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The contribution of ionic currents to changes in refractoriness of human atrial myocytes associated with chronic atrial fibrillation

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Abstract

Antony J Workman, Kathleen A Kane, Andrew C Rankin. The contribution of ionic currents to changes in refractoriness of human atrial myocytes associated with chronic atrial fibrillation. **Objective:** To investigate changes in human atrial single cell functional electrophysiological properties associated with chronic atrial fibrillation (AF), and the contribution to these of accompanying ion current changes. **Methods:** The whole cell patch clamp technique was used to record action potentials, the effective refractory period (ERP) and ion currents, in the absence and presence of drugs, in enzymatically isolated myocytes from 11 patients with chronic (>6 months) AF and 39 patients in sinus rhythm. **Results:** Stimulation at high rates (up to 600 beats/min) markedly shortened late repolarisation and the ERP in cells from patients in sinus rhythm, and depolarised the maximum diastolic potential (MDP). Chronic AF was associated with a reduction in the ERP at physiological rate (from 203±16 to 104±15 ms, $P<0.05$), and marked attenuation in rate effects on the ERP and repolarisation. The abbreviated terminal phase of repolarisation prevented fast rate-induced depolarisation of the MDP in cells from patients with AF. The density of L-type Ca^{2+} (I_{CaL}) and transient outward K^+ (I_{TO}) currents was significantly reduced in cells from patients with AF (by 60-65%), whilst the inward rectifier K^+ current (I_{K1}) was increased, and the sustained outward current (I_{KSUS}) was unaltered. Superfusion of cells from patients in sinus rhythm with nifedipine (10 micromol/L) moderately shortened repolarisation, but had no effect on the ERP (228±12 *vs* 225±11 ms). 4-aminopyridine (2 mmol/L) markedly prolonged repolarisation and the ERP (by 35%, $P<0.05$). However, the combination of these drugs had no effect on late repolarisation or refractoriness. **Conclusion:** Chronic AF in humans is associated with attenuation in adaptation of the atrial single cell ERP and MDP to fast rates, which may not be explained fully by accompanying changes in I_{CaL} and I_{TO}.

Keywords:

Discipline: experimental; Object of study: heart; Level of study: cellular; Field of study: electrophysiology, pathophysiology, pharmacology. Additional keywords: Supraventricular arrhythmia; arrhythmia (mechanisms); remodelling; myocytes; ion channels.

Introduction

Chronic atrial fibrillation (AF) causes electrophysiological changes in the atria which promotes the perpetuation of the arrhythmia [1-5], a process known as AF-induced atrial electrophysiological remodelling. AF in humans involves multiple intra-atrial reentrant circuits [6], the maintenance of which depends upon a shortened atrial effective refractory period (ERP), slowed conduction, or both. An increased vulnerability to develop atrial tachyarrhythmias was correlated, in patients, with a lack of adaptation of the atrial ERP to increasing rate [7]. More recently, studies on goats [1] and dogs [2] have demonstrated the importance of a shortened atrial ERP, and its impaired rate adaptation, to the perpetuation of artificially-induced AF. Similar abnormalities in refractoriness have since been measured in patients immediately after cardioversion of AF, contributing to the growing body of evidence that reversible remodelling also occurs as a result of chronic AF in humans [8-10].

The ERP is largely determined by the duration of final repolarisation, which depends upon the density, voltage-dependency and kinetics of a variety of sarcolemmal ion currents. Chronic pacing-induced atrial remodelling in dogs involves a progressive attenuation in ERP rate adaptation which is paralleled by a reduction in L-type Ca^{2+} (I_{CaL}) and transient outward K^+ (I_{TO}) currents, but no alteration in inward rectifier (I_{K1}) or various delayed rectifier K^+ (I_K) currents [2]. In humans, atrial I_{CaL} [11,12] and I_{TO} [12,13] are also reduced in AF, due to ion channel transcriptional down-regulation [14,15]. However, the pattern of clinical AF-induced ionic remodelling is far from clear, since available data on currents other than I_{CaL} and I_{TO} are conflicting [12,15] and often inconsistent with the canine study [2].

Moreover, the contribution of ionic remodelling to the alteration in functional electrophysiological properties such as the ERP and its rate adaptation, is also unclear. Indeed, the ERP has not been measured before in single atrial cells isolated from humans, with sinus rhythm (SR) or otherwise, and the pharmacological modulation of this important parameter has not yet been attempted in such cells.

During AF, the atria generally activate at rates >400 beats per min (bpm), and may reach 600 bpm [16]. Attenuation of action potential duration (APD) rate adaptation associated with human AF occurs at up to 240 bpm [12], but higher rates have not yet been studied. Fast atrial rate has been proposed as a potential trigger of electrophysiological remodelling by extending the depolarised time (and hence I_{CaL}

activity) per cardiac cycle [4]. However, data on effects of fast rates on the human atrial cell membrane potential, or any alteration by AF, are currently lacking.

We aimed to investigate, in human atrial isolated myocytes, changes in functional electrophysiological properties associated with chronic AF (including rate adaptation of the ERP and membrane potential) and, with pharmacological modulation, the contribution to these changes of observed alterations in ion currents.

Methods

Cardiac myocyte isolation

The tip of the right atrial appendage was obtained from 50 patients undergoing cardiac surgery, whose characteristics are shown in 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 [17]. The tissue was transported to the laboratory for processing within 5 min of excision. Atrial cells were isolated by enzymatic dissociation and mechanical disaggregation, using a modification of the chunk method, described in detail previously by Harding *et al* [18] and Escande *et al* [19]. Briefly, excised tissue (weight: 0.25±0.02 g) was sliced into chunks and shaken (130 strokes/min) at 37 ^{o}C, under O_2, in a nominally Ca^{2+}-free solution, containing (mM) NaCl (120.0), KCl (5.4), $MgSO_4$ (5.0), pyruvate (5.0), glucose (20.0), taurine (20.0), HEPES (10.0), nitrilotriacetic acid (5.0); pH 7.0. The chunks were incubated in this way for 12 min, with transfer to fresh Ca^{2+}-free solution every 3 min. Protease (Type XXIV, Sigma, 4 U/ml) and $CaCl_2$ (50 μM) were added, and tissues were shaken for a further 45 min. The protease was then substituted by collagenase (Type 1, Worthington, 400 U/ml), and partially digested tissues were incubated for consecutive periods of 15, 15 and 20 min. The cell suspension obtained after each incubation was filtered through nylon gauze (200 μm mesh, Barr & Wray, Lanark, UK) and centrifuged at 40 *g* for 2 min. The supernatant was removed and cells centrifuged again after their resuspension in 1 ml of a high $[K^+]$, low $[Ca^{2+}]$ solution ("KB" [20]), to wash off residual enzymes. Finally, the KB was replaced with

a 0.2 mM $CaCl_2$-containing physiological salt solution for the independent storage in petri-dishes of the 3 cell fractions, for up to 8 hours.

