A and B, Effects of isoproterenol on the outward K^+ current recorded at +30 mV in Isus-predominant RAA cells (6) obtained from an SR (**A**) and a CAF patient (**B**). **C to F,** Mean density-voltage relationships obtained by plotting I_{sus} density as a function of the membrane potential in LAA (**C and D**) and RAA myocytes (**E and F**) from SR (**C and E**) and CAF (**D and F**) patients, in control conditions and in the presence of isoproterenol (1 nM).

I_{to1}

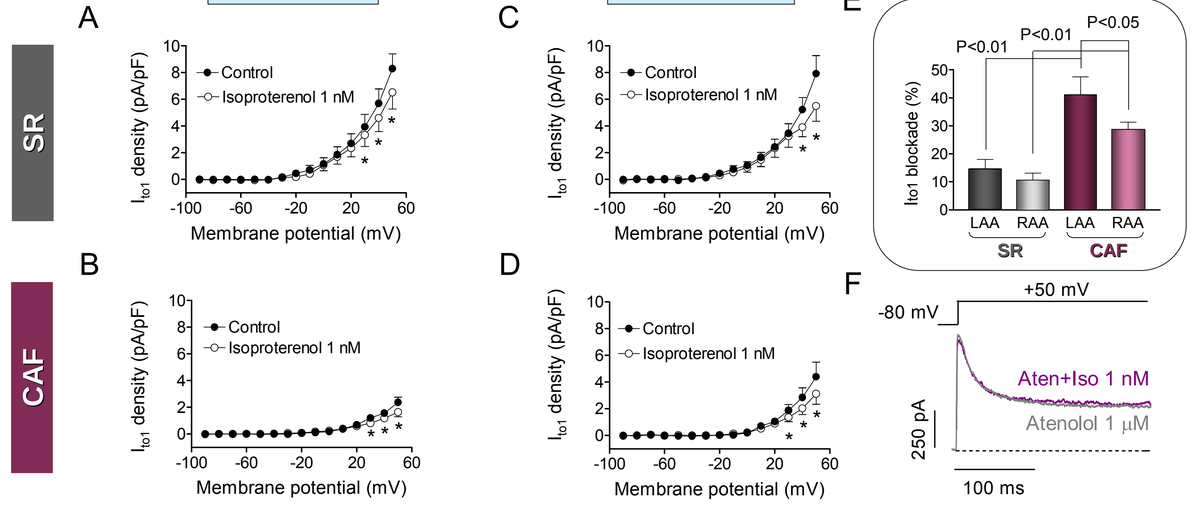

Figure 4. A to D, Mean density-voltage relationships obtained by plotting I_{to1} density as a function of the membrane potential in LAA (**A and B**) and RAA myocytes (**C and D**) from SR (**A and C**) and CAF (**B and D**) patients, in control conditions and in the presence of isoproterenol (1 nM). *P<0.05 vs. control. **E,** Effects of isoproterenol on I_{to1} density at +30 mV. **F,** Outward K^+ current traces recorded at +50 mV in a LAA myocyte from an SR patient, in the presence of atenolol (1 μM) and after atenolol+isoproterenol perfusion.

