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**Chronic beta-adrenoceptor blockade and human atrial cell electrophysiology: evidence of**

**pharmacological remodelling. Objective:** Chronic beta-adrenoceptor antagonist ( $\beta$ -blocker) treatment reduces the incidence of reversion to AF in patients, possibly via an adaptive myocardial response. However, the underlying electrophysiological mechanisms are presently unclear. We aimed to investigate electrophysiological changes in human atrial cells associated with chronic treatment with  $\beta$ -blockers and other cardiovascular-acting drugs. **Methods:** Myocytes were isolated enzymatically from the right atrial appendage of 40 consenting patients who were in sinus rhythm. The cellular action potential duration (APD), effective refractory period (ERP), L-type  $\text{Ca}^{2+}$  current ( $I_{\text{CaL}}$ ), transient ( $I_{\text{TO}}$ ) and sustained ( $I_{\text{KSUS}}$ ) outward  $\text{K}^{+}$  currents, and input resistance ( $R_i$ ) were recorded using the whole cell patch clamp. Drug treatments and clinical characteristics were compared with electrophysiological measurements using simple and multiple regression analyses.  $P < 0.05$  was taken as statistically significant. **Results:** In atrial cells from patients treated chronically with  $\beta$ -blockers, the  $\text{APD}_{90}$  and ERP (75 beats/min stimulation) were significantly longer, at  $213 \pm 11$  and  $233 \pm 11$  ms, respectively ( $n=15$  patients), than in cells from non- $\beta$ -blocked patients, at  $176 \pm 12$  and  $184 \pm 12$  ms ( $n=11$ ). These cells also displayed a significantly reduced action potential phase 1 velocity ( $22 \pm 3$  vs  $34 \pm 3$  V/s). Chronic  $\beta$ -blockade was also associated with a significant reduction in the heart rate ( $58 \pm 3$  vs  $69 \pm 5$  beats/min) and in the density of  $I_{\text{TO}}$  ( $8.7 \pm 1.3$  vs  $13.7 \pm 2.1$  pA/pF), an increase in the  $R_i$  ( $214 \pm 24$  vs  $132 \pm 14$  M $\Omega$ ), but no significant change in  $I_{\text{CaL}}$  or  $I_{\text{KSUS}}$ . The  $I_{\text{TO}}$  blocker 4-aminopyridine largely mimicked the changes in phase 1 and ERP associated with chronic  $\beta$ -blockade, in cells from non- $\beta$ -blocked patients. Chronic treatment of patients with calcium channel blockers or angiotensin converting enzyme inhibitors ( $n=11-13$  patients) was not associated with any significant changes in atrial cell electrophysiology. **Conclusion:** The observed atrial cellular electrophysiological changes associated with chronic  $\beta$ -blockade are consistent with a long term adaptive response, a type of “pharmacological remodelling”, and provide mechanistic evidence supportive of the anti-arrhythmic actions of  $\beta$ -blockade.

**Keywords:** Discipline: Experimental; Objective: Heart; Level: Cellular; Field: Electrophysiology, Pathophysiology, Pharmacology. Additional keywords: Antiarrhythmic agents; Arrhythmia (mechanisms); Remodeling; Myocytes; Ion channels.

## **Introduction**

Atrial fibrillation (AF) is the most common sustained cardiac rhythm disturbance. It is associated with a substantial decrease in the quality and expectancy of life, and is becoming more prevalent [1]. Beta-adrenoceptor antagonists ( $\beta$ -blockers) have a well established efficacy for controlling the ventricular rate during AF, by slowing atrioventricular conduction.  $\beta$ -blockade is also recognised to reduce the recurrence of adrenergically-mediated AF, for example after cardiothoracic surgery [2]. However, evidence is accumulating that  $\beta$ -blockade may also exert anti-arrhythmic actions in other groups of patients with AF. Metoprolol was more effective than placebo [3], and bisoprolol was as effective as sotalol [4], in preventing relapse into AF after cardioversion. Additionally, atenolol reduced the incidence and duration of paroxysmal AF [5].

