Rate-dependency of action potential duration and refractoriness in isolated myocytes from the rabbit AV node and atrium

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Running Head: Rate-dependency of APD and ERP in AV nodal cells.

Abstract

During atrial fibrillation, ventricular rate is determined by atrioventricular nodal (AVN) conduction, which in part is dependent upon refractoriness of single AVN cells. The aims of this study were to investigate the rate-dependency of the action potential duration (APD) and effective refractory period (ERP) in single myocytes isolated from the AV node and atrium of rabbit hearts, using whole cell patch clamping, and to determine the contribution of the 4-aminopyridine (4-AP)-sensitive current, I_{TO1} to these relations in the two cell types. AVN cells had a more positive maximum diastolic potential (*-60±1 vs -71±2mV*), lower V_{max} (*8±2 vs 144±17V/s*) and higher input resistance (420±46 *vs* 65±7MΩ [mean±S.E., $P<0.05$; n=9-33]), respectively, than atrial myocytes. Stepwise increases in rate from 75 beats/min (bpm) caused activation failure and Wenckebach periodicity in AVN cells (at around 400bpm), but 1:1 activation in atrial cells (at up to 600bpm). Rate reduction from 300 to 75bpm shortened the ERP in both cell types (from 155±7 to 135±11ms in AVN cells [$P<0.05$, n=6] and from 130±8 to 106±7ms in atrial cells [$P<0.05$, n=10]). Rate increase from 300 to 480 and 600bpm shortened ERP in atrial cells, by 12±4% (n=8) and 26±7% (n=7), respectively ($P<0.05$). By contrast, AVN ERP did not shorten at rates >300bpm. In atrial cells, rate reduction to 75bpm caused marked shortening of APD_{50} (from 51±6 to 29±6ms, $P<0.05$). 4-AP (1mM) significantly prolonged atrial APD_{50} at 75bpm ($P<0.05$, n=7), but not at 300 or 400bpm. In AVN cells, in contrast, there was less effect of rate change on APD, and 4-AP did not alter APD_{50} at any rate. 4-AP also did not affect APD_{90} or ERP in either cell type. In conclusion, a lack of ERP-shortening at high rates in rabbit single AVN cells may contribute to ventricular rate control. I_{TO1} contributed to the APD_{50} rate relation in atrial, but not AVN cells and did not contribute to the ERP rate relation in either cell type.

Keywords: Atrioventricular node; Rate; Isolated myocyte; Atrium; Action potential; Refractory period; Transient outward potassium current; Wenckebach periodicity; Rabbit.

List of Abbreviations used: 4-AP: 4-aminopyridine; APD: action potential duration; APD_{50} and APD_{90}: APD at 50 and 90% repolarisation, respectively; AV: atrioventricular; AVN: AV nodal; bpm: beats/min; ERP: effective refractory period; I_{CaL}: L-type Ca^{2+} current; I_K: delayed rectifier K^+ current; I_{K1}: inward rectifier K^+ current; *I_{Kr}: rapidly activating component of I_K; I_{Kur}: ultrarapid component of I_K; I_{KS}: slowly activating component of I_K;* I_{TO}: transient outward K^+ current; *I_{TO1}: 4-AP-sensitive component of I_{TO}; I_{TO2}: 4-AP-insensitive component of I_{TO}*; MDP: maximum diastolic potential; R_i: input resistance; V_{max}: maximum rate of rise of action

potential upstroke.

Introduction

The atrioventricular (AV) node of the heart has a protective role during high rate atrial arrhythmia, in particular atrial fibrillation, by periodically blocking the transmission of atrial impulses, and thus preventing life-threatening ventricular tachyarrhythmias. The mechanisms underlying the control of ventricular rate by the AV node, however, are not fully understood.[1,2] Rate-dependent activation failure of the AV node exhibits a periodic nature, known as Wenckebach periodicity.[3] This may be related to the refractoriness of the least excitable element in a heterogeneous AV nodal conduction pathway.[4] In support of this hypothesis, Wenckebach periodicity has been demonstrated in single cells of the AV node.[5] The effect on AV nodal refractoriness of increasing the stimulation rate may be variable, however, with reports of both prolongation[6,7] and reduction[8]. Such effects may depend upon interactions between AV nodal recovery, facilitation and fatigue.[9] The effective refractory period (ERP) of the intact AV node may also be influenced by the intra-nodal conduction velocity but is at least partly determined by the longest ERP among single refractory elements of the conduction pathway.[6] *In addition, we have previously shown differences in the refractory periods of AV nodal cells which may correspond to different cell types: "slow V_{max}" AV nodal cells[10] (which may be of mid-nodal origin,[11]) have a longer ERP than "fast V_{max}" cells,[10] and might therefore have the greatest influence on the intact nodal ERP.* It is therefore important to understand the relation between stimulation rate and the ERP in single cells of the AV node*, and this* has not previously been studied.

