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Inverse remodelling of $K_{2P}3.1$ K^+ channel expression and action potential duration in left ventricular dysfunction and atrial fibrillation: implications for patient-specific antiarrhythmic drug therapy

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Aims Atrial fibrillation (AF) prevalence increases with advanced stages of left ventricular (LV) dysfunction. Remote proarrhythmic effects of ventricular dysfunction on atrial electrophysiology remain incompletely understood. We hypothesized that repolarizing $K_{2P}3.1$ K^+ channels, previously implicated in AF pathophysiology, may contribute to shaping the atrial action potential (AP), forming a specific electrical substrate with LV dysfunction that might represent a target for personalized antiarrhythmic therapy.

Methods and results A total of 175 patients exhibiting different stages of LV dysfunction were included. Ion channel expression was quantified by real-time polymerase chain reaction and Western blot. Membrane currents and APs were recorded from atrial cardiomyocytes using the patch-clamp technique. Severely reduced LV function was associated with decreased atrial $K_{2P}3.1$ expression in sinus rhythm patients. In contrast, chronic (c)AF resulted in increased $K_{2P}3.1$ levels, but paroxysmal (p)AF was not linked to significant $K_{2P}3.1$ remodelling. LV dysfunction-related suppression of $K_{2P}3.1$ currents prolonged atrial AP duration (APD) compared with patients with preserved LV function. In individuals with concomitant LV dysfunction and cAF, APD was determined by LV dysfunction-associated prolongation and by cAF-dependent shortening, respectively, consistent with changes in $K_{2P}3.1$ abundance. $K_{2P}3.1$ inhibition attenuated APD shortening in cAF patients irrespective of LV function, whereas in pAF subjects with severely reduced LV function, $K_{2P}3.1$ blockade resulted in disproportionately high APD prolongation.

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Conclusion LV dysfunction is associated with reduction of atrial $K_{2P}3.1$ channel expression, while cAF leads to increased $K_{2P}3.1$ abundance. Differential remodelling of $K_{2P}3.1$ and APD provides a basis for patient-tailored antiarrhythmic strategies.

Keywords Arrhythmia • Atrial fibrillation • Electrical remodelling • Electrophysiology • Heart failure • $K_{2P}3.1$ channel

Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia, accounting for significant epidemiological and economical health burden. Current pharmacological or interventional treatments exhibit suboptimal effectiveness. The coexistence of heart failure (HF) worsens prognosis of AF patients and poses a particular therapeutic challenge.[1] Atrial effective refractory period and action potential duration (APD) are prolonged in AF complicated by reduced left ventricular ejection fraction (LVEF) in humans and animal models (a comprehensive overview of human data is provided in the Supplementary material online, *Table S1*).[2–7] Thus, HF-associated atrial arrhythmogenesis differs strikingly from patients without HF that show shortened APD [chronic (c)AF; i.e. persistent, long-standing persistent or permanent AF, defined according to current guidelines[8]] or no APD alterations [paroxysmal (p)AF[8]], respectively.[9] Two-pore-domain K^+ (K_{2P}) channels mediate transmembrane background currents that contribute to cardiac repolarization. Upregulation of atrial-selective $K_{2P}3.1$ (TASK-1, tandem of P domains in a weak inward rectifying K^+ channel-related acid-sensitive K^+ channel-1) expression and function promotes APD shortening in cAF patients, representing a novel target for antiarrhythmic AF therapy in this subgroup.[9] In contrast, $K_{2P}3.1$ channel downregulation has been detected in HF patients and animal models.[9,10] We hypothesized that differential $K_{2P}3.1$ channel remodelling contributes to a distinct atrial proarrhythmic substrate in patients with reduced LV function that would require tailored therapeutic approaches. The objective of this study was to elucidate the role of $K_{2P}3.1$ current dysregulation in LV dysfunction-related atrial AP changes and to evaluate the therapeutic significance of $K_{2P}3.1$ current blockade in AF patients with concomitant impairment of LV function.

Methods

A detailed description of the Methods is provided in the Supplementary material online.

Patients

A total of 175 patients (mean age, 69 ± 10 years; male/female, 131/44) with sinus rhythm (SR; $n = 89$), pAF ($n = 38$), and cAF ($n = 48$) undergoing open heart surgery for coronary artery bypass grafting, heart valve repair or valve replacement were included in the study (see Supplementary material online, *Tables S2* and *S3*). Patients were stratified according to LV dysfunction (preserved LVEF, ≥55%; mildly reduced LVEF, 45–54%; moderately reduced LVEF, 30–44%; severely reduced LVEF, <30%). Study patients were matched for baseline characteristics and medication to minimize any potential bias associated with these conditions, yielding minor remaining intergroup differences that require consideration when interpreting the present data. Importantly, patients receiving class I or class III antiarrhythmic were excluded from the study to exclude drug-associated APD alterations.

Molecular biology

Tissue samples were obtained from the right atrial (RA) or left atrial (LA) appendages. Quantitative real-time PCR (RT-qPCR) was performed using the StepOnePlus (Applied Biosystems, Foster City, CA, USA) PCR system according to the manufacturer's protocol (see Supplementary material online, *Table S4* for primer details).

Biochemistry

Protein immunodetection was performed by sodium dodecyl sulfate (SDS) gel electrophoresis and Western blotting using primary antibodies directed against study channels as reported.[6,9]

Cellular electrophysiology

Human atrial myocytes were isolated freshly. Electrophysiological recordings were carried out at room temperature (21–25 °C) using the whole-cell patch clamp configuration.[9]

Computational modelling

The Grandi *et al.*[11] computational model of the human atrial cardiomyocyte, including Na^+-dependent regulation of I_{K1} and $I_{K,ACh}$ and a formulation for the $K_{2P}3.1$ current,[9] was adapted to investigate the role of $K_{2P}3.1$ channels in patients with cAF, LV dysfunction, or both conditions. Multicellular simulations in homogeneous, isotropic virtual tissue in the absence or presence of $K_{2P}3.1$ current inhibition were performed using Myokit software.[12]

Statistics

Data are expressed as mean ± SD. Statistical significance between means of continuous variables was evaluated using Student's *t*-test (two-sample *t*-test; equal variances not assumed). $P < 0.05$ was considered statistically significant. The Bonferroni adjustment was used to correct for multiple testing. Please refer to Supplementary material online, *Supplementary Statistical Data*, for detailed statistical test results. Analysis of covariance (ANCOVA) was used to test how $K_{2P}3.1$ expression was affected by the factors *LA dilatation*, *male sex*, *elevated body mass index (BMI)*, and *smoking*, and covariates *AF status* and *LV dysfunction*. Linear regression analysis was applied to assess the relation between LVEF and $K_{2P}3.1$ mRNA, protein, and current, as well as atrial APD_{90}.