The mechanisms of these  $\beta$ -blocker effects are presently unclear, but may involve a long term adaptive change in myocardial electrophysiology. Animal studies have indicated that chronic  $\beta$ -blockade prolonged the action potential duration (APD) in atria and ventricles [6]. However, the effects of chronic  $\beta$ -blockade on human myocardial repolarisation are presently unclear, with conflicting reports in the ventricle (lengthening [7] and shortening [8]) and no available data, to our knowledge, in the atrium.

AF is maintained by intra-atrial reentry, facilitated by a shortened atrial effective refractory period (ERP). Atrial electrophysiological remodelling due to AF contributes to stabilisation of the arrhythmia, and is associated with shortening of the APD and ERP, as confirmed recently in our laboratory in human atrial myocytes [9]. It is conceivable, therefore, that the anti-fibrillatory effects of  $\beta$ -blockers might involve, through an adaptive response to their chronic use, a prolongation of the atrial cell APD and ERP. We wished to test this hypothesis, examine potential ionic mechanisms involved, and assess, for comparison, electrophysiological changes associated with chronic use of other drugs acting on the cardiovascular system.

The objective, therefore, was to investigate changes in human atrial cell action potentials, ERP and ion currents associated with chronic use of  $\beta$ -blockers and other cardiovascular-acting drugs.

## Methods

### *Cell isolation*

Right atrial appendage tissue was obtained from 40 patients undergoing corrective cardiac surgery (Table 1). Procedures approved by the institutional research ethics committee were followed, and each patient's informed consent was obtained. The investigation conforms with the principles outlined in the Declaration of Helsinki [10]. Atrial cells were isolated enzymatically with protease (Type XXIV, Sigma, 4U/ml) and collagenase (Type 1, Worthington, 400 U/ml) using the method described previously [9].

### *Electrical recording*

Action potentials and ion currents were recorded using the whole cell patch clamp technique, with an Axopatch-1D amplifier (Axon Instruments). Cells were superfused at 37°C with a physiological salt solution containing (mM): NaCl (130.0), KCl (4.0), CaCl<sub>2</sub> (2.0), MgCl<sub>2</sub> (1.0), glucose (10.0) and HEPES (10.0). The pipette solution contained (mM): K-aspartate (110.0), KCl (20.0), MgCl<sub>2</sub> (1.0), EGTA (0.15), Na<sub>2</sub>ATP (4.0), Na<sub>2</sub>GTP (0.4) and HEPES (5.0). Series resistance and capacitive transients were compensated electronically prior to recording. Action potentials were stimulated using 5 ms duration current pulses of 1.2x threshold strength, after current clamping the resting potential to -75 mV and keeping the holding current constant thereafter. The ERP was measured using a standard S<sub>1</sub>-S<sub>2</sub> stimulation protocol, with an 8-pulse conditioning train (75 beats/min), and S<sub>1</sub> and S<sub>2</sub> pulses of equal magnitude. The ERP was defined as the longest S<sub>1</sub>-S<sub>2</sub> interval failing to elicit an S<sub>2</sub> action potential of amplitude >80% of the preceding S<sub>1</sub> action potential. The APD was calculated as the interval between the action potential upstroke and repolarisation to the level of both 50 and 90% (APD<sub>50</sub> and APD<sub>90</sub>, respectively). The voltage clamp was used to record the cells' input resistance (R<sub>i</sub>), the L-type Ca<sup>2+</sup> current (I<sub>CaL</sub>), the transient outward K<sup>+</sup> current (I<sub>TO</sub>) and the sustained outward K<sup>+</sup> current (I<sub>K<sub>SUS</sub></sub>). R<sub>i</sub> was determined using a linear voltage ramp (increasing from -120 mV to +50 mV in 7 s) from the slope of the ohmic portion of the resulting current-voltage relationship, usually between -120 mV and -100 mV. I<sub>CaL</sub> was stimulated from a holding potential of -40 mV, with 100 ms pulses (0.33 Hz), increasing from -30 mV to +50 mV in 10 mV steps. I<sub>CaL</sub> was measured at each voltage step as the peak inward deflection minus the current at the end of the pulse. I<sub>TO</sub> and I<sub>K<sub>SUS</sub></sub> were stimulated from a holding potential of -60 mV, with 100 ms pulses (0.33 Hz), increasing from -50 mV to +60 mV in 10 mV steps. I<sub>K<sub>SUS</sub></sub> was measured as the steady-state (end-pulse) current, and I<sub>TO</sub> as the peak positive deflection