I_{Ks}

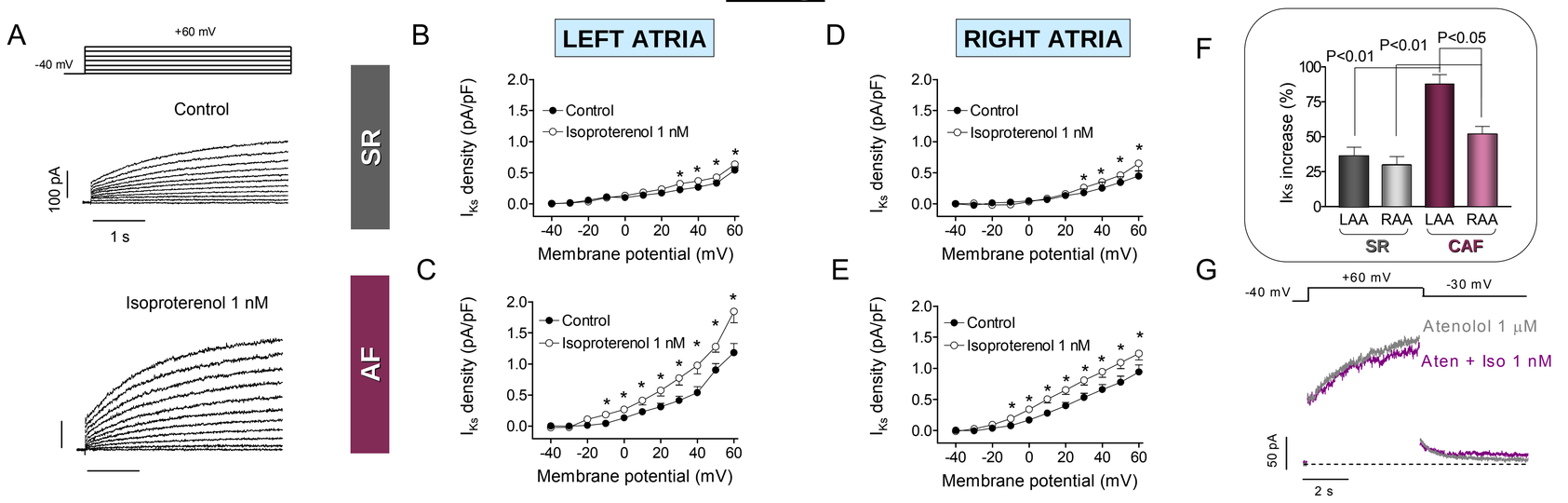

Figure 5. A, Representative I_{Ks} traces obtained after 4-s pulses from -40 mV to potentials ranging -40 and +60 mV in a RAA myocyte from a CAF patient, in control conditions and in the presence of isoproterenol (1 nM). **B to E,** Mean density-voltage relationships obtained by plotting I_{Ks} density as a function of the membrane potential in LAA (**B and C**) and RAA myocytes (**D and E**) from SR (**B and D**) and CAF (**C and E**) patients, in control conditions and in the presence of isoproterenol (1 nM). *P<0.05 vs. control. **F,** Effects of isoproterenol on I_{Ks} density at +30 mV. **G,** I_{Ks} traces recorded at +60 mV and after repolarization to -30 mV in a RAA myocyte from a CAF patient, in the presence of atenolol (1 μM) and after atenolol+isoproterenol perfusion.