Electrical recording technique

Action potentials and ion currents were recorded using the whole cell patch clamp technique, with an Axopatch-1D amplifier (Axon Instruments). Cells were superfused (37 ^oC) at 1.5 ml/min (RC-24E fast exchange perfusion chamber, Warner) with a physiological salt solution containing (mmol/L): NaCl (130.0), KCl (4.0), $CaCl_2$ (2.0), $MgCl_2$ (1.0), glucose (10.0), HEPES (10.0), pH 7.4, with added nifedipine (10 μmol/L) and/or 4-aminopyridine, 4-AP (2 mmol/L) when required. Microelectrodes were constructed from thin walled, filamented borosilicate glass (Clark Electromedical) using a micropipette puller (Narishige PP-83), and heat polished to resistances of 5-10 MΩ. Electrodes were filled with an aspartate-based solution which contained (mM): L-aspartic acid (110.0), KCl (20.0), $MgCl_2$ (1.0), EGTA (0.15), Na_2ATP (4.0), Na_2GTP (0.4), HEPES (5.0), pH 7.3. Using this solution, a liquid junction potential of +7±0.3 mV (*n*=6) was measured (bath relative to pipette) and was compensated for prior to seal formation [21]. Only single, isolated myocytes were selected for electrical recording, and both the cells' series resistance and capacity (4.5±0.3 MΩ and 77±3 pF, respectively; *n*=97 cells) were routinely compensated electronically prior to recording. The software program WinWCP (J Dempster, Strathclyde University) was used both to stimulate and record electrical activity. Current and voltage signals were low pass filtered at 5 kHz and digitised (Digidata 1200 A-D converter, Axon Instruments) prior to storage on magnetic and compact discs.

Experimental protocols

Action potentials were stimulated at various rates (75-600 bpm) using 5 ms current pulses of 1.2 x threshold strength, after current clamping resting cells at -75 mV and keeping the holding current constant thereafter. Only those cells which required a holding current of <150 pA immediately after establishing the whole cell configuration were used, and this frequently could be reduced following stimulation with a train of depolarising pulses, as reported previously in human atrial cells [22]. In subsequent action potential recordings, there was no significant difference in the holding current

between the groups of patients with SR and AF, at -66±6 and -64±19 pA (*n*=12 and 11 patients), respectively. The stimulus threshold current amplitude was initially determined in each cell by stimulating repetitively with trains of 3 current pulses, the 1st and 2nd being of equal, suprathreshold amplitude, and the 3rd pulse increasing progressively (from zero, in steps of 50 pA) until it produced a regenerative action potential. The ERP was measured using a standard S_1-S_2 stimulation protocol, with an 8-pulse conditioning train delivered at various rates, and S_1 and S_2 pulses of equal magnitude. The S_1-S_2 interval was shortened in 10 ms steps, and the ERP was defined as the longest S_1-S_2 interval which failed to elicit an S_2 action potential of amplitude >80% of the preceding S_1 action potential. The voltage clamp technique was used to record the voltage-dependency of I_{CaL}, I_{TO}, the sustained outward current (I_{KSUS}) and I_{K1}, and the frequency-dependency of I_{CaL}. The same electrode filling solution was used as for recording action potentials, permitting the recording and comparison of all ion currents and action potentials under constant ionic conditions, and often in the same cell. To minimise, as far as possible, contamination from K^+ currents, I_{CaL} and I_{TO} were measured after subtraction of steady-state (end-pulse) currents. The input resistance (R_i) was measured in each cell from the slope of the linear part of the current (usually recorded at around -100 mV) recorded in response to a linear voltage ramp (from -120 mV to +50 mV in 7 s). I_{CaL} voltage-dependent activation was measured from a holding potential of -40 mV, with 100 ms duration voltage pulses (0.33 Hz), increasing in amplitude from -30 mV to +50 mV in steps of 10 mV. The rate-dependency of I_{CaL} was measured with trains of 20 pulses (50 ms duration) from -40 mV to +10 mV, delivered at progressively increasing rates from 75 to 600 bpm, with a 5 s rest between trains. I_{TO} and I_{KSUS} were stimulated from a holding potential of -60 mV, with 100 ms pulses (0.33 Hz), increasing from -50 mV to +60 mV in steps of 10 mV. To study I_{K1}, steady-state currents were measured at the end of 500 ms duration pulses (0.33 Hz), increasing in 10 mV steps from -120 mV, with a holding potential of -40 mV. When superfusing cells with drugs, ion currents, action potentials and/or the ERP were recorded 90 s after drug addition, and again at 90 s intervals after drug removal until the reversal of any drug effect was confirmed.

Data analysis and statistics

Patients were grouped according to their cardiac rhythm at the time of surgery. Only those in SR, or in AF which had persisted for at least the previous 6 months, were included. Patient data were analysed using Access software (Microsoft), and voltage and current data, using WinWCP. I_{CaL} and action potential data were included only if the post cell-access time was ≤10 min, to avoid distortion by current "run-down". Cells were included for APD or ERP analysis only if the control APD_{90} was ≤290 ms, consistent with Type 3 human atrial cells [23]. All currents were normalised to the cell's capacity. Values (means+SEM) were compared using two-tailed paired or unpaired Student's *t* tests, as appropriate. $P<0.05$ was regarded as statistically significant.

Results

Fast rate-induced depolarisation and APD-shortening was attenuated in cells from patients with AF

Figure 1 shows that at the physiological rate of 75 bpm (top traces), action potentials recorded from a single atrial cell isolated from a patient with chronic AF displayed a markedly different morphology to those recorded in a cell from a patient in SR. Repolarisation was more linear in form than in the cell from the SR patient, with a less pronounced phase 1 and a markedly shorter late repolarisation. When the stimulation rate was increased to 300 bpm (cycle length: 200 ms) in the cell from the patient in SR (Figure 1, left hand panel), the upstroke of the second and subsequent action potentials impinged on the final stages of repolarisation of the respective preceding action potentials. This prevented full recovery of the resting potential following each response, causing depolarisation of the maximum diastolic potential (MDP). This depolarising effect became more pronounced as the rate was increased further, was marked at the fastest rates encountered during AF (ie: up to 600 bpm) and was fully reversible upon returning to 75 bpm (bottom trace). Despite such depolarisation at the highest rates, regenerative responses occurred but with reduced action potential amplitude (63 mV). This was substantially higher than that of the electrotonic ("just subthreshold") responses (17 mV) measured at the start of the experiment. The stepwise increase in rate was accompanied by a progressive reduction in steady-state

APD at late repolarisation, with, for example, a 39% shortening in APD_{90} upon increasing from 75 to 300 bpm stimulation. By contrast, in the cell from the patient with chronic AF (Figure 1, right hand panel), increasing the stimulation rate to 300 bpm produced a negligible change in the MDP, since the final phase of repolarisation of the post-rest action potential was sufficiently abbreviated in this cell not to be encroached upon by the reduced basic cycle length at this rate. Furthermore, even at rates as high as 600 bpm, such as these cells might have encountered *in-vivo*, the degree of MDP depolarisation was smaller than that seen in the cell from the patient in SR.

Figure 2 shows mean action potential data which confirmed that, at the physiological rate of 75 bpm, chronic AF was associated with a marked and significant abbreviation in late (APD_{90}), but not in early (APD_{50}) repolarisation (panel A). The APD_{50} exhibited a moderate variation between cells, particularly in those from patients in SR, owing to the high sensitivity of this measurement to the height of the end of phase 1. The mean data also confirmed that increases in rate to 300 bpm and higher in cells from patients in SR produced both substantial shortening in late repolarisation, and depolarisation of the MDP (panel B). By contrast, in the cells from the patients with chronic AF, whilst a small depolarisation of the MDP and shortening in APD_{90} were evident at supraphysiological rates (Figure 2), the rate adaptation in these measurements was clearly markedly attenuated in comparison with that seen in the cells from the patients in SR.