minus the end-pulse current at each voltage step. The same electrode filling solution was used for recording action potentials and ion currents, permitting their comparison under constant ionic conditions, and often in the same cell. Contamination of  $I_{CaL}$  and  $I_{TO}$  by  $K^+$  currents was minimised as far as possible, by the subtraction of the steady-state currents. Each current's density was calculated as the peak amplitude (ie: at +10 mV for  $I_{CaL}$ , +60 mV for  $I_{TO}$  and +60 mV for  $I_{KSUS}$ ) divided by the cell's capacity, prior to the statistical analyses and graphing of mean data. Action potentials and ion currents were stimulated, recorded and measured using "WinWCP" software, donated by J Dempster, Strathclyde University.

### ***Data analysis and statistics***

Details of each patient's clinical characteristics and drug treatments were obtained from the case notes. The cardiac rhythm and heart rate were assessed from the pre-operative electrocardiogram. Only those patients in sinus rhythm at the time of surgery were included. Patient and associated cellular electrophysiological data were stored in a database (Access, Microsoft) for subsequent analysis. Univariate electrophysiological measurements were compared between pairs of various subgroups of patients using 2-sided, 2-sample unpaired Student's *t* tests. Univariate data are expressed as cell means  $\pm$  standard error (SE). Multiple linear regression [11] was then used to further investigate associations between patients' atrial electrophysiology and drug treatments or clinical characteristics. This provided an estimate of the difference in each electrophysiological measurement between the levels of the factor of interest, according to 8 variables considered to be of particular importance. These were the patients' drug treatments ( $\beta$ -blockers, calcium channel blockers [CCBs] and angiotensin converting enzyme [ACE] inhibitors), age (for a 5 year increase), sex, heart rate (for a 5 beats/min increase), and the presence or absence of coronary artery bypass graft (CABG) surgery or previous myocardial infarction. Two multiple regressions were fitted: firstly, by taking into account all covariables, and secondly (when comparing patients taking a drug with those not), by taking as covariables only those drugs additional to the one under consideration. All analyses were performed using "SAS 8.2 for Windows NT" software. No adjustment was made for multiple comparisons.  $P < 0.05$  was regarded as statistically significant.

## Results

### *Patients' drug treatments*

The patients' characteristics are shown in Table 1. Drugs were administered chronically, with each patient receiving their routine cardiac drugs on the day of surgery. All patients undergoing  $\beta$ -blockade were treated with cardiac selective  $\beta_1$ -adrenoceptor antagonists, with 88% on atenolol, 8% on metoprolol and 4% on bisoprolol. No patients were administered sotalol (which has additional class III activity). Of the patients treated with CCBs, 33% were receiving diltiazem, a predominantly cardiac-acting drug. The remaining 67% were taking drugs with mixed vascular and cardiac actions, but with vascular activity predominating, including amlodipine (44%), nifedipine (17%) and nicardipine (6%). Twelve patients were taking both  $\beta$ -blockers and CCBs. The ACE inhibitors administered included lisinopril (50%), captopril (21%), ramipril (21%) and enalapril (7%). Of the 14 patients on ACE inhibitors, 9 were on  $\beta$ -blockers, and 6 on CCBs. All cardio-active drugs had been administered for a minimum duration of 3 months.