The rate-dependency of atrial refractoriness is also of importance, since this may influence the maximum rate of impulses reaching the AV node. There have been several studies in intact atria and atrial tissues on the rate-relation of both the action potential duration (APD) and the ERP. These have generally shown that, similarly to the ventricle, increasing rate causes shortening of both APD[12] and ERP.[13] However, reports on the relation between stimulation rate and action potential configuration in atrial single isolated myocytes are few,[14] and only rates of 120 beats/min (bpm) or lower have been studied. Moreover, to our knowledge, the rate-dependency of the ERP has not been studied in atrial isolated myocytes.

The ionic mechanisms underlying APD-shortening at fast rates are not fully understood.[15] However, APD-shortening resulting from low rates of stimulation, as demonstrated in hearts of both the rabbit[16] and rat,[17] has been correlated with a slow reactivation of *I_{TO}, the 4-aminopyridine (4-AP)-sensitive component of the transient outward K^+ current, I_{TO}.*[14,18,19] The reactivation kinetics of I_{TO} is species-dependent,[20] which may

explain the lack of APD-shortening at low rates in human,[12] in contrast to rabbit myocardium. Moreover, in the rabbit, the contribution of I_{TO} to the APD rate-relation in the AV node might be expected to be less than in the atrium, due to the relatively small I_{TO} in mid-nodal cells.[11,21] *Additionally, ionic mechanisms which may influence late repolarisation secondarily to I_{TO} modulation,[22,23] might be expected to influence the ERP*. An investigation into the contribution of I_{TO} to the rate relation of either the APD or the ERP has not yet been carried out in AV nodal cells.

The aims of the present study were, firstly, to investigate the rate-dependency of the APD and ERP in single myocytes isolated from both the AV node and atrium of rabbit hearts and, secondly, to determine and compare the contribution to these relations in the two cell types, of the 4-AP-sensitive current, I_{TO1}.

Materials and methods

Isolation of single AV nodal and atrial myocytes

The investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Cells were isolated from the AV nodal region and the left atrium of rabbit hearts, using a modification of methods described previously.[10,24] Briefly, male New Zealand White rabbits (2.0-2.8kg) were administered a lethal dose of anaesthetic (sodium pentobarbitone, 80mg/kg) with added heparin (335U/kg), by intravenous injection. The heart was excised and retrogradely perfused via the aorta for 4min at 20ml/min, with a solution containing (mM): NaCl (130), KCl (4.5), $CaCl_2$ (0.75), $MgCl_2$ (3.5), NaH_2PO_4 (0.4), HEPES (5), glucose (10); bubbled with 100% O_2; pH 7.25; 37^{o}C. $CaCl_2$ was included for the first 4min to aid blood clearance through myocardial contraction, but was substituted with EGTA (100μM) for a further 8min perfusion, to reduce $[Ca^{2+}]_o$. The heart was then perfused for 4min with collagenase (Type 1, Worthington: 216U/ml) and protease (Type XIV, Sigma: 0.54U/ml), with EGTA re-substituted by 240μM $CaCl_2$. The coronary effluent was recirculated after 1min of perfusion with enzymes. The heart was then pinned out on a dissecting dish and the right atrium opened. The partially digested AV nodal region was removed using anatomical landmarks: the tricuspid valve inferiorly, the tendon of Todaro superiorly, the coronary sinus posteriorly and the central fibrous body anteriorly. The left atrial appendage was removed at this stage and treated identically to, but separated from, the AV nodal region. The tissue to be digested was chopped into approximately 1mm^3 pieces and placed into a flask containing 1.5ml of recirculated enzyme solution with 2% bovine albumin (Fraction V, Sigma). Cells were then mechanically disaggregated using a shaking water bath (Grant OLS 200), with orbital shaking (130revs/min) at 37^{o}C, for periods of 5, 10 and 15min. After each stage, the cell suspension was filtered through 200μm nylon mesh into 1.5ml of a high $[K^+]$, low $[Ca^{2+}]$ solution ("KB"[25]), containing 3% BSA, before being centrifuged at 80*g* for 2min. The supernatant was then replaced with 1ml KB (20^{o}C) and the cells centrifuged for a further 1min, before being resuspended in KB for 30min sedimentation. Finally, cells were stored for up to 12hr in a physiological salt solution containing 2mM $CaCl_2$, as used to superfuse cells during recording (see below).