Atrial cell ERP was shortened and its rate adaptation attenuated in myocytes from AF patients

The human atrial cell ERP was measured initially at the physiological basic rate of 75 bpm, as shown in the upper trace of Figure 3A, by the superimposed action potentials recorded in a single cell obtained from a patient in SR. A four-fold increase in the stimulation rate in this cell produced a marked reduction in the ERP, from 200 to 140 ms, as shown in the trace beneath. This effect was fully reversible upon returning to 75 bpm stimulation. By contrast, in a single cell obtained from a patient with chronic AF, the ERP was dramatically abbreviated at the physiological rate. Moreover, an increase in the rate to 300 bpm produced only a small degree of further ERP-shortening, of 20 ms (Figure 3A, lower trace pair). The high time resolution of these ERP recordings permitted accurate measurement of the maximum upstroke velocity, V_{max}, which decreased from 203±11 to 159±20 V/s, when increasing

from 75 to 300 bpm (P<0.05, n=9 patients). A similar rate dependent reduction in V_{max} occurred in the cells from the patients with AF (from 231±16 to 180±8 V/s; P<0.05, n=5 patients). Moreover, there was no significant difference in V_{max} at the physiological rate, between the two patient groups. Figure 3B shows mean data on the ERP, obtained from each patient group. This confirmed that the ERP at physiological rates was markedly and significantly shorter in single atrial cells isolated from patients with chronic AF, than in those from patients in SR, by 49%. Additionally, this data confirmed a substantial adaptation of the ERP to a four-fold increase in rate in cells from patients in SR, with a significant and reversible shortening of mean ERP, by 27% (Figure 3B, left hand histogram). In cells from patients with chronic AF, by contrast (Figure 3B, right hand histogram), there was attenuation of the adaptation of the ERP to an increase in rate, with no significant shortening at higher rate.

I_{CaL} was reduced at physiological rates in atrial cells from patients with AF

The current traces in Figure 4A show that the amplitude of I_{CaL} was substantially reduced in a cell isolated from a patient with chronic AF, compared with that recorded in a cell from a patient in SR. Mean I_{CaL} density-voltage relationship data in each patient group confirmed that chronic AF was associated with a marked and significant reduction in the density of I_{CaL} at all potentials between -10 and +30 mV, with a maximum reduction in peak I_{CaL} of 63% (Figure 4A). This reduction in I_{CaL} occurred without any alteration of its voltage-dependency, since the mean current-voltage relationship from each patient group superimposed when I_{CaL} was expressed as a function of the maximum peak value obtained in each cell. The amplitude of peak I_{CaL} was also measured at physiological rates and faster. Figure 4B (top trace) shows currents evoked by a train of voltage pulses delivered at 75 bpm in a cell from a patient in SR. There was an immediate and progressive reduction in peak I_{CaL} following initiation of stimulation in this cell. Steady-state was reached by the eighth pulse, with an amplitude of only 58% of the post-rest value. This change in the Ca^{2+} current included a potentially interfering influence from outward currents, but this was minimised by the subtraction of end-pulse currents from peak I_{CaL}. The trace beneath shows that a four-fold increase in rate, in the same cell, produced a rapid and substantial reduction in steady-state I_{CaL}. In a cell from a patient with AF (Figure 4B, lower trace pair), both the post-rest and steady-state I_{CaL} amplitudes were markedly smaller at 75 bpm, than in the

cell from the patient in SR. However, the effect of rate increase was similar in both cell types, with a diminution of I_{CaL} at supraphysiological rates. Figure 4C shows mean data, including rates of up to 600 bpm, which confirmed these changes and indicated that the contribution of the reduction in I_{CaL} associated with chronic AF would be smaller at physiological rates and higher, than at sub physiological rate.

I_{TO} was reduced, I_{KSUS} was unaltered and I_{K1} was increased in atrial cells from patients in AF

Figure 5A shows that the rapidly activating, transient outward currents evoked by voltage pulses to between +10 and +60 mV, which represent I_{TO}, were markedly reduced in amplitude in a cell isolated from a patient with chronic AF, compared with those recorded in a cell from a patient in SR. In contrast, the steady-state (end-pulse) currents, I_{KSUS}, were similar in both cells. The mean I_{KSUS} and I_{TO} density-voltage relationships were calculated, from the end pulse currents and by subtraction of these from the transient peaks at each voltage step, respectively. These confirmed that chronic AF was associated with a marked and significant reduction in I_{TO}, by 65% (Figure 5A, upper graph), but with no significant alteration in I_{KSUS} (Figure 5A, lower graph). The reduction in I_{TO} included no alteration in its voltage-dependency, since mean current density-voltage plots in cells from patients in SR and AF, respectively, superimposed when I_{TO} was normalised to the peak value in each cell. The original traces in Figure 5B show that the amplitude of time-independent inward currents elicited at substantially hyperpolarised potentials (to more negative than approximately -100 mV), was larger in a cell isolated from a patient with AF, than in one from a patient in SR. Mean current density-voltage data confirmed this (Figure 5B, left hand panel), with a significantly larger inward current at -120 mV in the cells from patients with chronic AF (by 91%), but with no significant alteration at more positive potentials. The reversal potential of the steady-state currents was around -30 mV, as reported previously [12], and consistent with the low resting potential typically observed in these cells. Nevertheless, the slope of the current-voltage relationship between -120 and -100 mV indicated that it was largely representative of I_{K1}. This indicated, therefore, that chronic AF was associated with an increase in the density of I_{K1}. Quasi-steady-state current-voltage relationship data obtained to determine each cell's input resistance supported this, since the current measured at -115 mV was significantly larger in cells from patients

with AF, at -6.3±0.9 pA/pF (*n*=11 patients) compared with -3.6±0.2 pA/pF in cells from patients in SR (*P*<0.05; *n*=39 patients).

Effects of independent and combined pharmacological inhibition of I_{CaL} and I_{TO} on APD and ERP

Nifedipine (10 μmol/L) virtually abolished I_{CaL}, with a reduction in the peak from -10.5±3.1 to -0.2±0.2 pA/pF (*P*<0.05, *n*=4). Figure 6A shows the corresponding effects on action potentials and the ERP at physiological rate, in an atrial cell isolated from a patient in SR. Nifedipine had no effect on early repolarisation, but produced a small reduction in the plateau (left hand panel of Figure 6A) accompanied by a shortening in APD_{75} and APD_{90}, but with little effect, however, on final repolarisation. The right hand panel of Figure 6A shows that in the same cell, the ERP was hardly affected by nifedipine, with a shortening of 10 ms. Table 2 shows mean data which confirmed that, in cells from patients in SR, I_{CaL} inhibition, which had no effect on the MDP or APD_{50}, produced a small but statistically significant reduction in both APD_{75} and APD_{90} (by 29 and 17%, respectively), but with no accompanying effect on the ERP. Superfusion of cells from patients in SR with 4-AP (2 mmol/L) reduced peak I_{TO} from 12.7±3.4 to 5.9±1.0 pA/pF (*P*<0.05, *n*=4), and I_{KSUS} from 7.0±1.3 to 3.3±0.6 pA/pF (*P*<0.05, *n*=4). The left hand panel of Figure 6B shows that 4-AP suppressed phase 1 and substantially prolonged subsequent repolarisation. The right hand panel of Figure 6B shows that 4-AP also produced a marked lengthening in the ERP in a single cell, by 60 ms. Mean data confirmed these drug effects, with a marked and significant prolongation of all levels of repolarisation, and of the ERP by 33% (Table 2). Figure 6C illustrates the actions of the combination of 10 μmol/L nifedipine and 2 mmol/L 4-AP in an atrial cell from a patient in SR. The left hand panel shows that whilst phase 1 was suppressed, in contrast with the effects of 4-AP on its own, subsequent repolarisation was not prolonged. Indeed, there was a slight shortening effect on late repolarisation in this cell. However, the ERP, recorded in the same cell (Figure 6C, right hand panel) was unaffected by this drug combination. Mean data (Table 2) confirmed that there was a negligible and statistically insignificant effect on either late repolarisation or the ERP, despite substantial prolongation in APD_{50} (by 177%), in the presence of combined pharmacological inhibition of I_{CaL} and I_{TO}.