Results

LV dysfunction and AF are primary determinants of atrial $K_{2P}3.1$ abundance

$K_{2P}3.1$ K^+ channel levels have previously been implicated in atrial AP regulation of cAF patients, and preliminary observations suggested HF-associated $K_{2P}3.1$ suppression.[9] We performed a comprehensive

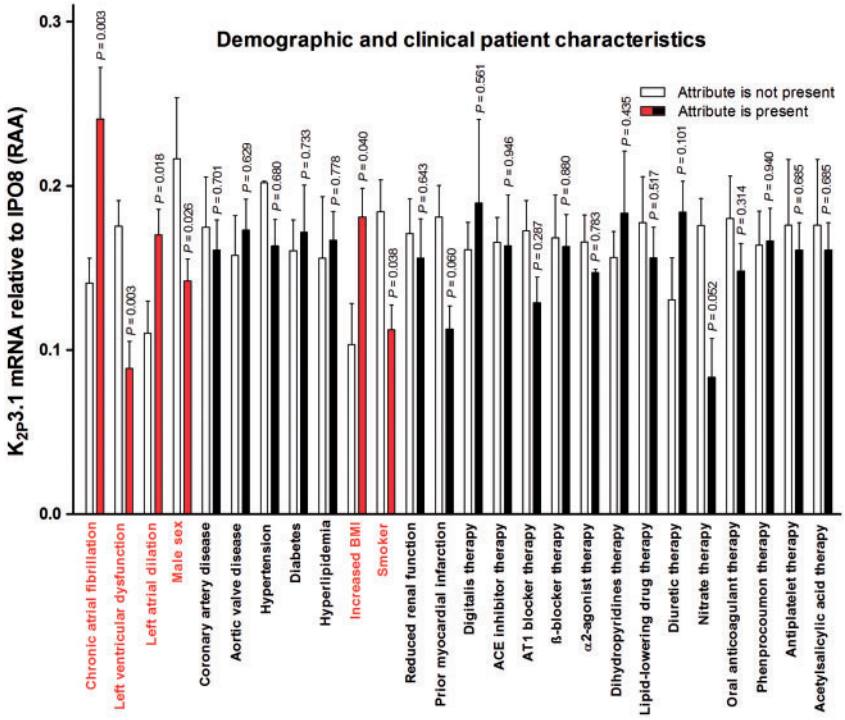

Figure 1 Demographic and clinical determinants of $K_{2P}3.1$ channel expression. The association of patient characteristics with right atrial appendage (RAA) $K_{2P}3.1$ channel mRNA expression was assessed by one-way analysis of variance in a cohort of 39 patients. Please note that $K_{2P}3.1$ expression data associated with factors chronic atrial fibrillation and left ventricular dysfunction (i.e. severely reduced left ventricular function) represent an extension of a previously reported analysis.[9] Data are expressed as mean ± SD. Unadjusted *P*-values were obtained from pairwise comparisons. ACE, angiotensin-converting enzyme; AT, angiotensin; BMI, body mass index; IPO8, importin 8.

analysis to delineate the association of cAF and severely reduced LV function with atrial $K_{2P}3.1$ mRNA regulation based on two-sample *t*-tests (*Figure 1*; Supplementary material online, *Figure S1, Table S5*). Besides cAF and severe LV dysfunction, RA $K_{2P}3.1$ levels depended on LA dilatation (>40 mm diameter; Supplementary material online, *Figure S2*), male sex, elevated BMI (defined as BMI ≥27), and smoking. $K_{2P}3.1$ expression was increased in AF and with increased LA diameters or high BMI, whereas reduced $K_{2P}3.1$ levels were associated with LV dysfunction, male sex, and smoking.

However, differences in $K_{2P}3.1$ levels were most significant for AF and LV dysfunction, indicating that cAF and severe LVEF impairment were important regulators of $K_{2P}3.1$ expression. Parameters such as LA dilatation or male gender might be directly associated with cAF and LV dysfunction. To test whether dependencies between LA dilatation, male sex, elevated BMI or smoking, and $K_{2P}3.1$ levels were confounded by cAF and impaired LVEF, we performed ANCOVAs in which we combined either one of the factors *LA dilatation*, *male sex*, *elevated BMI*, and *smoking* or combinations of these factors with covariates *AF status* (SR, pAF, or cAF) and *LV dysfunction* (none, mild, moderate, or severe). Effects of LA dilatation, male sex, elevated BMI or smoking were not significant when they were modeled together with AF status and LV dysfunction as covariates in any combination tested. These results indicate that associations of $K_{2P}3.1$ levels with LA dilatation, male sex, elevated BMI, and smoking were indeed confounded by AF status and LV dysfunction. We therefore conclude that cAF and severe LV dysfunction were primary regulators of $K_{2P}3.1$ levels, while LA dilatation, male sex, elevated BMI, and smoking were secondary regulators (Supplementary material online, *Figure S3*).

Atrial K_{2P} channel expression is decreased in patients with reduced LV function

Study patients were screened for mRNA levels of additional K_{2P} channels with confirmed expression in human atrium[9] to assess specificity of $K_{2P}3.1$ channel regulation by LV dysfunction and cAF. $K_{2P}3.1$ mRNA showed downregulation by severely reduced LVEF and antagonistic upregulation by cAF in RA and LA (*Figure 2A*). In contrast, other K_{2P} channels were uniformly suppressed ($K_{2P}2.1$, $K_{2P}5.1$, $K_{2P}13.1$, and $K_{2P}17.1$) or not markedly affected ($K_{2P}1.1$ and $K_{2P}6.1$) in patients with reduced LV function or cAF.