Electrical recording technique

Action potentials and ion currents were recorded using the whole cell patch clamp technique, with an Axopatch-1D amplifier (Axon Instruments). Aliquots of cell suspension were transferred to a fast exchange (<10s) perfusion chamber of 200μl volume (RC-24E, Warner), mounted on the stage of an inverted microscope (TMS, Nikon). Cells were allowed to sediment for 10min, before being superfused under gravity at 1.5ml/min, with a physiological salt solution containing (mM): NaCl (130), KCl (4), $CaCl_2$ (2), $MgCl_2$ (1), glucose (10), HEPES (10), pH 7.36. This was heated to 35-37^{o}C, using an in-line solution heater (SH-27G, Warner). Microelectrodes were pulled by gravity from thin walled, filamented borosilicate glass tubes (Clark Electromedical) to resistances of 4-8MΩ, using a vertical micropipette puller (Narishige PP-83) and fire-polished. The pipette solution contained (mM): L-aspartic acid (110), KCl (20), $MgCl_2$ (1), EGTA (0.15), Na_2ATP (4), Na_2GTP (0.4), HEPES (5), pH 7.25. With this solution, a liquid junction potential of +7±0.3mV (bath relative to pipette, *n*=6) was measured, and compensated for prior to seal formation.[26] Action potentials and ion currents were both stimulated and recorded by current- and voltage-clamping, respectively, using the software program "WinWCP" (John Dempster, Strathclyde University). Current and voltage signals were filtered at 5kHz, monitored on an oscilloscope (Farnell DTS 20), and digitised (Digidata 1200, Axon Instruments), for storage on the hard disc of a computer.

Experimental protocols

Identification of AV nodal cells. The cell input resistance (R_i) was initially measured in each cell, by voltage clamping with a voltage ramp (from -120mV to +50mV in 7s), from the slope of the linear part of the resulting whole cell current, usually between -90 and -120mV. The AV nodal sample inevitably contained right atrial cells also, which may show similar morphology and electrophysiology to atrio-nodal AV nodal cells.[11] Since AV nodal cells have a high R_i (due to the absence of inward rectifier K^+ current, I_{K1}), this was used to distinguish AV nodal cells from other cell types. As reported previously, based on our own experience[10] and that of others,[27] we accepted cells as AV nodal if their R_i was >180MΩ. *We studied only those cells in which V_{max} at 300bpm stimulation ≤15V/s (defined previously[10] as "slow V_{max}" AV nodal cells) since these are most likely of mid-nodal origin.*

Action potential recording. The stimulus current magnitude required to cause a regenerative action potential response (the threshold current) was determined in each cell by progressively increasing the amplitude of a 5ms

duration current pulse, from zero, in steps of 50pA (with pulses delivered at 2s intervals) until an action potential occurred. All subsequent action potential stimulation protocols used stimulus pulses of 5ms duration and 1.2x threshold strength, with the exception of selected cells (detailed in Results), in which "just-threshold" pulses were used. The stimulus strength was kept constant for control, intervention and recovery protocols.

ERP measurement. The ERP was determined by stimulating cells with trains of 8 conditioning current pulses (S_1), delivered at pre-determined rates (between 75 and 600bpm), with each train being followed by a premature pulse (S_2) of the same magnitude. The S_1-S_2 interval was shortened by 10ms intervals 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. Rate-dependent activation failure was investigated by stimulating with trains of 20 S_1 pulses delivered at various rates between 75 and 600bpm, but with no test pulses.