Discussion

The ERP was measured, for the first time to our knowledge in human atrial isolated myocytes, and both its value at physiological rate and its adaptation to an increase in rate were in close agreement with those recorded in the right atrium of patients, including the appendage [8,9,24,25]. Moreover, in line with clinical studies [8,10], we demonstrated that chronic AF was associated with shortening of the ERP and APD, and attenuation in their adaptation to the high rates frequently encountered during AF. The magnitude of ERP reduction at physiological rate was substantial, and larger than in some studies in which the ERP was measured in patients after cardioversion of AF [8]. However, the reduction in late repolarisation was similar to that reported in human atrial cells [12].

Rates up to 600 bpm caused marked adaptation of both the MDP and APD in cells from patients in SR. Rate dependent APD shortening, and its attenuation by AF, has been reported at up to 240 bpm in human atrial cells [11,12]. Rate-dependent depolarisation of the MDP was reported in atrial trabeculae from dogs [26] and humans [23,27] at up to 270 bpm, but in human atrial cells, neither higher rates, effects of rate on the MDP, nor associated effects of AF, were reported [11,12]. Our data suggest that in unremodelled atrial cells exposed to sufficiently high rate, as might occur during paroxysmal AF, depolarisation of the MDP may result. This is consistent with the hypothesis that fast rate-induced depolarisation acts as a trigger of electrical remodelling in human atria, by contributing to intracellular Ca^{2+} overload [4]. Moreover, in cells from patients with chronic AF, abbreviation of late repolarisation, by permitting full recovery of the resting potential, largely attenuated such depolarisation, even at the highest rates encountered during AF [16]. In the canine model, AF also attenuated depolarisation [26]. These results support the notion that remodelling may be a cellular adaptive response to keep the APD short, and thus oppose Ca^{2+} overload, but at the expense of reentry-promoting ERP-shortening.

We demonstrated a marked rate-dependent reduction in I_{CaL}, consistent with a contribution to the APD rate response. Chronic AF was associated with a substantial reduction (by 60-65%) in the fully reactivated I_{CaL} and I_{TO}, with no alteration in their voltage-dependency. The peak density of these currents in cells from patients in SR, and the magnitude of their reduction associated with AF, agreed with previous studies [2,11]. However, at physiological rate, I_{CaL} was partially inactivated, due to its

slow reactivation kinetics [28], suggestive of a relatively reduced contribution of I_{CaL} reduction to AF-induced APD- and ERP-shortening. AF was also associated with an increased density of inwardly rectifying current at hyperpolarised potentials, representative of I_{K1}. Consistent with this, an increase in human right atrial I_{K1} was associated with AF in one study [12], but an increase in I_{K1} in left, but not right, atrial cells has also been reported [15], and there was an absence of change in I_{K1} in canine atria [2]. The lack of a substantial change in I_{K1} over physiological potentials in the present study suggested only a limited involvement of this current in the shortening of APD and ERP. Human atrial I_K includes the rapidly and slowly activating currents, I_{Kr} and I_{Ks}, respectively [29], and the ultra-rapidly activating, I_{Kur} [30], the main component of I_{KSUS} [31]. I_{KSUS} was similar in magnitude to that reported previously [12] and was not different between cells from patients in SR and AF. This is consistent with reports of a lack of change in both human atrial I_{KSUS} [12] and I_{Kur} [13], as well as in canine atrial I_{Kur} [2], but is in conflict with other reports of a reduction in human atrial I_{Kur} or I_{KSUS} [15,32]. We found that V_{max}, measured at physiological rate, was similar in cells from patients with and without chronic AF, consistent with a lack of alteration in the Na^+ current density reported in human atrium [12].

In cells from patients without AF, nifedipine significantly shortened the action potential, particularly at around 75% repolarisation. This indicated an important contribution of I_{CaL} to the APD and its rate adaptation [2,11,28] suggestive of an involvement of the reduction in I_{CaL} associated with AF to the accompanying abbreviation in APD. However, the effect of nifedipine on the APD was relatively modest, as reported previously at physiological [2,28], in contrast with sub-physiological [2], rates, and particularly at final repolarisation. Moreover, the ERP, mainly determined by final repolarisation, was hardly affected by nifedipine, in contrast with the substantial shortening associated with AF. These data suggest that an exclusive reduction in I_{CaL} would be insufficient to explain AF-induced remodelling of action potentials and the ERP in human atrial myocytes. Inhibition of I_{TO} using 4-AP produced, by contrast, marked prolongation of both the single cell ERP and all levels of repolarisation. 4-AP shortened late repolarisation in canine atrial cells [2], presumably by prolonging the plateau and consequently enhancing voltage-dependent K^+ currents [33], but this was also only reported at low rate (6 bpm) [2]. Moreover, the combination of nifedipine and 4-AP, despite prolonging early repolarisation at physiological rate, had no significant effect on either late repolarisation or the

ERP. Thus, the simultaneous reduction in atrial I_{CaL} and I_{TO} associated with human AF may not be sufficient to fully account for the marked shortening in late repolarisation and ERP which results from this arrhythmia. In support, a recent mathematical model [34] suggested that AF-induced remodelling of canine atrial I_{CaL} and I_{TO} cannot wholly explain associated changes in action potential morphology. Atrial electrophysiological remodelling by chronic AF, therefore, probably involves changes in additional ion channel, and/or exchanger or pump currents.

Potential limitations: 1) Studies on human cells involve inherently uncontrolled differences in patient drug regime, gender and disease state. Several cardiac diseases, often associated with AF, display distinct patterns of remodelling, eg: down regulation of atrial I_{TO} in hypertrophy and heart failure [33], and of I_{CaL}, I_{TO}, I_{Ks} and I_{Kr} in myocardial infarction [35]. The majority of patients with AF underwent mitral valve surgery, alone or with other surgical procedures, whilst most patients in SR underwent coronary revascularisation only. Mitral valve disease is usually accompanied by atrial dilation, which may involve down regulation of I_{TO} and I_{CaL} [36]. Nevertheless, the dilation is most severe in the left atrium [37], and we studied only right atrial cells. 2) The chunk method of cell isolation may potentially disrupt ionic currents, eg: I_K in canine atria [38]. 3) The majority of action potentials studied were of Type 3 [23], ie: with a plateau of relatively low amplitude. However, "spike and dome" (Type 1) action potentials [23,27], and those with extremely prolonged repolarisation [23] have also been reported, and these could have differing ERP and MDP rate responses, potentially contributing to electrical heterogeneity. Additionally, the ERP was recorded in the absence of the electrotonic influences and structural and electrophysiological heterogeneity which are present *in-vivo*. 4) The results with 4-AP must be interpreted with caution, since whilst this is the best currently available I_{TO} blocker, it is not entirely selective [39], blocking I_{Kur} at low concentration [40], and without effect on the Ca^{2+}-dependent component of I_{TO} [19]. Whilst I_{Kur} blockade could potentially prolong APD, a recent mathematical model [41] suggested, however, that inhibition of I_{Kur} by 50% (ie: similarly to that produced by 4-AP), would lengthen late repolarisation by only a small degree. 5) Of the patients who were in pre-operative SR, 29% developed AF post-operatively within the first week, and the possibility that cells from patients in SR included those with a pre-operative propensity for post-operative AF cannot be excluded.

In conclusion, chronic AF in humans is associated with shortening, and attenuation in rate adaptation, of the ERP in single atrial cells. The accompanying abbreviation in late repolarisation may largely prevent excessive depolarisation at high rates. Associated alterations in I_{CaL} and I_{TO} are probably insufficient to fully account for such changes. Our data may have important implications in the potential therapeutic modulation of AF-induced electrophysiological remodelling.

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Table and Figure legends

Table 1. Patients' clinical characteristics.