### *Atrial cellular electrophysiological changes associated with chronic $\beta$ -blockade*

Figure 1A shows original action potential traces and ERP measurements, from single atrial cells representative of patients not treated (top trace) and treated (bottom trace) with  $\beta$ -blockers. Chronic  $\beta$ -blockade was associated with a lengthening of both the ERP and late repolarisation. Univariate analysis (simple regression) indicated that the mean APD<sub>90</sub> and ERP were markedly and significantly longer in atrial cells from the patients receiving  $\beta$ -blockers, at  $213 \pm 11$  ms ( $n=21$  cells, 15 patients) and  $233 \pm 11$  ms ( $n=16$  cells, 11 patients), respectively, than in the non-treated patients, at  $176 \pm 12$  ms ( $n=20$  cells, 11 patients) and  $184 \pm 12$  ms ( $n=17$  cells, 10 patients), respectively (Figure 1B). The univariate means within each patient group, and the probability values of differences between groups, were similar whether analysed at cell or subject (patient) level. The changes in APD and ERP associated with chronic  $\beta$ -blockade were independent of cell capacity or holding current, each of which were similar in  $\beta$ -blocker-treated and non-treated patients, at  $74 \pm 4$  pF ( $n=36$  cells, 21 patients) vs  $81 \pm 3$  pF ( $n=28$  cells, 15 patients,  $P=0.22$ ), and  $57 \pm 7$  pA ( $n=30$  cells, 18 patients) vs  $60 \pm 7$  pA ( $n=16$  cells, 9 patients,  $P=0.70$ ), respectively. The mean action potential overshoot, amplitude and maximum upstroke velocity ( $V_{\max}$ ) were not significantly different between the cells from patients receiving  $\beta$ -blockers ( $48 \pm 2$  mV,  $126 \pm 3$  mV and  $149 \pm 16$  V/s;  $n=21$  cells, 15 patients, respectively)

and those who were not ( $53\pm 2$  mV,  $130\pm 2$  mV and  $176\pm 15$  V/s;  $n=20$  cells, 11 patients, respectively). The heart rate was significantly slower in the patients undergoing  $\beta$ -blockade ( $58\pm 3$  vs  $69\pm 5$  beats/min;  $P=0.02$ ,  $n=25$  and 15, respectively). Multiple regression analysis was used to further investigate associations between patients' characteristics and electrophysiological changes. Table 2 provides an example of this analysis with respect to the atrial cell ERP. With ACE inhibitors and CCBs taken as covariables ("Drugs"), the direction, magnitude and probability value of the estimated difference in ERP between  $\beta$ -blocker-treated and non-treated patients were similar to that found without adjusting for these covariables. When all covariates were considered ("All"), the direction and approximate magnitude of the difference were again maintained, although the difference was no longer significant. A similar analysis of the  $APD_{90}$  also provided data supportive of the univariate association between  $\beta$ -blockade and APD-lengthening, with an estimated prolongation of 40 ms with drugs as covariables. Simple and multiple regression analysis revealed no significant associations between atrial cell ERP and the patient's age, heart rate, or presence of previous myocardial infarction (Table 2). However, simple (but not multiple) regression indicated that both CABG surgery and male sex were associated with prolongation of the ERP.

The amplitude and density of  $I_{TO}$  were markedly smaller in atrial cells from the patients treated with  $\beta$ -blockers than in those from untreated patients, as shown by the representative traces of Figure 2A, and the univariate analysis in Figure 2D. Additionally, the  $R_i$  was higher in the atrial cells from patients who underwent  $\beta$ -blockade than in untreated patients. This is illustrated in Figure 2C (lower trace), by the relatively shallow slope of the ohmic portion of the current-voltage relationship, and confirmed by univariate analysis (Figure 2D). The tangent to the ohmic portion intersected zero current at  $-81\pm 3$  mV ( $n=15$  cells), compared with the predicted  $K^+$  equilibrium potential of -93 mV. This suggested that  $R_i$  was contributed to by the inward rectifier  $K^+$  current ( $I_{K1}$ ). The density of atrial  $I_{KSUS}$  was not significantly different between patients who were, and who were not, treated with  $\beta$ -blockers, at  $8.81\pm 1.74$  pA/pF ( $n=10$  cells, 7 patients) and  $7.37\pm 1.14$  pA/pF ( $n=8$  cells, 4 patients, respectively;  $P=0.77$ ), as represented in Figure 2A. The density of  $I_{CaL}$  also was not significantly different between treated and untreated patients, at  $-6.76\pm 1.14$  pA/pF ( $n=17$  cells, 8 patients) and  $-4.77\pm 0.99$  pA/pF ( $n=6$  cells, 4 patients, respectively ( $P=0.34$ ), as represented in Figure 2B. These data for  $I_{TO}$ ,  $R_i$ ,  $I_{KSUS}$  and  $I_{CaL}$  were supported by multiple regression: with the "Drugs" model, the estimated magnitude of the reduction in  $I_{TO}$  was 6.9 pA/pF ( $P=0.03$ ). In this analysis, the magnitude (but not