Resting and action potential measurements. The maximum diastolic potential (MDP) was measured between the end of the 7th and the start of the 8th S_1 action potentials. In contrast to some spontaneously active AV nodal cells, all atrial cells were quiescent, and the maximum diastolic and resting potentials, respectively, of these cell types, is referred to throughout as the MDP. The maximum rate of rise of the action potential upstroke (dV/dt)max (referred to throughout as V_{max}) was measured from the action potential evoked by the 8th S_1 pulse, after any initial upstroke artifact caused by the current pulse. The Axopatch-1D is a current follower, which accurately measures V_{max} of ≤60V/s. However, V_{max} measurements >60V/s may have been underestimated by the use of this amplifier. The APD was measured at both 50 and 90% repolarisation (APD_{50} and APD_{90}, respectively).

Effects of 4-AP. Cells were superfused with 4-AP (1mM), and recordings were made 90s after drug addition, and again 180s after drug removal. This concentration of 4-AP was chosen because previous work has shown it to produce 90% block of I_{TO} in rabbit atrial isolated myocytes.[14]

Data analysis and statistics

Action potential recordings were analysed using the WinWCP software. Electrophysiological changes due to interventions were included in the analysis only if these could be shown to be reversible on removal of the intervention. Values were expressed as mean±S.E.. Differences between values were assessed using two-tailed paired or unpaired Student's *t* tests, as appropriate. $P<0.05$ was regarded as statistically significant.

Results

Differences between AV nodal and atrial isolated cells

AV nodal and left atrial cells displayed distinct electrophysiological characteristics, which were consistent with previously reported data from our laboratory.[10] For example, AV nodal cells *(which had a significantly higher R_i: 420±46 MΩ, n=23, than the atrial cells: 65±7 MΩ n=33, consistent with the AV nodal cell selection criteria used)* had a significantly more positive MDP than left atrial cells, *at -60±1 compared to -71±2 mV*, and a slower V_{max}, *at 8±2 compared to 144±17 V/s* (P<0.05, n=11 and *19*, respectively; *all cells stimulated at 300 bpm*).

Comparison of rate-dependency of activation in AV nodal and atrial cells

The effect of increasing the stimulation rate on the pattern of action potential activation was investigated in single AV nodal and left atrial cells. In each cell studied, the rate of a succession of 20 current pulses (1.2x threshold strength) was changed from 75 to 300, 400, 480, 520, 560 and 600bpm. Typical patterns of action potential responses that resulted are shown in Fig 1. In a single atrial cell (left hand traces), during steady-state activation, action potentials were evoked by each of the stimuli (ie: 1:1 activation), even at the fast rate of 600bpm. A similar activation pattern was seen in 3 other atrial cells studied, with 1:1 activation up to rates of 560 or 600bpm. Additional rate-related effects were observed, with an initial transient rise in the action potential amplitude, a sustained rise in the plateau amplitude, and at fast rates, a subsequent fall in the action potential amplitude.

The AV nodal cells, by contrast, displayed activation failure at rates as low as 400bpm. The AV nodal cell shown in Fig 1 (right hand traces) beat spontaneously at approximately 260bpm, but stimulation at 300bpm resulted in 1:1 activation. When the rate was increased to 480bpm, the 9th action potential of the train failed, and there was subsequent regular 2:1 activation failure. Further rate increases to 520, 560 and 600bpm resulted in the same pattern of activation failure. On returning to 300bpm, 1:1 activation was regained. Similar patterns of rate-dependent activation failure were observed in 2 other AV nodal cells. In a further 2 AV nodal cells, the effect of stimulation with "just-threshold" current pulses was examined, and under these conditions, periodic rate-dependent activation failure was clearly demonstrated (Fig 2). There was 1:1 activation at 300bpm, but on changing to 400bpm (lower trace), there was a progressive reduction in the V_{max} of successive action potentials

following the first response. This was associated with prolongation of activation delay (latency) until the response to the 4th pulse failed. The responses stabilised, with 3:2 activation failure. Upon returning to 300bpm, 1:1 activation resumed.