LV dysfunction-related K_{2P} channel remodelling was next specifically assessed in SR subjects. RA $K_{2P}3.1$ mRNA downregulation by 49% with progressive decline of LV function has previously been indicated.[9] This key finding was studied at the protein level (*Figure 2B*), revealing 54% suppression ($n = 5$; $P = 0.049$) associated with severely impaired LVEF compared with patients exhibiting preserved cardiac function ($n = 5$). In LA tissue, $K_{2P}3.1$ mRNA (-59%; $n = 17$; $P = 0.030$)

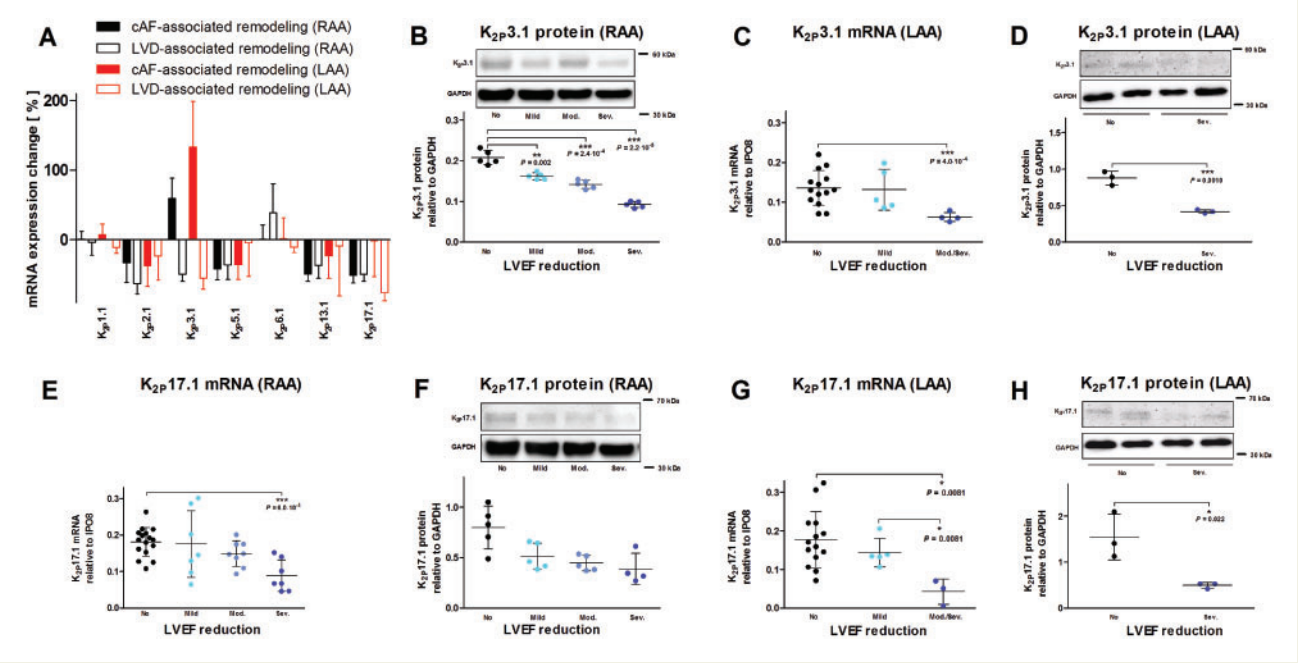

Figure 2 Left ventricular dysfunction-related remodelling of atrial K_{2P} channel mRNA and protein. (*A*) Transcriptional analysis of human atrial K_{2P} channels with relevant overall mRNA levels. Changes in right (RAA) or left atrial appendage (LAA) mRNA expression associated either with left ventricular dysfunction (LVD) characterized by severely reduced left ventricular ejection fraction (LVEF) irrespective of rhythm status ($n = 41$), or with chronic atrial fibrillation (cAF; $n = 28$ patients with normal or reduced LVEF) were normalized to importin 8 (IPO8) and are shown relative to mRNA expression in patients not exhibiting the respective attribute. (*B–H*) $K_{2P}3.1$ (*C*) and $K_{2P}17.1$ (*F*, *G*) mRNA expression in human RAA (*E*) and LAA (*C*, *G*) were quantified and normalized to IPO8 in SR patients. Analysis of RAA samples included patients with preserved LVEF (*E*; $n = 17$) and mildly (*E*; $n = 7$), moderately (*E*; $n = 8$), or severely reduced LVEF (*E*; $n = 8$). Owing to low numbers of patients with reduced LVEF in the LAA group, mRNA analyses were limited to three subgroups: no LVEF reduction (*C*, *G*; $n = 14$), mildly reduced LVEF (*C*, *G*; $n = 4$), and moderately or severely impaired LVEF (*C*, *G*; $n = 3$). Representative $K_{2P}3.1$ and $K_{2P}17.1$ immunoblots and mean optical density values normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) are shown for human RAA (*B*, *F*) and LAA tissue (*D*, *H*). RAA samples were obtained from patients with normal LV function (*B*, *F*; $n = 5$) and mildly (*B*, *F*; $n = 5$), moderately (*B*, *F*; $n = 5$) or severely reduced LVEF (*B*, *F*; $n = 5$). LAA protein analyses were limited to two groups: no LVEF reduction (*D*, *H*; $n = 3$) and severely reduced LVEF (*D*, *H*; $n = 3$). Data are provided with mean ± SD. Unadjusted *P*-values are given. Levels of significance (*$P < 0.05$; **$P < 0.01$) include adjustments for multiple comparisons (except panel *H*) (see Supplementary material online, *Supplementary Statistical Data* for details).

and protein (-51%; $n = 6$; $P = 0.040$) were similarly diminished in patients with moderate to severe LVEF reduction compared with study subjects showing normal LVEF (*Figures 2C* and *D*).

Among functional K_{2P} channels, $K_{2P}17.1$ subunits show second highest expression in human atrium following $K_{2P}3.1$ and are downregulated in cAF (*Figure 2A*).[9] Atrial $K_{2P}17.1$ mRNA (RA: -51%; $n = 24$; $P = 0.002$; LA: -76%; $n = 17$; $P = 0.017$) and protein (RA: -38%; $n = 10$; $P = 0.033$; LA: -70%; $n = 6$; $P = 0.049$) were significantly decreased in patients with advanced LVEF reduction (*Figure 2E–H*).

$K_{2P}3.1$ K^+ currents are suppressed in patients with reduced LV function

Functional consequences of $K_{2P}3.1$ downregulation were studied in RA myocytes obtained from patients with different stages of LV dysfunction. Study subjects were further stratified according to rhythm status (SR, pAF, and cAF). Among SR subjects, $K_{2P}3.1$ current density at +40 mV was reduced by 63% in patients with severely reduced LVEF ($n = 16$ cells obtained from $N = 5$ individuals) compared with cases with preserved LV function ($n/N = 18/7$; $P = 0.0001$) (*Figure 3A* and *B*). Patients with pAF or cAF exhibited $K_{2P}3.1$ current reduction associated with severe LV dysfunction to similar extent by 58% (pAF; $n/N = 6/3$; $P = 0.052$; *Figure 3C* and *D*) and by 56% (cAF; $n/N = 5/3$; $P < 0.0001$; *Figure 3E* and *F*) relative to subjects with identical rhythm status but preserved LVEF (pAF, $n/N = 14/5$; cAF, $n/N = 16/5$).