Values are total numbers of patients in SR or chronic AF with selected clinical characteristics (*n* and % of total within rhythm group, respectively), except where parameter mean±SEM is shown. CABG=coronary artery bypass graft, AVR=aortic valve replacement, MVR=mitral valve replacement, ASD=atrial septal defect, VSD=ventricular septal defect, CCB=Calcium channel blocker, ACE=angiotensin converting enzyme, LVF=left ventricular function.

Table 2. Effects of I_{CaL} and I_{TO} blockade on action potentials and ERP in atrial cells from patients in SR. Nif=nifedipine (10 μmol/L), 4-AP=4-aminopyridine (2 mmol/L). Stimulation rate=75 bpm in all cells. MDP=maximum diastolic potential (mV); APD_x=action potential duration at x% repolarisation (ms); ERP=effective refractory period (ms).Values are means±SEM (*n* cells). Control data pooled from all drug experiments, but for statistical analyses, intervention data compared only with appropriate corresponding controls (ie: in same cells); *=$P<0.05$ *vs* corresponding control group (paired *t*-test).

Figure 1. Effect of rate increase on action potentials and MDP in cells from patients with or without AF. Action potentials from a single atrial cell isolated from a patient in SR (left side) and from a single atrial cell from a patient with chronic AF (right), produced by stimulus trains of progressively increasing rate (labelled), with a 10 s pause between each train, before returning to 75 bpm stimulation. Dotted line=0 mV.

Figure 2. Rate-dependency of mean action potential parameters in cells from patients in SR and AF.

A, Action potential duration (APD) at 50 and 90% repolarisation (labelled). B, Maximum diastolic potential (MDP). ○=SR (*n*=5 patients, 6 cells), ●=AF (5 patients, 7 cells). Values, measured from the 15th action potential of a stimulus train, are mean±SEM; *=$P<0.05$ *vs* corresponding SR value at same rate, †=$P<0.05$ *vs* corresponding 75 bpm value of same action potential parameter. Recovery of changes upon rate decrease is shown following axis break (//).

Figure 3. Effect on the ERP of increasing rate in atrial cells from patients with and without AF.

A, Superimposed action potentials recorded in response to the 7th and 8th of a train of 8 conditioning pulses of rates shown, followed by the responses to increasingly premature (in steps of -10 ms) test pulses in a single cell from a patient in SR, and in a single cell from a patient with chronic AF. Solid bars=ERP. B, Changes in mean (±SEM) ERP on increasing rate from 75 bpm (open columns labelled "C": Control) to 300 bpm (filled columns) and returning to 75 bpm (open column labelled "R": Recovery) in single cells from patients in SR or AF (n=8-13 cells, 6-9 patients and 4-7 cells, 2-5 patients/rate, respectively). *=P<0.05 *vs* control rate, within rhythm; †=P<0.05 between control rates.

Figure 4. Changes in atrial L-type Ca^{2+} current associated with rate increase and/or chronic AF.

○ and ●=data from patients in SR and AF, respectively; *=P<0.05 *vs* corresponding SR value. A, Mean (±SEM) steady-state peak I_{CaL} density-voltage relationship, with representative traces in right hand panel (n=24 cells, 12 patients in SR and 18 cells, 7 patients with AF). Holding potential: -40 mV; voltage steps: 100 ms duration, 0.33 Hz. B, I_{CaL} recorded in response to trains of voltage pulses (to +10 mV, 50 ms duration) of rate shown, with each response number labelled above. //=break in trace. C, Rate-dependency of mean (±SEM) peak I_{CaL} (n=7 cells, 6 patients in SR and 4 cells, 3 patients with AF). Rec=recovery. Post-rest value=response to first pulse of stimulus train, following a 5 s rest. Subsequent measurements taken from 15th response of each train.

Figure 5. Changes in human atrial K^+ currents associated with chronic AF.

○ and ●=data from patients with SR and AF, respectively; *=P<0.05 *vs* corresponding SR value. A, Mean (±SEM) current density-voltage relationship for I_{TO} (upper left panel) and I_{KSUS} (lower left panel); n=18 cells, 11 patients in SR and 9 cells, 5 patients with AF). Representative currents shown in right hand panel. Holding potential: -60 mV; voltage steps: 100 ms duration, 0.33 Hz. B, Mean (±SEM) I_{K1} density-voltage relationship (n=8 cells, 6 patients in SR and 7 cells, 5 patients with AF). Holding potential: -40 mV; voltage steps: 500 ms duration, 0.33 Hz. Right hand panel shows representative currents recorded at 20 mV steps.

Figure 6. Effects of nifedipine and 4-aminopyridine on action potentials and the ERP.

Action potentials recorded in single atrial cells isolated from patients in SR in the absence (Control) and presence (after 90 s superfusion) of (A) nifedipine at 10 μmol/L (Nif), (B) 4-aminopyridine at 2 mmol/L (4-AP) or (C) their combination. Left hand panels show superimposed action potentials recorded at steady-state (7th response of 75 bpm train) to illustrate drug effects. Right hand panels show superimposed action potentials evoked by the 7th and 8th of trains of 8 conditioning current pulses (rate: 75 bpm) followed by responses to increasingly premature test pulses, in the absence (upper traces) and presence (lower traces) of drugs. Solid bars=ERP.

Table 1

	SR		AF	
	n	%	*n*	%
Patients	39		11	
Male/female	31/8	79/21	3/8	27/73
Age	59±2		65±2	
CABG	30	77	1	9
AVR	4	10	0	0
MVR	0	0	5	45
AVR+MVR	0	0	2	18
CABG+AVR	2	5	0	0
CABG+MVR	0	0	3	27
ASD repair	2	5	0	0
VSD repair	1	3	0	0
CCB	18	46	2	18
ß-blocker	25	64	1	9
Digoxin	2	5	10	91
ACE inhibitor	14	36	3	27
Nitrate	25	64	1	9
Diuretic	9	23	10	91
Lipid lowering	27	69	2	18
Aspirin	24	62	2	18
Warfarin	2	5	10	91
LVF:				
Normal	18	46	8	73
Mild/moderate	17	44	2	18
Severe dysfunction	4	10	0	0

Table 2

	Control	Nif	4-AP	Nif+4-AP
MDP	-79±1 (24)	-79±1 (16)	-76±2 (6)*	-77±2 (8)
APD_{50}	22±4 (24)	20±3 (16)	76±15 (6)*	43±6 (8)*
APD_{75}	118±9 (24)	83±7 (16)*	176±18 (6)*	108±11 (8)
APD_{90}	203±10 (24)	170±10 (16)*	262±24 (6)*	186±16 (8)
ERP	221±10 (18)	225±11 (11)	282±19 (5)*	210±4 (4)