Rate-dependency of ERP in atrial isolated myocytes

Fig 3A illustrates the effect on the ERP of increasing the stimulation rate from 75bpm, in a single isolated left atrial cell. Superimposed action potentials evoked by the last 2 conditioning (S_1) pulses are shown, followed by responses to the increasingly premature test (S_2) pulses. The longest S_1-S_2 interval at which the S_2 action potential failed (the ERP) is shown at each conditioning pulse rate. The ERP lengthened when the rate was increased from 75 to 300bpm, and then shortened as the rate was increased further to 600bpm, the latter effect reversing on return to 300bpm. The rate-induced changes of ERP, APD_{90} and APD_{50} in the same cell are shown in Fig 3B. There was a biphasic rate-relation of these parameters with shortening at both low and high rates. The fast rate-induced shortening of ERP and APD_{90} was more pronounced than that of APD_{50}.

To avoid effects of current "run-down" during the relatively long ERP stimulation protocols, the ERP was measured in the majority of cells using only one rate change per cell. A control rate of 300bpm was chosen, since the longest ERP and APD values were recorded at this rate, and the recovery of any ERP-shortening intervention would then be most clearly detectable after returning to 300bpm. *By reducing the total time spent recording from each cell in this manner, all effects of rate change on ERP, APD_{90} and APD_{50} were reversible upon returning to 300 bpm stimulation.* Fig 4 shows the effect on the mean ERP of a rate change to either 75, 400, 480 or 600bpm (filled columns), compared to 300bpm, investigated in groups of 7-10 left atrial cells. A rate reduction to 75bpm caused significant and fully reversible shortening of the ERP, from 130±8 to 106±7ms (P<0.05, n=10). An increase of rate to 400bpm had no significant effect on the atrial cell ERP. However, further rate increases, to 480 and 600bpm, respectively, caused significant and reversible ERP-shortening to below the 300bpm value. This histogram clearly confirms the biphasic nature of the ERP rate-relation in these cells, with ERP-shortening at rates both faster and slower than 300 to 400bpm.

Rate-dependency of ERP in AV nodal isolated cells

The effect on the ERP of both decreasing and increasing the stimulation rate from 300bpm was determined in groups of AV nodal cells, in the same way as for the atrial cells. An example of the effect of such an intervention in a single isolated AV nodal cell is shown in Fig 5, in which rate reduction to 75bpm reversibly shortened the ERP. The effects of rate change on the mean ERP data, from groups of 5-6 AV nodal cells (1 group/rate intervention) are shown in Fig 6. Similarly to the atrial cells, rate reduction (from 300 to 75bpm) caused significant ERP-shortening (from 155±7 to 135±11ms, $P<0.05$; $n=6$). Studies of the rate-dependency of ERP at higher rates in AV nodal cells were restricted by activation failure. In those cells allowing 1:1 activation, a rate increase to 400bpm had no effect on the ERP, and, in contrast to the atrial cells, a further rate increase to 480bpm did not cause significant shortening of the ERP ($P=0.25$, $n=5$). An effect on the ERP of a rate increase to 600bpm could not be measured in AV nodal cells, since 1:1 activation could not be achieved during the conditioning train. It may be noted that the tendency to shortening of the control (300bpm) ERP values with the fast intervention rate groups was more marked in these AV nodal cells compared to that observed in atrial cells (Fig 4). For example, with the AV nodal cells, the control ERP in the 480bpm intervention group was significantly different from that of the 75bpm group ($P<0.05$), whereas in atrial cells, the differences were not significant. This supports the difference in rate-response of refractoriness between the cell types, with a lack of shortening of ERP in AV nodal cells preventing stimulation at high rates. Only those cells with inherent relatively shorter refractoriness could therefore be included in the studies of higher intervention rates. For example, the mean ERP at 300bpm in the AV nodal cells (of the 75bpm intervention group) was 155±7ms, ($n=6$) which would require to shorten to allow stimulation at 480bpm, which is a cycle length of 125ms. The rate-dependency of the ERP in AV nodal isolated cells, with shortening at the low rate of 75bpm, but a lack of significant shortening at rates ≥400bpm, *is clearly different from the biphasic ERP rate relation observed in the atrial cells.*

Rate-dependency of APD and other action potential parameters in AV nodal and atrial isolated cells

The effects of rate change on various action potential measurements, in the same AV nodal and atrial cells used for the ERP measurements, are shown in Table 1. The rate relation of the APD_{90} was similar in both cell types to that of the ERP, with significant shortening in response to a reduction in rate from 300 to 75 bpm (by

15±4% and 13±5%, in AV nodal and atrial cells, respectively). There was also a marked and significant shortening of APD_{90} below the 300 bpm value (by 27±5% at 600 bpm), in the atrial cells stimulated at the fastest rates, but less marked changes in APD_{90} in the AV nodal cells. However, AV nodal and atrial cells differed in another respect: in response to rate decrease, the degree of shortening of the APD_{50} was much greater in the atrial than the AV nodal cells. These changes in absolute APD_{50} are shown in Table 1, and the reduction in the atrial cells, of 46±7%, was significantly greater ($P<0.05$) than the 20±5% reduction seen in the AV nodal cells.