Furthermore, baseline current densities were increased in patients with cAF by 3.1-fold (normal LVEF; $n/N = 16/5$; $P < 0.0001$) and by 3.6-fold (severely impaired LVEF; $n/N = 5/3$; $P < 0.0001$) compared with SR subjects ($n/N = 18/7$ and 16/5, respectively) (*Figure 3G*), reflecting arrhythmia-related $K_{2P}3.1$ augmentation.[9] Analysis of $K_{2P}3.1$ levels without stratification according to underlying atrial rhythm in study patients further illustrated a progressive reduction of $K_{2P}3.1$ current with increasing stages of LV dysfunction (*Figure 3H* and *I*; see Supplementary material online, *Figure S4*), consistent with corresponding decline of atrial $K_{2P}3.1$ mRNA and protein content (*Figure 2B–D*).

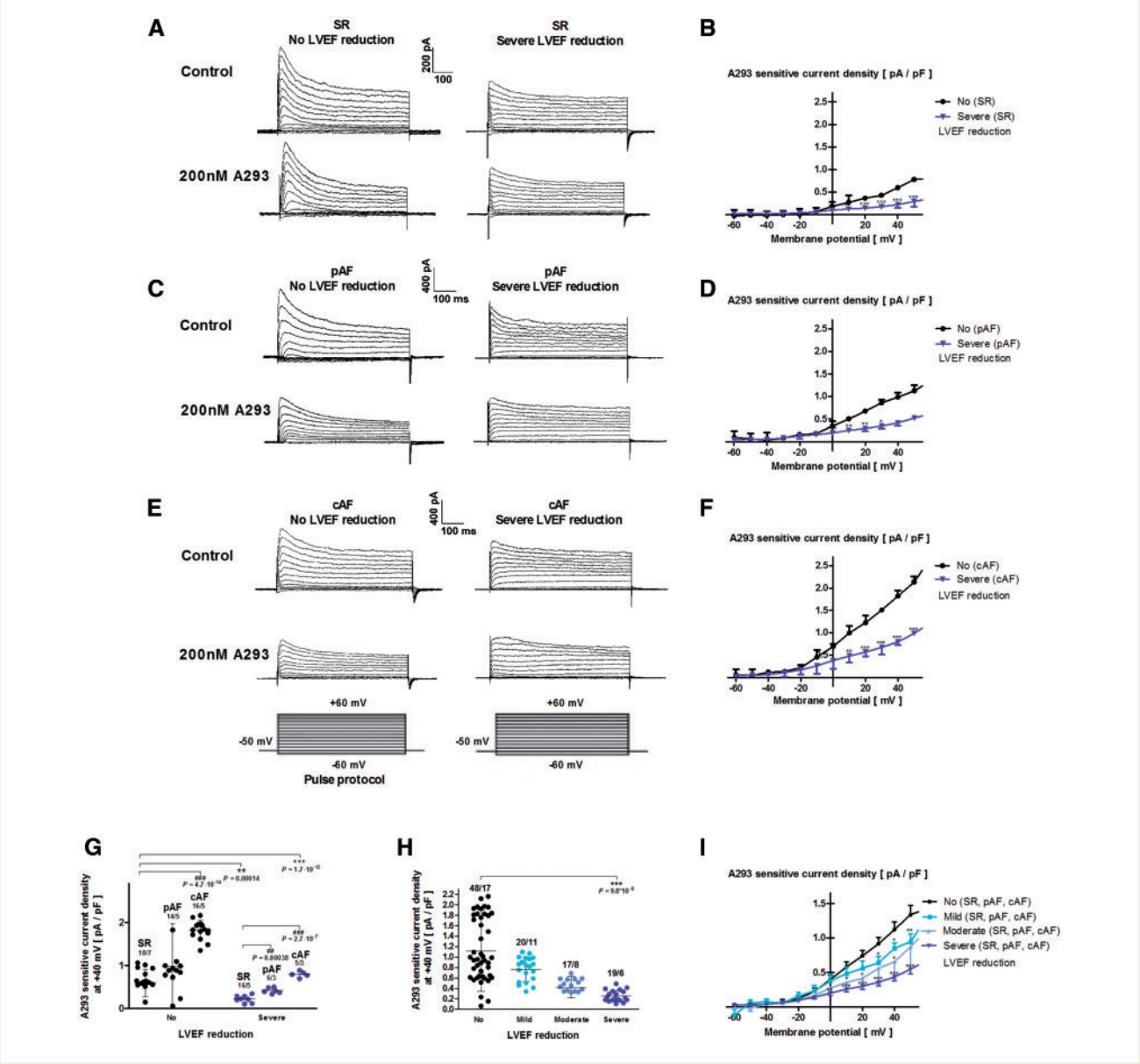

Figure 3 $K_{2P}3.1$ current characteristics in patients with left ventricular dysfunction. (*A–F*) Representative K^+ currents (*A*, *C*, *E*) recorded from human right atrial myocytes of patients with normal left ventricular (LV) function and with severely reduced LV ejection fraction (LVEF), and mean step current density (*B*, *D*, *F*) are displayed vs. respective test potentials for sinus rhythm (SR; A, B), paroxysmal atrial fibrillation (pAF; C, D), and chronic atrial fibrillation (cAF; E, F), respectively. $K_{2P}3.1$ currents were acquired with indicated voltage protocols and isolated using the specific inhibitor A293. (*G*) A293-sensitive current density, corresponding to data presented in *A–F*. (*H*) Left ventricular dysfunction-related reduction of $K_{2P}3.1$ current density among study subjects with indicated LVEF, irrespective of the underlying rhythm (SR, pAF, or cAF). (*I*) Current-voltage relationships of mean A293-sensitive current density are depicted for patients with increasing degrees of LV dysfunction in comparison to individuals showing preserved cardiac function. Data are provided with mean ± SD; *n*/*N*, number of myocytes/patients. Unadjusted *P*-values are given. Levels of significance ($^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. patients with preserved LVEF and similar rhythm status; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$ vs. patients with sinus rhythm) include adjustments for multiple comparisons in panels G and H (see Supplementary material online, *Supplementary Statistical Data* for details).