the probability value) of the increase in  $R_i$  was also maintained. The absence of significant change in  $I_{CaL}$  and  $I_{KSUS}$  was also confirmed, whether the analysis incorporated all of the covariables, or the drugs alone. Since a reduction in atrial  $I_{TO}$  has also been associated with congestive heart failure [12], a sub-analysis of electrophysiological data was performed in patients with and without significant (moderate or severe) left ventricular dysfunction (LVD). Eleven of the 25  $\beta$ -blocker-treated patients (44%) had significant LVD, and 4 of the 15 non- $\beta$ -blocker-treated patients (27%) had significant LVD. There was no significant difference in the proportion of patients with significant LVD between the two patient sub-groups ( $P=0.28$ ). Within both of these sub-groups, there was no significant difference between patients with and without LVD, in the atrial cell ERP,  $APD_{90}$ ,  $I_{TO}$  or  $R_i$ .

The potential contribution of the observed reduction in atrial  $I_{TO}$  to associated changes in repolarisation and refractoriness was evaluated. Chronic  $\beta$ -blockade, in addition to prolonging the ERP (Figure 1), was associated with a significant slowing of the action potential maximum downstroke velocity, phase 1  $V_{max}$  (Figure 3A). The  $I_{TO}$  blocker 4-aminopyridine (4-AP) produced a similar reduction in phase 1  $V_{max}$  in cells from non- $\beta$ -blocker-treated patients (Figure 3B), which was also accompanied by a significant prolongation in the ERP (Figure 3C).

#### ***Lack of electrophysiological changes associated with calcium channel blockade or ACE inhibition***

Chronic calcium channel blockade or ACE inhibition was not associated with any significant changes in the APD or ERP, as indicated by univariate mean data, shown in Figure 4. The action potential overshoot, amplitude and  $V_{max}$  were  $49\pm 2$  mV,  $128\pm 2$  mV and  $160\pm 17$  V/s, respectively, in atrial cells from patients not treated with CCBs, and  $51\pm 2$  mV,  $128\pm 3$  mV and  $164\pm 15$  V/s, respectively, in cells from patients not treated with ACE inhibitors. Chronic treatment with either drug was not significantly associated with a change in any of these values. A comparison of data from patients receiving CCBs acting predominantly on the myocardium with those exerting mixed actions but mainly affecting the vasculature, indicated no significant differences in atrial cell electrophysiology between the drug sub-types. For example, the ERP in patients receiving cardiac- and vascular-acting drugs was  $223\pm 13$  and  $201\pm 26$  ms, respectively, not significantly different from each other or from the combined mean ( $214\pm 13$  ms). There were also no significant differences in the magnitudes of  $I_{CaL}$ ,  $I_{TO}$ ,  $I_{KSUS}$ , or the  $R_i$  between patients treated and not treated with either CCBs or ACE inhibitors. Multiple regression analysis supported the lack of association between action

potential configuration, duration, the ERP, ion currents, and treatment with either CCBs or ACE inhibitors. Table 2 provides an example of this, indicating a lack of statistical significance of any estimated difference in the ERP between non-treated and treated patients, at either level of analysis, ie: with drugs alone, or with consideration of all covariates.

## Discussion

We report the novel finding that chronic  $\beta$ -blockade was associated with prolongation of action potentials and the ERP in human atrial cells. These changes represent an adaptive response, a type of “pharmacological remodelling”, whereby chronic blockade of the  $\beta$ -adrenoceptors resulted in alterations of ion current density, recorded independently of receptor occupancy, ie: in the absence of drugs. This response may contribute to the anti-fibrillatory actions of  $\beta$ -blockade by prolonging refractoriness, and thus lengthening the minimum path length required for reentry. Moreover, during  $\beta$ -blocker therapy, such actions may be enhanced by the presence of the drug itself and would also persist in patients who miss a dose, which may offset the potentially pro-arrhythmic effects of increased atrial sensitivity to catecholamines and 5-HT [13-15].