Figure 1

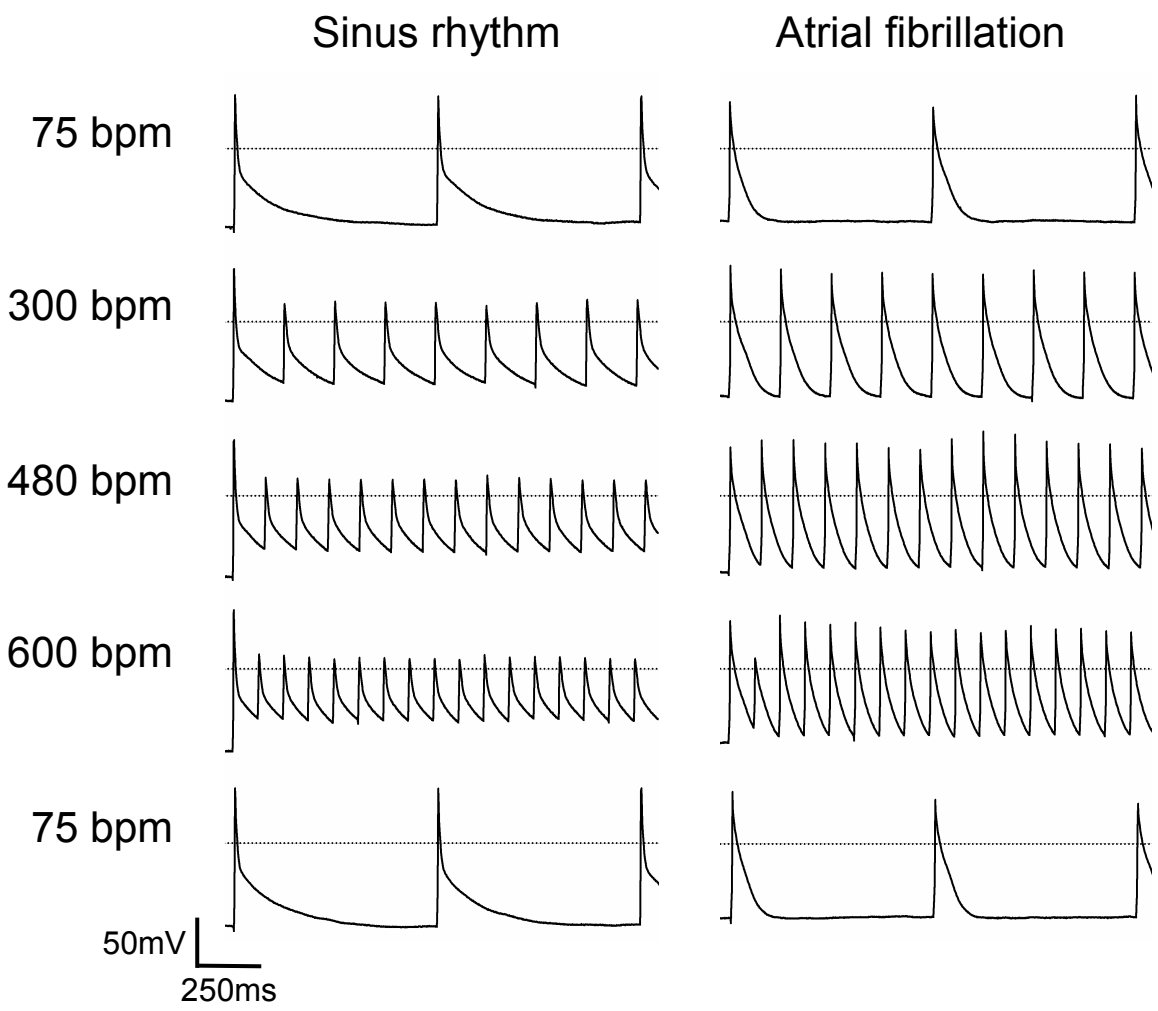

Sinus rhythm
Atrial fibrillation
75 bpm
300 bpm
480 bpm
600 bpm
75 bpm
50mV
250ms

Figure 2

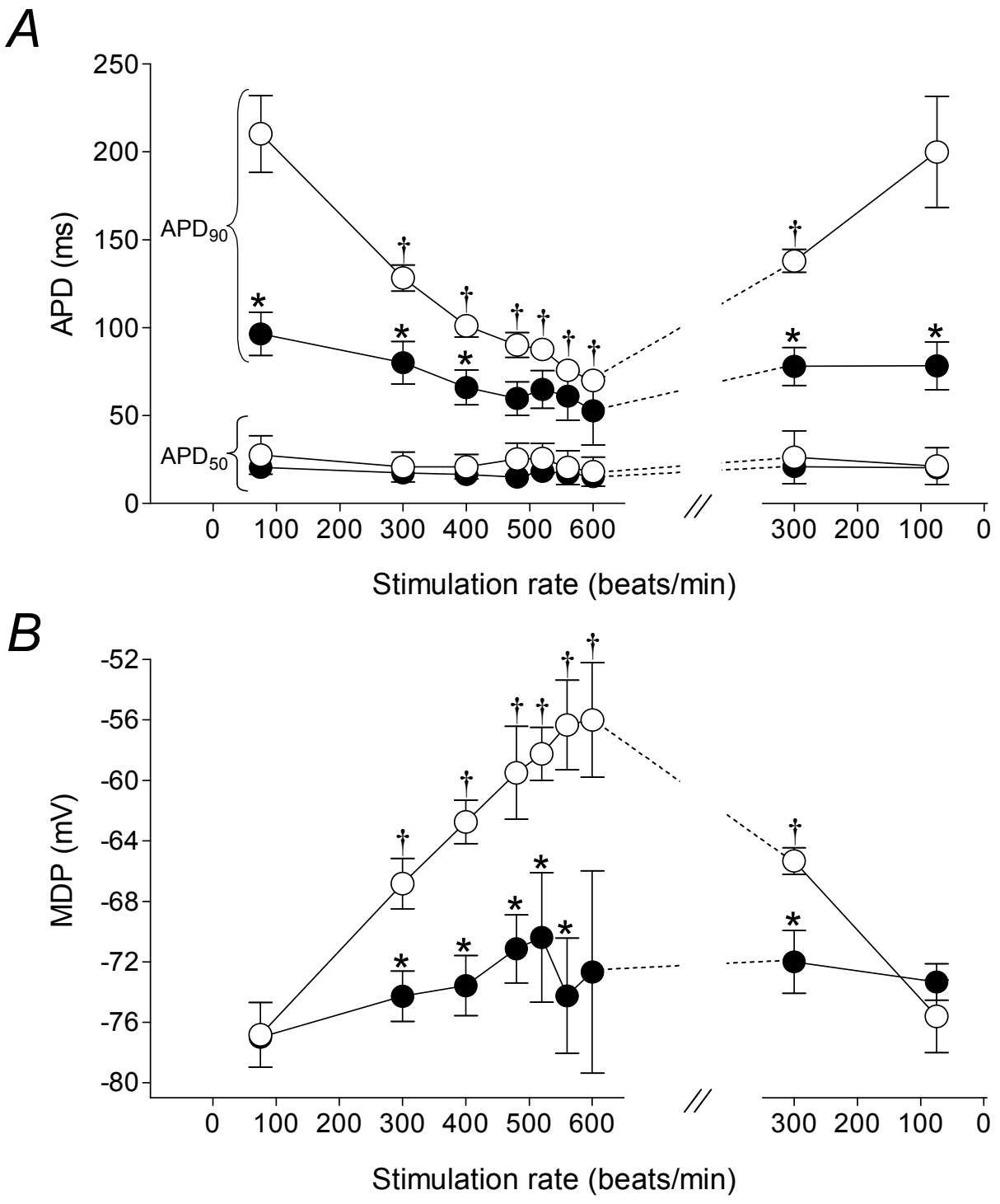

A
APD (ms)
APD90
APD50
Stimulation rate (beats/min)
B
MDP (mV)
Stimulation rate (beats/min)