Functional $K_{2P}3.1$ downregulation causes APD prolongation

Suppression of repolarizing atrial $K_{2P}3.1$ channels is expected to result in APD prolongation in LV dysfunction patients. Indeed, in SR subjects, atrial APD at 90% repolarization (APD_{90}) was increased by 34% from 190 ± 19 ms (preserved LVEF; *n*/*N* = 10/6) to 254 ± 20 ms (severely impaired LVEF; *n*/*N* = 5/2; *P* = 0.0003) (*Figure 4A* and *G*). Patients with cAF exhibited shorter APD_{90} levels at baseline compared with SR

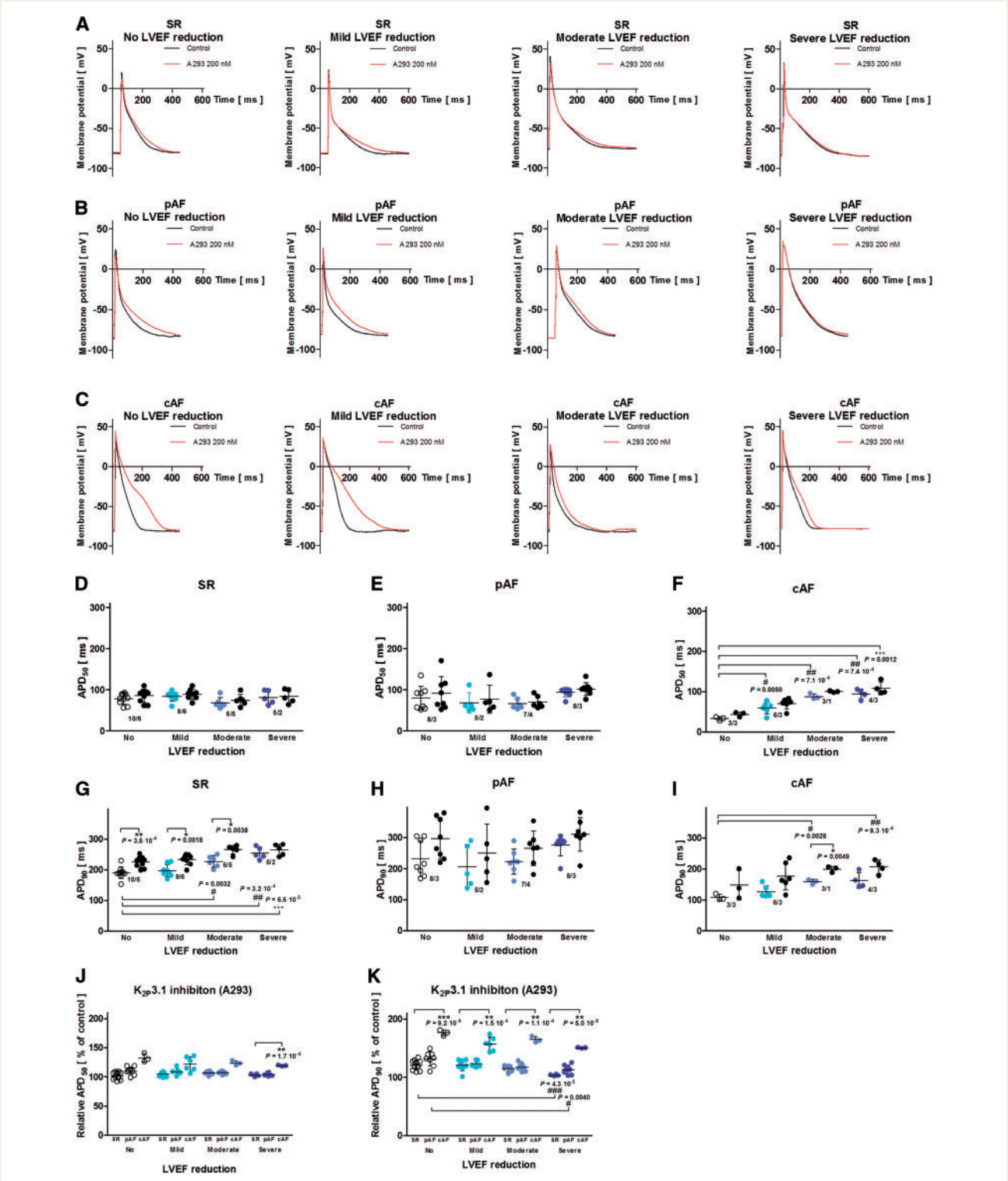

Figure 4 Atrial action potentials and effects of $K_{2P}3.1$ inhibition. (*A–C*) Representative action potentials (AP) recorded at 0.2 Hz in the absence or presence of A293 are shown for sinus rhythm (SR; A), paroxysmal AF (pAF; B) and chronic AF (cAF; C) patients with different left ventricular ejection fraction (LVEF). (*D–I*) Corresponding mean AP durations at 50% (APD_{50}; D-F) and 90% repolarization (APD_{90}; G-I) at baseline and following specific $K_{2P}3.1$ inhibition with 200 nM A293. (*J*, *K*) Relative APD_{50} and APD_{90} after application of A293 in atrial myocytes obtained from patients with indicated LVEF and cardiac rhythm. Values were normalized to respective baseline APD in the absence of A293. Data are provided with mean ± SD; *n*/*N*, number of myocytes/patients. Unadjusted *P*-values are given. Levels of significance ($^*P < 0.05$, $^{**}P < 0.01$ vs. drug-free control conditions and similar rhythm status; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. preserved LVEF; $^{+++}P < 0.001$ vs. preserved LVEF in the absence of A293) include adjustments for multiple comparisons (see Supplementary material online, *Supplementary Statistical Data* for details).

(*Figure 4C* and *I*) owing to cAF-associated $K_{2P}3.1$ upregulation.[9] Relative APD_{90} prolongation associated with LVEF reduction was similarly observed in cAF patients (*Figure 4I*). The correlation between the extent of LV dysfunction and $K_{2P}3.1$ expression and function was confirmed by linear regression analysis (see Supplementary material online, *Figure S5*). Finally, baseline APD_{90} remained virtually unchanged in pAF patients with preserved cardiac function compared with SR subjects (*Figure 4B, G,* and *H*), consistent with previous clinical observations.[9] In pAF patients with reduced LVEF, a similar tendency towards prolonged APD_{90} was noted, confirming a general role for ventricular dysfunction in APD prolongation (*Figure 4B* and *H*). Patients with SR, pAF, or cAF and mildly to moderately impaired LVEF exhibited intermediate APD_{90} prolongation (*Figure 4A–C* and *G–I*) that did not reach statistical significance. Furthermore, there was no significant modulation of APD at 50% of repolarization (APD_{50}), with the exception of cAF patients characterized by rhythm-dependently shortened APD_{50} at baseline that increased with worsening of functional LV impairment (*Figure 4A–F*).