Our results are the first, to our knowledge, to be obtained from human atrium, and are consistent with previous studies in animals [6,16]. Thus, chronic metoprolol treatment in rabbits lengthened the atrial APD, as well as the ventricular APD and ERP, and the QT<sub>c</sub> interval [6], and the atrial effects were similar in magnitude to those reported here. In humans, only effects on the ventricle have been studied, and chronic  $\beta$ -blockade prolonged both QT interval and monophasic action potentials [7,17] especially during exercise [18]. However, an absence of change [19,20] or even a shortening [8], in QT interval or ERP have also been reported. Differences between human atrial and ventricular responses may be expected, in view of, for example, the presence of a sustained outward current in human atrium but not ventricle, and the relatively slow recovery kinetics of atrial I<sub>TO</sub> [21]. Additionally, pharmacological remodelling by  $\beta$ -blockade in the rabbit was more rapid in the atrium than in the ventricle [6].

The present study also addressed ionic changes underlying the effects of chronic  $\beta$ -blockade in human myocardium. We demonstrated a reduction in atrial I<sub>TO</sub> associated with chronic  $\beta$ -blockade, and provided evidence to suggest that this may be a contributory ionic mechanism for the lengthening of the action potential and ERP. Thus, the velocity of phase 1, which is dependent on the magnitude of both I<sub>TO</sub> and I<sub>CaL</sub>, was reduced by chronic  $\beta$ -blockade, in the absence of a change in I<sub>CaL</sub>. Furthermore,  $\beta$ -blocker-induced changes in both phase 1 and ERP were largely mimicked, in cells from non- $\beta$ -blocker-treated patients, by the I<sub>TO</sub> blocker, 4-AP. However, it is recognised that 4-AP, whilst the most specific I<sub>TO</sub> blocker currently available, also blocks the ultra-rapid delayed rectifier K<sup>+</sup> current, I<sub>Kur</sub>, at concentrations well below those

required to block  $I_{TO}$ , and, therefore, that the effects of 4-AP on both the APD and ERP should be interpreted with caution. A further potential limitation is that a reduced density of atrial  $I_{TO}$  has been demonstrated in dogs with congestive heart failure, associated with an increase in cell capacity, but with no change in APD at physiological rate [12]. In the present study, however, the effects of chronic  $\beta$ -blockade to reduce  $I_{TO}$  density were independent of cell capacity, which was similar in treated and untreated groups. Moreover, the atrial cell APD, ERP,  $I_{TO}$  density and  $R_i$  were all similar in the patients with and without left ventricular dysfunction. Chronic  $\beta$ -blockade was associated with an increase in atrial  $R_i$ , which may be expected to have been due, at least in part, to a decrease in  $I_{K1}$ . However, it is acknowledged that  $I_{K1}$  was not measured directly, and that a change in  $I_{K1}$  might have occurred within the physiological voltage range, rather than at the potentials at which changes in  $R_i$  were observed. Nevertheless, our data raise the possibility that  $I_{K1}$  may decrease as a result of chronic  $\beta$ -blockade, and as such, a contribution to the associated lengthening in the APD and ERP cannot be excluded.

The mechanisms of these electrophysiological changes associated with chronic  $\beta$ -blockade are presently unclear. A chronically slowed heart rate by  $\beta$ -blockade might lengthen the atrial APD and ERP recorded at constant stimulation rate, analogous to their shortening resulting from chronic fast atrial rate [9]. However, the multiple regression analysis indicated that the observed prolongation of APD and ERP was independent of heart rate. An alternative mechanism is a direct regulation of expression of ion channels by chronic  $\beta$ -receptor modulation. Chronic  $\beta$ -blockade alone has not been studied, but chronic  $\beta$ -stimulation has been shown to alter ion channels, via mechanisms including cAMP-regulated modification of transcription [22,23]. In guinea pig cultured ventricular myocytes, exposure to isoproterenol for 48 hours reduced the density of  $I_{K1}$ ,  $I_{CaL}$ , and the delayed rectifier current  $I_{Ks}$ , which was reversed by co-incubation with propranolol [22]. Additionally, chronic  $\beta$ -stimulation increased the density of  $I_{TO}$  in canine cultured ventricular myocytes [24], and an increase in both  $I_{TO}$  and  $I_{K1}$  occurred during sympathetic innervation of newborn rat hearts [25]. If one speculated that chronic  $\beta$ -blockade might produce the opposite effects, then a reduced  $I_{TO}$  and  $I_{K1}$ , as reported here, would be predicted by some [24,25], but not all [22], studies.