Figure 3

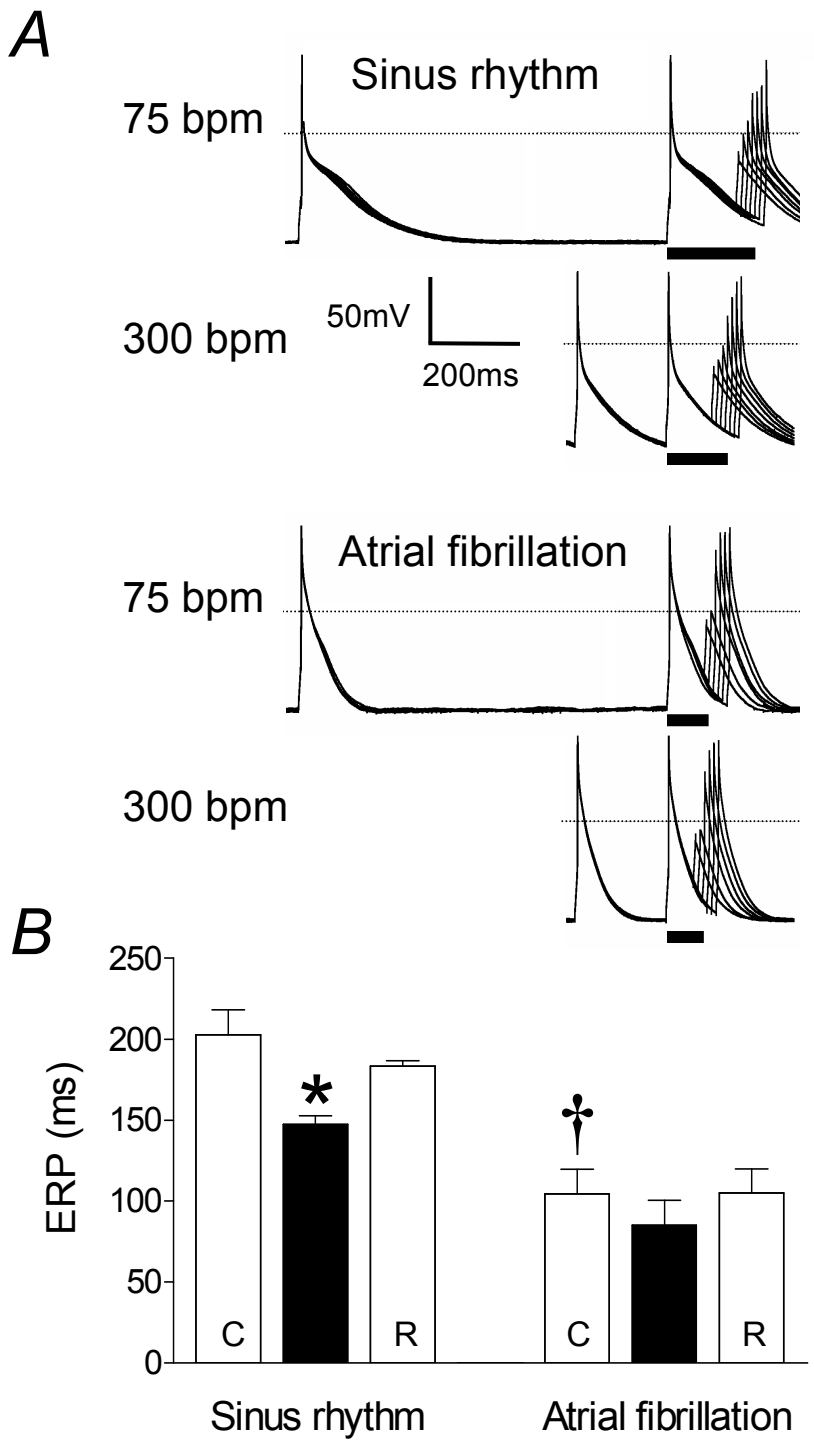

A
Sinus rhythm
75 bpm
300 bpm
50mV
200ms
Atrial fibrillation
75 bpm
300 bpm
B
ERP (ms)
250
200
150
100
50
0
*
†
C
R
C
R
Sinus rhythm
Atrial fibrillation

Figure 4

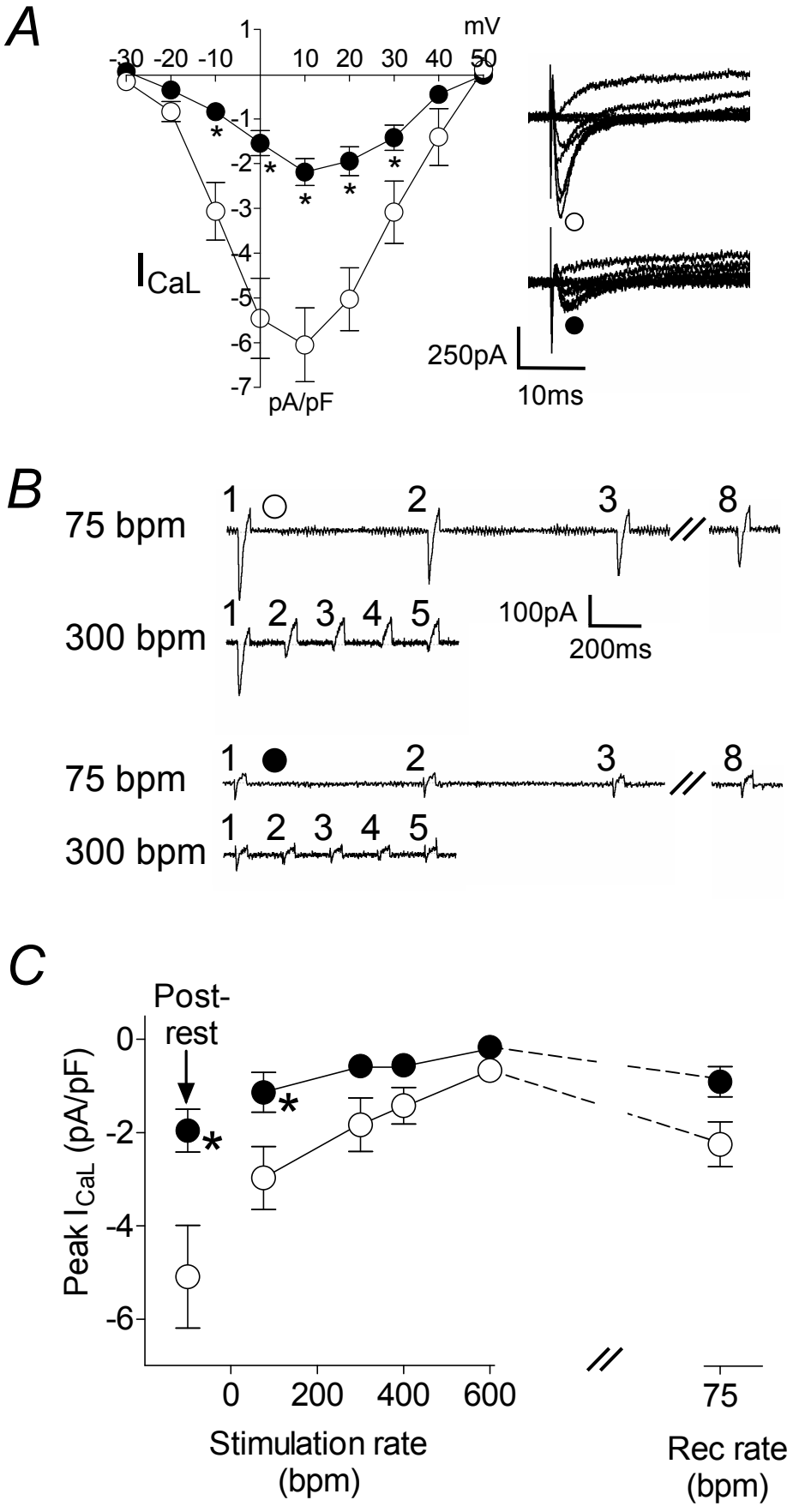

A
mV
1
-30 -20 -10
10 20 30 40 50
-1
-2
-3
-4
-5
-6
-7
ICaL
pA/pF
250pA
10ms
B
75 bpm
1
2
3
8
300 bpm
1 2 3 4 5
100pA
200ms
75 bpm
1
2
3
8
300 bpm
1 2 3 4 5
C
Post-
rest
0
-2
-4
-6
Peak ICaL (pA/pF)
0
200
400
600
75
Stimulation rate
(bpm)
Rec rate
(bpm)