Patient-specific cellular antiarrhythmic efficacy of $K_{2P}3.1$ channel inhibition depends on LVEF and rhythm status

Differential remodelling of $K_{2P}3.1$ levels and APD suggests a need for patient-tailored antiarrhythmic strategy planning. The experimental $K_{2P}3.1$ inhibitor A293 (200 nM) was employed to evaluate patient-specific effects of $K_{2P}3.1$ blockade on APD. We first confirmed that pharmacological $K_{2P}3.1$ inhibition successfully attenuated APD shortening in cAF patients in the absence of LV dysfunction. APD_{90} was prolonged by 74% ($n/N = 3/3$; $P = 0.14$) to 148 ± 49 ms, thus approaching levels observed among SR subjects at baseline (190 ± 19 ms; $n/N = 10/6$) and indicating class III antiarrhythmic efficacy[9] in this AF subgroup (*Figures 4G, I,* and *K*). Furthermore, study subjects with cAF and impaired LV function showed APD_{90} prolongation following $K_{2P}3.1$ inhibition, suggesting an extension of beneficial effects to cAF cases with concomitant LV dysfunction (*Figure 4I* and *K*). $K_{2P}3.1$ inhibition prolonged APD_{90} in patients with mildly, moderately, or severely reduced LVEF by 53% to 170 ± 44 ms ($n/N = 6/3$; $P = 0.037$), by 61% to 190 ± 10 ms ($n/N = 3/1$; $P = 0.004$), and by 48% to 202 ± 22 ms ($n/N = 4/3$; $P = 0.037$), respectively. In addition, we observed a tendency towards APD_{50} prolongation in these patients (*Figure 4D, F,* and *J*). In contrast, $K_{2P}3.1$ blockade had little effect on APD_{90} in pAF patients (*Figure 4H* and *K*), in line with weak $K_{2P}3.1$ and APD remodelling. Of note, APD_{90} markedly exceeded SR levels in the presence of A293 when pAF and concomitant severe LVEF reduction were present (*Figure 4H*). Finally, a direct comparison of A293 effects between preserved LV function and mildly, moderately, or severely reduced LVEF irrespective of rhythm status indicated progressive attenuation of class III antiarrhythmic APD prolongation following $K_{2P}3.1$ blockade in patients with more advanced stages of LV dysfunction (Supplementary material online, *Figure S6*).

AP simulations confirm mechanistic significance of $K_{2P}3.1$ channels in atrial repolarization and antiarrhythmic potential of $K_{2P}3.1$ inhibition

We employed computational modelling to assess the causal role of $K_{2P}3.1$ channel remodelling in atrial repolarization. Four representative models of rhythm status and LV function that primarily affected $K_{2P}3.1$ levels and APD in patient-derived atrial myocytes (i.e. SR vs. cAF in combination with preserved vs. severely reduced LVEF) reproduced human $K_{2P}3.1$ current *I–V* relationships, showing upregulation of $K_{2P}3.1$ current in cAF and downregulation with severely reduced LVEF based on experimental voltage-clamp data (*Figure 5A*). Under baseline conditions reflecting intracellular and extracellular solutions used for experimental AP recordings, the four models with $K_{2P}3.1$ channel formulations exhibited distinct AP morphologies and duration (*Figure 5B* and *C*). Consistent with previous human and computational studies, cAF was associated with pronounced APD shortening.[9,11] Conversely, the model predicted that electrical remodelling associated with LV dysfunction results in APD prolongation (*Figure 5B* and *C*), in line with experimental AP recordings (*Figure 4G*).

Furthermore, simulated inhibition of $K_{2P}3.1$ channels prolonged APD at 50 and 90% repolarization in all four groups, with the latter being most pronounced (*Figure 5C*). APD prolongation was largest in the cAF group with preserved LV function and smallest in the SR group with severe LVEF reduction. Due to the opposing regulation of $K_{2P}3.1$ channels by both cardiovascular pathologies, cAF in combination with LV dysfunction showed intermediate prolongation in the model.

We investigated the role of $K_{2P}3.1$ channel remodelling in APD differences during steady-state pacing at various pacing frequencies (*Figure 5D*, solid lines) by comparing simulations incorporating all ionic changes for a given pathology to those omitting changes in $K_{2P}3.1$ current (i.e. employing 'SR with preserved LVEF' formulations of the $K_{2P}3.1$ current; *Figure 5D*, dashed lines). These simulations indicated that remodelling of $K_{2P}3.1$ plays a major role in APD shortening during cAF, both in the absence or presence of LV dysfunction, and a minor role in LV dysfunction-dependent APD prolongation in SR. In addition, the changes in APD are expected to modulate repolarization dynamics such as the occurrence of APD alternans (*Figure 5D*) and may contribute to atrial arrhythmogenesis. Similar results were obtained using the Courtemanche et al.[13] human atrial cardiomyocyte model (see Supplementary material online, *Figure S7*), indicating that the results are not model dependent.

Finally, the antiarrhythmic potential of $K_{2P}3.1$ current inhibition was investigated in multicellular simulations. An S1-S2 protocol induced 5980 ms of re-entry in virtual tissue with cAF-associated electrical properties (conduction velocity, 50 cm/s). In contrast, re-entry could not be induced under these conditions in the presence of $K_{2P}3.1$ inhibition (*Figure 5E;* Supplementary material online, *Videos S1 and S2*). In the setting of combined cAF and LV dysfunction, $K_{2P}3.1$ inhibition reduced re-entry duration from 4345 ms to 3075 ms (conduction velocity of 35 cm/s to simulate increased fibrosis and chosen to match re-entry duration in the cAF model), but did not prevent it (*Figure 5F;* Supplementary material online, *Videos S3 and S4*), suggesting reduced antiarrhythmic efficacy of $K_{2P}3.1$ current inhibition with LV dysfunction.