Chronic calcium channel blockade or ACE inhibition were not associated with adaptive changes in atrial ion currents, action potentials or the ERP. Human atrial  $I_{CaL}$  was increased by acute ACE inhibition [26], but no studies could be found with chronic ACE inhibition. In a single report of effects of chronic CCB

treatment on human atrial electrophysiology [26], a reduction in both the  $APD_{50}$  and  $I_{CaL}$  was observed. Whilst the ERP was unchanged, consistent with our study, it is unclear why our results differ with respect to  $APD_{50}$  and  $I_{CaL}$ . Animal studies of chronic calcium channel blockade have shown an absence of change in several myocardial electrophysiological and related measurements, including reports of no change [27], and even an increase [28] in  $I_{CaL}$ . Consistent with the presently observed absence of associated atrial electrophysiological changes, neither chronic calcium channel blockade nor ACE inhibition has a proven efficacy in the treatment of AF [29].

Pharmacological intervention is the mainstay of current treatment for AF. However, the use of anti-arrhythmic agents with established efficacy for treating AF, ie: from classes 1C and III, may be precluded by the presence of associated heart disease and risk of serious side effects [29]. The role of  $\beta$ -blockers in the treatment of patients with AF is becoming increasingly recognised [3-5]. The presently observed atrial cellular adaptive electrophysiological responses to chronic  $\beta$ -blockade provide mechanistic evidence supportive of the anti-arrhythmic actions of  $\beta$ -blockade.

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## Table and Figure legends

### Table 1. Patients' characteristics.

Values are numbers of patients (*n* and % of total, respectively) with selected clinical characteristics, except for age (mean±SE). CCB=calcium channel blocker, CABG=coronary artery bypass graft surgery, AVR=aortic valve replacement, ASD=atrial septal defect, VSD=ventricular septal defect, LV=left ventricular, MI=myocardial infarction. All patients were in sinus rhythm at the time of surgery.

### Table 2. Multiple regression analysis of the atrial cell refractory period.

ERP=effective refractory period, ACEI=ACE inhibitors, BB=β-blockers, HR=heart rate. The model is either "Alone" (univariate), "Drugs" (adjusting for all drugs) or "All" (adjusting for all covariates). ERP estimate=estimated difference in mean ERP between highest (eg: drug-treatment) and lowest (eg: non-drug-treatment) level of variable, or male minus female. Values are subject (patient) means±standard error (SE); \*=*P*<0.05.

### Figure 1.

Atrial cell action potential and refractory period changes associated with chronic β-blocker treatment.

A, Superimposed action potentials in response to the 7th and 8th of a train of 8 conditioning, *S*<sub>1</sub>, pulses (basic rate: 75 beats/min) followed by responses to an increasingly premature test, *S*<sub>2</sub>, pulse ( in an atrial cell from a patient not treated (○) and treated (●) with a β-blocker. The effective refractory period (ERP), indicated in each panel by a solid bar, was calculated as the longest *S*<sub>1</sub>-*S*<sub>2</sub> interval failing to elicit an *S*<sub>2</sub> response of amplitude >80% of the preceding *S*<sub>1</sub> action potential. In each case, the *S*<sub>2</sub> response thus used to measure this interval is labelled (⚡). B, Mean (±SE) atrial cell action potential duration measured at 50 and 90% repolarisation (APD<sub>50</sub> and APD<sub>90</sub>, respectively) and ERP in cells from patients not undergoing (□: *n*=17-20 cells, 10-11 patients) or undergoing (!: *n*=16-21 cells, 11-15 patients) chronic β-blockade. \*=*P*<0.05 between patient groups.