Figure 5

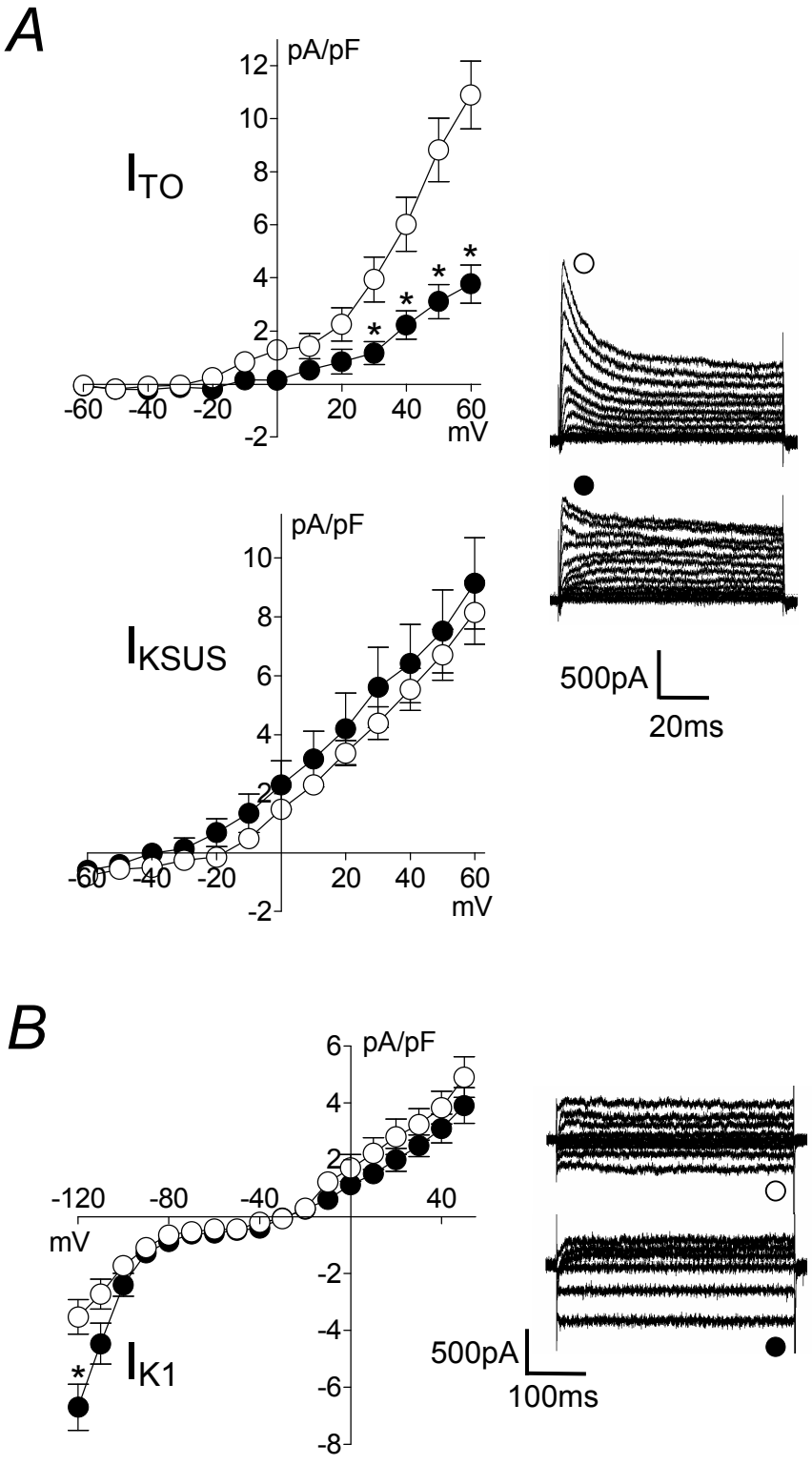
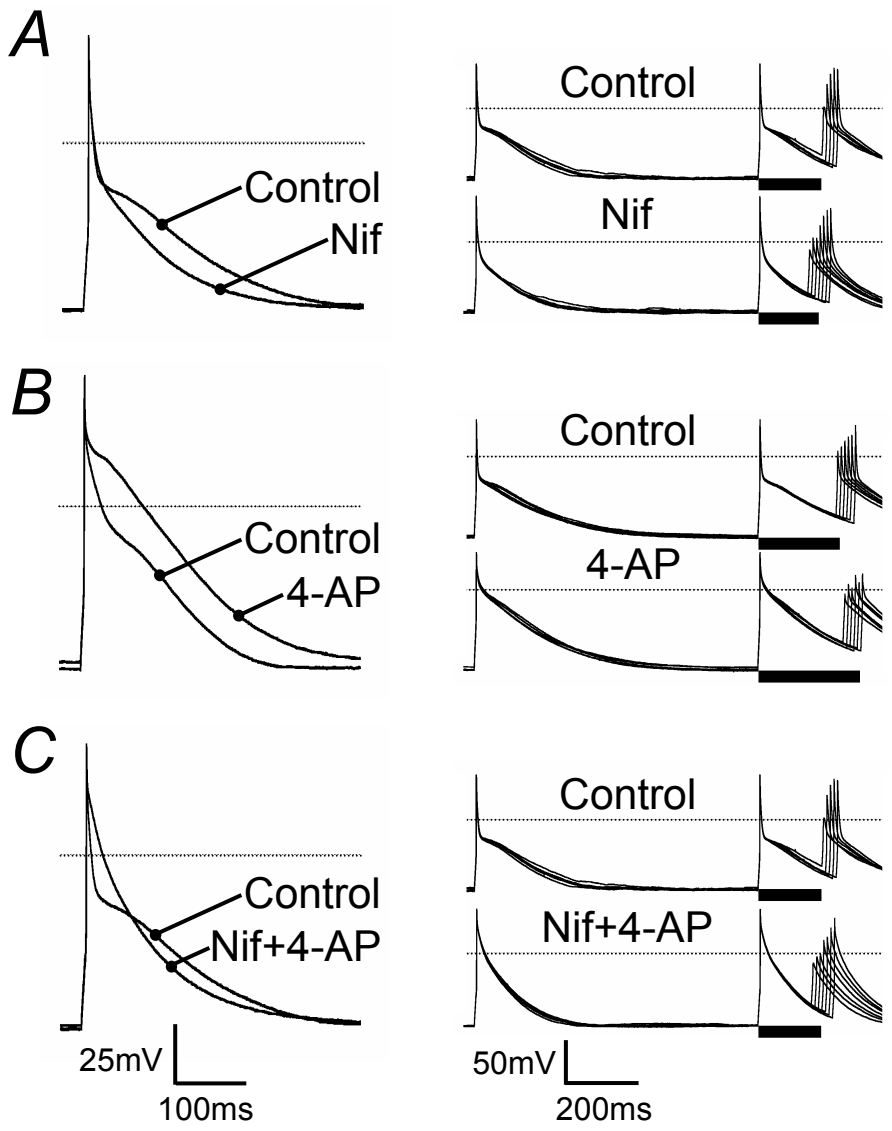

A
pA/pF
12
10
8
6
4
2
-2
I_{TO}
-60
-40
-20
20
40
60
mV
*
pA/pF
10
8
6
4
2
-2
I_{KSUS}
-60
-40
-20
20
40
60
mV
500pA
20ms
B
pA/pF
6
4
2
-2
-4
-6
-8
-120
-80
-40
40
mV
*
I_{K1}
500pA
100ms

Figure 6


A
Control
Nif
Control
Nif
B
Control
4-AP
Control
4-AP
C
Control
Nif+4-AP
Control
Nif+4-AP
25mV
100ms
50mV
200ms