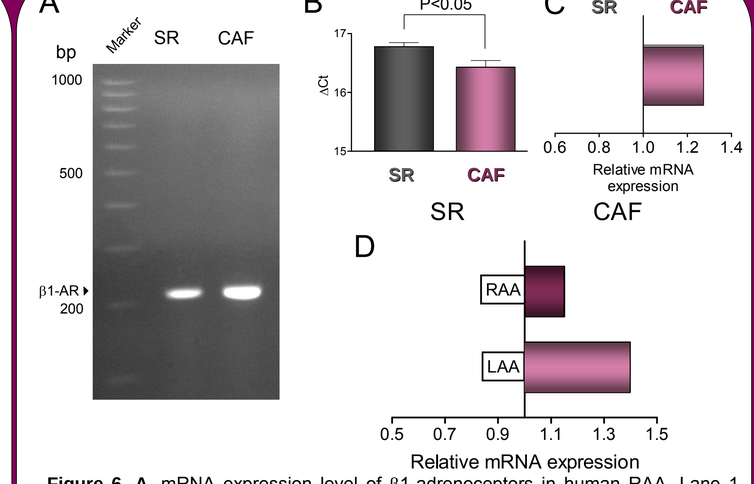

Figure 6. A, mRNA expression level of β1-adrenoceptors in human RAA. Lane 1 shows molecular weight markers (1000-100 bp). Lanes 2 and 3 show β1-adrenoceptor (209 bp) mRNA expression in samples from SR and CAF patients, respectively. **B and C,** Delta cycles to threshold (ΔCt) values (**B**) and relative expression level (**C**) of β1-adrenoceptor mRNA measured by qPCR in samples obtained from SR and CAF patients (pooled data). Relative expression level was calculated by transforming Ct values to equivalent fold differences using: Fold Difference (SR-CAF)=$2^{(CtSR-CtCAF)}$. **D,** Relative expression level of β1-adrenoceptors mRNA in SR and CAF samples when considering LAA and RAA separately.

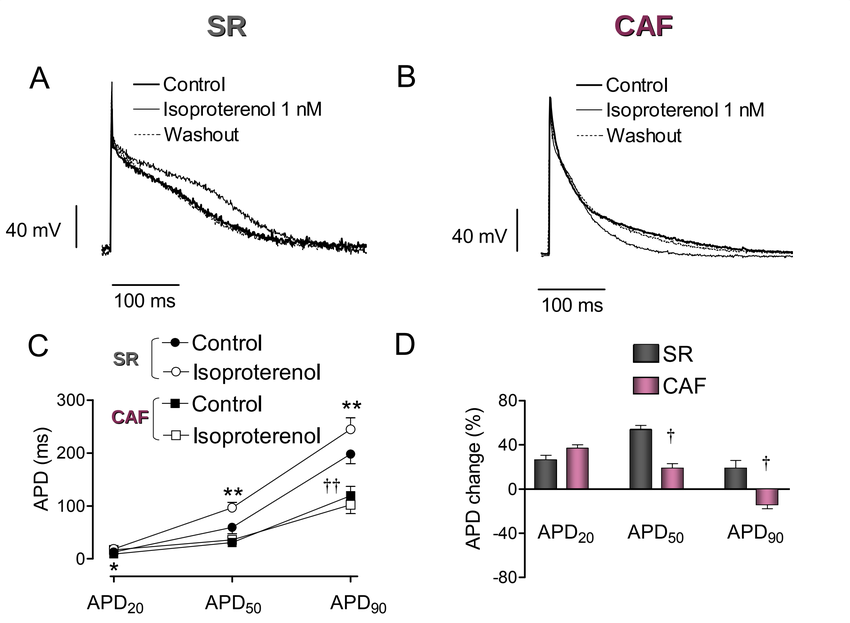

Figure 7. A and B, Action potentials recorded in two different RAA myocytes obtained from an SR (**A**) and a CAF (**B**) patient in control conditions and in the presence of isoproterenol (1 nM). **C,** APD measured at 20%, 50%, and 90% of repolarization in RAA myocytes from SR and CAF patients in the absence and presence of isoproterenol. *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.

CONCLUSIONS

- **CAF potentiates the β-adrenergic-induced inhibition of the I_{to1}, this effect being greater in LAA than in RAA myocytes.**
- **CAF potentiates the β-adrenergic-induced increase of the I_{Ks}. Again, this effect was greater in LAA than in RAA myocytes.**
- **β-adrenergic stimulation does not modify the I_{Kur} either in SR or in CAF myocytes.**
- **The CAF-induced potentiation of the β-adrenergic effects on human atrial K^+ currents can be attributed to an increase in the β1-adrenoceptor expression. Moreover, the mRNA expression of the β1-adrenoceptor is higher in LAA than in RAA samples.**
- **The increase in β1-adrenoceptor expression as well as the ion channel derangements produced by CAF, could account for the different effects produced by the β-adrenoceptor stimulation on the APD in myocytes from SR (prolongation) and CAF patients (shortening).**
- **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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GRANTS

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