**Figure 2.**

Ion current changes associated with chronic  $\beta$ -blockade.

A, Transient outward  $K^+$  currents,  $I_{TO}$  (positive deflections), and sustained outward  $K^+$  currents,  $I_{KSUS}$  (end-pulse currents); B, L-type  $Ca^{2+}$  currents,  $I_{CaL}$  (negative deflections); C, Pseudo steady-state current-voltage relationships, in cells from patients not treated (○) and treated (●) with a  $\beta$ -blocker. D, Mean ( $\pm$ SE)  $I_{TO}$  peak density and cell input resistance,  $R_i$ , in cells from non- $\beta$ -blocker-treated patients (□:  $n=8$  cells, 4 patients for  $I_{TO}$  and 28 cells, 13 patients for  $R_i$ , respectively) or treated patients (!:  $n=10$  cells, 7 patients for  $I_{TO}$  and 36 cells, 18 patients for  $R_i$ , respectively).  $*=P<0.05$  between patient groups.

**Figure 3.**

Contribution of transient outward current to chronic  $\beta$ -blocker effects on action potentials and refractoriness.

A, Atrial action potential phase 1 maximum downstroke velocity (Phase 1  $V_{max}$ ) in cells from patients not treated (□:  $n=17$  cells, 10 patients) or treated (!:  $n=16$  cells, 11 patients) with  $\beta$ -blockers. B, Phase 1  $V_{max}$ , and C, Effective refractory period (ERP) in cells from non- $\beta$ -blocker-treated patients, in the absence (open bars) and presence (striped bars) of 2 mM 4-aminopyridine (90 s superfusion;  $n=4$  cells, 2 patients). All values in A-C are means ( $\pm$ SE), with asterisks denoting  $P<0.05$  between groups.

**Figure 4.**

Lack of change in APD or ERP associated with chronic calcium channel blockade or ACE inhibition.

Mean ( $\pm$ SE) atrial cell action potential duration measured at 50 and 90% repolarisation (APD<sub>50</sub> and APD<sub>90</sub>, respectively) and effective refractory period (ERP) in cells from patients not treated (open bars,  $n=17-21$  cells, 12-13 patients) or treated (hatched bars,  $n=18-20$  cells, 11-13 patients) with A, Calcium channel blockers, or B, ACE inhibitors. NS= $P>0.05$ .

**Table 1**

		<i>n</i>	%
Patient details	Male/female	32/8	80/20
	Age (years)	58±2	-
Drug treatments	Lipid lowering	27	68
	β-blocker	25	63
	Nitrate	25	63
	CCB	18	45
	ACE inhibitor	14	35
	Diuretic	9	23
	Digoxin	2	5
Operation type	CABG	30	75
	AVR	4	10
	AVR+CABG	3	7
	ASD repair	2	5
	VSD repair	1	3
LV function	Normal	19	47
	Mild/moderate	17	43
	Severe dysfunction	4	10
Disease	Angina	33	82
	Hyperlipidaemia	26	65
	Hypertension	18	45
	Previous MI	16	40
	Diabetes	4	10

**Table 2**

Variable	Multiple regression model	ERP estimate (ms)	ERP SEM	<i>P</i>
ACEI	Alone	+13.7	22.1	0.54
	Drugs	+11.3	18.9	0.56
	All	-9.3	18.1	0.62
BB	Alone	+55.8	18.1	0.01*
	Drugs	+55.8	18.7	0.01*
	All	+38.5	21.4	0.09
CCB	Alone	+10.5	22.0	0.64
	Drugs	+13.7	18.7	0.47
	All	+15.1	17.2	0.40
Age	Alone	0.0	4.6	0.99
	All	-4.8	3.9	0.24
Sex	Alone	+86.8	19.9	0.01*
	All	+51.9	45.3	0.27
CABG	Alone	+73.2	19.8	0.01*
	All	+50.1	44.4	0.28
MI	Alone	+13.3	22.6	0.56
	All	-20.1	18.8	0.30
HR	Alone	-4.4	3.1	0.16
	All	-0.8	2.6	0.76

Figure 1