Contribution of rate-dependency of I_{TO} to rate-response of APD and ERP in AV nodal and atrial cells

Fig *7*A shows superimposed recordings of action potentials, each stimulated by the last pulses of the conditioning train (delivered at either 75bpm [left panels] or 300bpm [right panels]) in both the presence and absence of 4-AP (1mM). In the atrial cell stimulated at 75bpm (upper left panel), the action potential had a fast phase 1 repolarisation, associated with a short APD_{50}, and 4-AP caused marked APD-prolongation at this level of repolarisation. In the same atrial cell in the absence of 4-AP, the APD_{50} was already prolonged at the faster rate (upper right panel), and 4-AP had no additional effect. In contrast, in the AV nodal cell stimulated at 75bpm (lower left panel), APD_{50} was longer than in the atrial cell and 4-AP had no effect on action potential repolarisation. Similarly to the atrial cell, 4-AP had no effect on repolarisation of the action potential stimulated at 300bpm. *A small hyperpolarisation of MDP was observed in some cells.* The mean *APD* data from atrial cells are illustrated in Fig *7*B. 4-AP caused prolongation of APD_{50} in atrial cells stimulated at 75bpm (from 7±1 to 22±4ms, n=7; P<0.05), but had no significant effect at 300bpm (Fig *7*B, lower panel). There was no effect of 4-AP on APD_{90} at either rate (upper panel). In a group of 4 left atrial cells stimulated at 400bpm, 4-AP was also shown to have no significant effect on repolarisation: APD_{50} was 41±5 and 37±3ms, in the absence and presence, respectively, of 1mM 4-AP. In AV nodal cells 4-AP had no effect on APD_{50} or APD_{90} at either rate (n=5). The rate-dependency of effects of 4-AP on other action potential characteristics, including refractoriness, was investigated in the same cells, at stimulation rates of 75, 300 and 400bpm. Despite the marked effect of 4-AP on the APD_{50} in atrial cells stimulated at 75bpm, 4-AP had no significant effect on the ERP in either atrial or AV nodal cells, at any of the rates studied.

Discussion

This study has shown, for the first time, that isolated AV nodal cells exhibit unique rate-dependent properties, which are consistent with the role of the intact AV node in the control of ventricular response to high atrial rates. The lack of shortening of action potential duration and refractory period at high stimulation rates in AV nodal cells was in contrast to the shortening of these parameters observed in atrial cells. This difference was reflected in the failure of activation shown at high rates in AV nodal, but not atrial, myocytes. Thus, the refractoriness of single AV nodal cells may contribute to the block of conduction through the AV node. However, it is likely that the relationship between the properties of individual cells and the function of the intact AV node is complex.

The steady-state values of ERP recorded from isolated AV nodal myocytes in this study were similar to those reported from intact superfused AV nodal preparations from the rabbit heart.[6] The effect of rate increase on refractoriness in the intact AV node, however, may be variable: previous reports indicate no change, shortening, or prolongation of the ERP.[6-8] The refractoriness of the intact AV node is influenced by conduction time, and identification of the effects of rate is complicated by associated changes in this.[28] Moreover, the effects of rate increase on AV nodal conduction and refractoriness are dependent upon interactions between the processes of recovery, facilitation and fatigue,[9] the cellular counterparts of which are incompletely understood.