Discussion

$K_{2P}3.1$ K^+ channels regulate atrial electrophysiology in LV dysfunction and AF

The present study links downregulation of repolarizing $K_{2P}3.1$ channels to atrial AP prolongation that was identified as

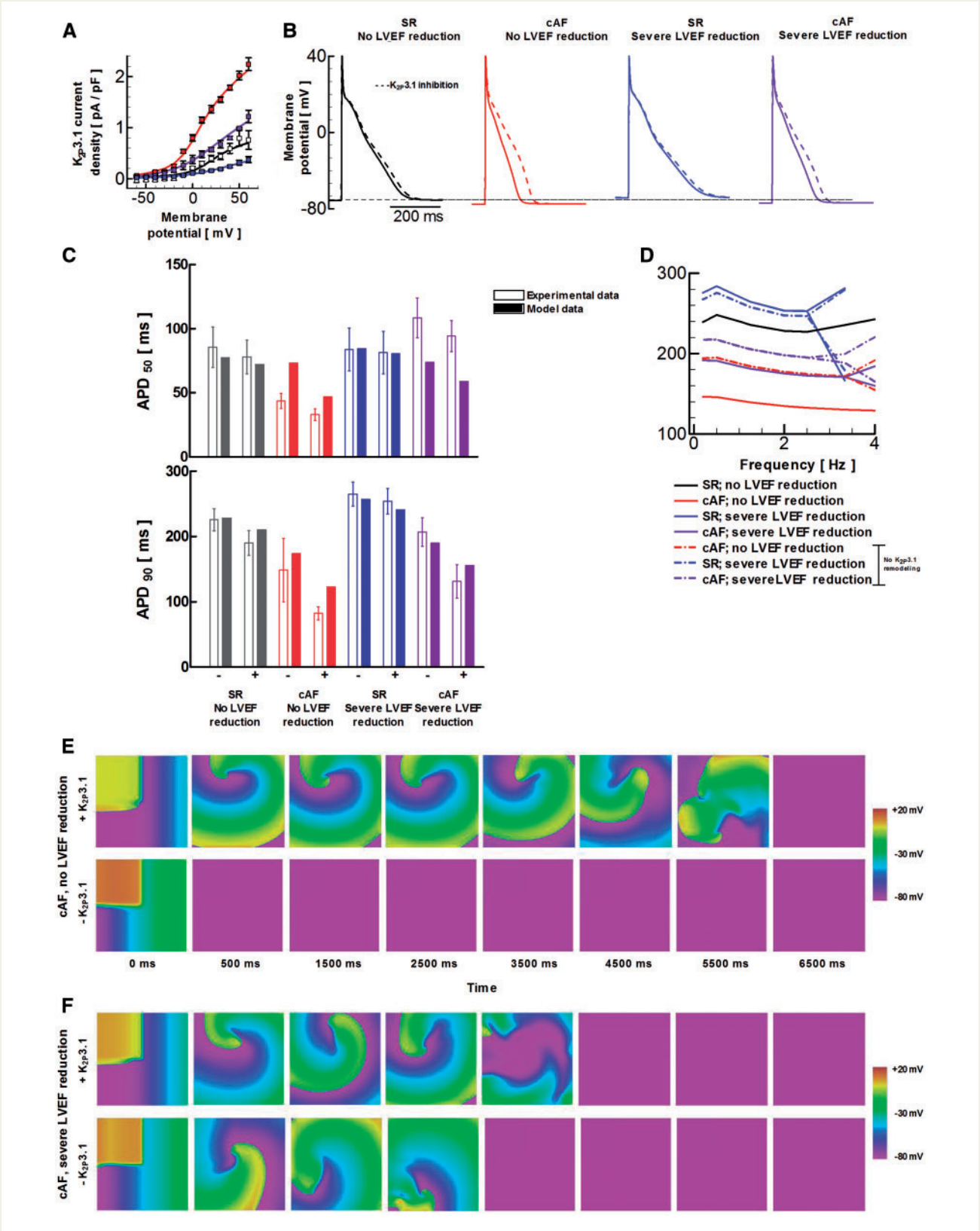

Figure 5 Computational analysis of $K_{2P}3.1$ channel remodelling. (*A*) Validation of $K_{2P}3.1$ model formulations for sinus rhythm (SR) in the absence of left ventricular (LV) dysfunction (SR; black color scheme), chronic atrial fibrillation (cAF) and preserved cardiac function (red), SR in the presence of severely reduced LV ejection fraction (LVEF) (blue), and cAF with concomitant severe LVEF reduction (purple), using A293-sensitive current-voltage relationships obtained from human atrial cardiomyocytes (data indicated by symbols). (*B*) Effects of the presence (solid lines) or absence (i.e.

electrophysiological characteristic of patients with impaired LVEF. Reduced atrial $K_{2P}3.1$ expression at mRNA, protein and functional levels in patients with impaired LV function is a novel key finding of this work and provides a mechanistic explanation of prolonged APD in atrial cardiomyocytes of HF patients.

Statistical analysis revealed that besides LV dysfunction, only cAF independently determined atrial $K_{2P}3.1$ levels among multiple demographic and clinical factors. In patients with preserved cardiac function, cAF was associated with increased $K_{2P}3.1$ and APD shortening, as demonstrated previously.[9] Given the common concurrence of AF and LV dysfunction, the net effect of cAF and LV dysfunction on $K_{2P}3.1$ abundance and APD is clinically relevant. The patient subgroup with concomitant functional LV impairment and cAF exhibited higher $K_{2P}3.1$ current levels and shorter APD compared with individuals with SR and normal LVEF, indicating a predominant electrophysiological effect of cAF over LV dysfunction.

The investigation further provides novel insights into atrial pathophysiology in pAF patients with concomitant LV dysfunction. Severe LVEF reduction was linked to reduced $K_{2P}3.1$ currents, resulting in a tendency towards APD prolongation. These findings were corroborated by our novel computational tool, which also adds mechanistic insight into the relative contribution of $K_{2P}3.1$, extends the data to a wider range of experimental conditions, shows the antiarrhythmic potential of $K_{2P}3.1$ inhibition, and provides a platform for future mechanistic and interventional studies. In contrast, pAF in the absence of LV dysfunction had no effect on either $K_{2P}3.1$ or APD. Thus, LV dysfunction emerges as primary determinant of atrial $K_{2P}3.1$ remodelling in pAF patients.

Implications for patient-specific antiarrhythmic management

The coexistence of LV dysfunction poses a clinically significant therapeutic challenge that is attributed to a distinct atrial substrate[2–4] that was here studied in detail. We elucidated differential remodelling of $K_{2P}3.1$ by LV dysfunction and cAF, indicating that $K_{2P}3.1$ constitutes a molecular marker of atrial electrical dysfunction that may enable more specific antiarrhythmic management in the future based on the underlying disease mechanism (*Figure 6*). The present study suggests that clinical factors LV dysfunction and AF type (pAF vs. cAF), and associated $K_{2P}3.1$ expression changes should be considered in antiarrhythmic therapy planning. Decreased $K_{2P}3.1$ and APD prolongation predict reduced effectiveness of $K_{2P}3.1$ blockade in LV dysfunction patients. Specifically, a patient subgroup characterized by LV dysfunction and pAF exhibited prolonged baseline APD and therefore may be less sensitive or resistant to anti-$K_{2P}3.1$ interventions that further prolong the atrial AP. In contrast, cAF patients with different stages of LV dysfunction consistently showed enhanced $K_{2P}3.1$ current and shortened APD, suggesting beneficial effects of $K_{2P}3.1$ K^+ current inhibition (*Figure 5*).[9] $K_{2P}3.1$ antagonists are currently not available for clinical application in humans. Therapeutic targeting of $K_{2P}3.1$

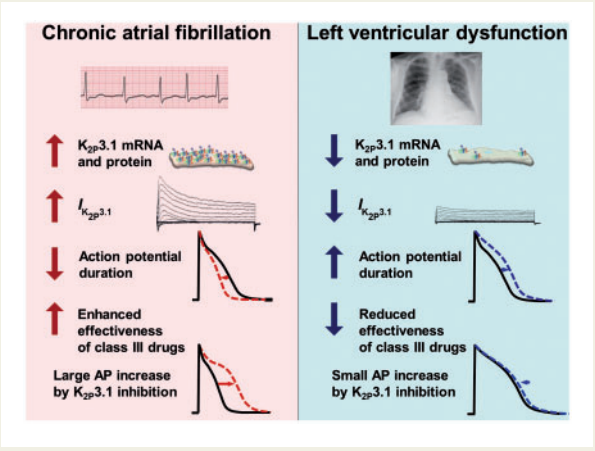

Figure 6 The role of $K_{2P}3.1$ K^+ channels in atrial arrhythmogenesis and antiarrhythmic therapy. Chronic atrial fibrillation (cAF) and left ventricular (LV) dysfunction antagonistically determine atrial action potential duration (APD) via changes in $K_{2P}3.1$ subunit abundance and current density. In chronic atrial fibrillation patients, increased $K_{2P}3.1$ levels accelerate action potential repolarization and shorten APD. In contrast, LV dysfunction is associated with reduced atrial $K_{2P}3.1$ expression and function, resulting in prolongation of repolarization and APD. Patient-specific remodelling of $K_{2P}3.1$ and APD affects antiarrhythmic therapy: action potential prolongation in patients with reduced LV function reduces effectiveness of $K_{2P}3.1$ blockade, whereas $K_{2P}3.1$ K^+ current inhibition is expected to be particularly effective in patients with cAF and shortened action potential.

complete pharmacological inhibition; dashed lines) of $K_{2P}3.1$ current on action potential (AP) morphology in respective computational models during 0.2 Hz pacing. (*C*) Effect of presence (+) or absence (inhibition; -) of $K_{2P}3.1$ current on AP duration (APD) at 50% (top) and 90% (bottom) repolarization in the model (filled bars) compared with experimental data (open bars). (*D*) Role of $K_{2P}3.1$ channel remodelling in rate-dependent APD changes. Steady-state APD_{90} at various pacing frequencies was determined for indicated models (solid lines), and in the absence of $K_{2P}3.1$ changes for three pathological settings (cAF; severely reduced LVEF; combined conditions cAF and severe LVEF impairment) to illustrate the contribution of $K_{2P}3.1$ (dashed lines). Rate-dependent APD shortening was incomplete for the SR with preserved LVEF model, due to impaired recovery of I_{Na} causing reduced upstroke of the AP and limiting voltage-dependent activation of repolarizing K^+ currents, resulting in a longer APD at a pacing frequency of 3.3 Hz compared with 2.5 Hz. (*E*) Snapshots of various time points of re-entry induced by an ectopic stimulus in the top-left quadrant at S1–S2 interval of 230 ms in virtual atrial tissue (8 cm × 8 cm) with cAF characteristics in the absence (+ $K_{2P}3.1$) or presence of $K_{2P}3.1$ inhibition (-$K_{2P}3.1$). (*F*) Similar to panel E for tissue with combined 'cAF and LV dysfunction' electrophysiological characteristics (S1–S2 interval, 290 ms).

channels that are predominantly expressed in human atria is expected to confer 'atrial selectivity' by limiting the electropharmacological action to atrial tissue, thereby reducing the risk of proarrhythmic effects in the ventricles. Further development of optimized $K_{2P}3.1$ inhibitors is warranted, with particular focus on mechanism-based antiarrhythmic treatment of patients with cAF and normal or reduced LVEF that are characterized by enhanced $K_{2P}3.1$ currents and shortened atrial APD.

Potential limitations

The assessment of atrial ionic remodelling was limited to RA and LA appendage tissue. Thus, extrapolation of the present results to other atrial regions cannot be readily supported by experimental data owing to limited availability of human tissue. In addition, the lack of freshly isolated LA cells precluded any direct electrophysiological assessment of LA $K_{2P}3.1$ currents and APD in this work. While altered ion channel expression at transcriptional and protein level in cardiac tissue may reflect other cell types in addition to cardiomyocytes, electrophysiological recordings provide confirmation of functional $K_{2P}3.1$ and APD remodelling in atrial myocytes. $K_{2P}17.1$ channels exhibited significant atrial expression and downregulation in patients with cAF and LV dysfunction, suggesting a potential contribution to atrial ionic remodelling. However, the lack of a specific $K_{2P}17.1$ inhibitor precluded the functional analysis of the relative $K_{2P}17.1$ contribution to atrial AP regulation. In addition to electrical remodelling, structural alterations of atrial tissue may contribute to the initiation and maintenance of AF. Structural remodelling was not specifically addressed as the present study focused on the role of $K_{2P}3.1$ current dysregulation in electrical remodelling. Prior to translation of the present findings into clinical application, inter-subject and time-dependent variability of $K_{2P}3.1$ expression remain to be studied. Finally, clinical antiarrhythmic efficacy of $K_{2P}3.1$ inhibition needs to be investigated in future studies.

Conclusions

The present study identifies LV dysfunction as a clinical key factor in remote remodelling of atrial electrophysiology. LV dysfunction and cAF inversely determine atrial AP duration through functional regulation of $K_{2P}3.1$ K^+ current levels. Specific $K_{2P}3.1$ blockade exerted cellular class III antiarrhythmic effects in patients with cAF irrespective of LV function, while in pAF subjects with concomitant impairment of LV function APD prolongation exceeded normal levels observed among individuals with SR. Mechanistic findings from this work may serve to guide and optimize future, individualized antiarrhythmic therapy planning: cAF patients characterized by shortened APD and increased atrial $K_{2P}3.1$ levels are predicted to benefit from the use of anti-$K_{2P}3.1$ interventions for rhythm control.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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