In the present study, rate-related responses were observed in single AV nodal cells that may correlate with rate-related properties of the intact node. Decreasing recovery times with short coupling intervals or high stimulation rates was associated with increased latency between the stimulus and the upstroke of the action potential in AV nodal, but not atrial, cells. In AV nodal cells, activation failure occurred which exhibited a beat-to-beat increase in activation delay (latency) until failure occurred, with subsequent repetition of the cycle, i.e. Wenckebach periodicity. This phenomenon is most commonly observed in the AV nodal transmission system, and has been reported from a single previous study of ellipsoid quiescent cells from the region of the AV node.[5] It can also be demonstrated in other cardiac tissues, provided that the recovery of excitability follows a slow time course that outlasts repolarisation. Indeed, production of such post-repolarisation refractoriness, by using critically timed stimuli of low amplitude, allowed the demonstration of Wenckebach periodicity in single ventricular myocytes.[29] In the present study, the AV nodal cells displayed marked post-repolarisation refractoriness, and activation failure was detected at cycle lengths of about 400bpm. This was

consistent with the stimuli (delivered at a cycle length of 150ms) encroaching on the refractory period of these cells (155ms at 300bpm). The phenomenon of increasing latency, although observed during standard protocols (e.g. figure 1), was most apparent when "just threshold" stimuli were used (figure 2), since refractoriness in cardiac cells is a function of the amplitude of the test pulse.[4] Prolongation of activation delay by the premature pulse reduced the recovery time for the subsequent beat, which lead to an even longer activation delay until, eventually, activation failure occurred.

The mechanisms underlying the function of the AV node in the control of ventricular rate during atrial fibrillation are complex and multiple, of which refractoriness of single AV nodal cells is just one component. Rate-dependent heart block requires adjacent regions of AV nodal tissue with differing electrical properties such that, at critical cycle lengths, impulse delay occurs at the boundary between such regions. This delay is determined by the recovery of excitability of the less excitable element or elements in the tissue.[4] The lack of shortening of the AV nodal cell ERP in the present study may, therefore, be considered likely to play an important mechanistic role in the limitation of excessively high ventricular rates during fast atrial rate[2]. However, anatomical and electrophysiological properties of the intact AV node, including the *presence of multiple* atrial inputs to the node,[2] cell-to-cell coupling[4] and electrotonic modulation of propagation,[30] are also important.

In the atrial myocytes, in contrast to those from the AV node, we demonstrated that the ERP and APD shortened as the rate was increased, and stimulation at cycle lengths even as short as 100ms was possible. This is consistent with studies on the rate relation of APD, measured in intact atrial[12] and ventricular[31,32] preparations and also in isolated myocytes, from both the atrium[33] and ventricle[34]. The rate relation of refractoriness had been measured previously only from intact myocardium and, in line with the changes in the APD, was found to shorten at fast rates, both in the atria of dogs[13] and the ventricles of patients[7]. The ability of the atrial APD and ERP to shorten at fast rates is of importance, allowing propagation of rapid rate impulses originating from a high rate ectopic focus, or a sufficient reduction in the minimum path-length of reentrant circuits to sustain multiple reentrant tachyarrhythmias such as atrial fibrillation.

In the present study, there was a biphasic rate relation of both the ERP and APD in the atrial isolated myocytes, with shortening at rates slower, as well as faster, than 300-400bpm, which is the upper end of the normal range for the rabbit.[35] Shortening of the APD at low rates has been reported previously from studies on

intact myocardium, both atrial[16,36,37] and ventricular,[38] and more recently from isolated myocytes, again atrial[14] and ventricular.[17] In addition to demonstrating shortening of the APD at slow rate, in the present study we showed that the ERP shortened also. However, importantly, we found that in the atrial cells, there was a significantly greater degree of shortening of the APD_{50}, than of either the ERP or late repolarisation.

It has been shown that I_{TO} is responsible for phase 1 repolarisation, and shortening of APD at low rates has previously been correlated with the kinetics of recovery from inactivation of this current.[14,19] Slow reactivation of I_{TO} is species-dependent and accounts for the APD-shortening at slow rates which occurs in those tissues of certain species (rabbit and rat) in which I_{TO} is a major repolarising current.[14,20] Since I_{TO} both activates and inactivates rapidly in the potential range of phase 1, it might not be expected to contribute to later phases of repolarisation. Nevertheless, *previous studies have indicated that* I_{TO} may influence late repolarisation indirectly, *through secondary effects on additional ion currents, such as the L-type* Ca^{2+} *current,* I_{CaL},[22] *and* K^+ *currents.*[23] In the present study, there is evidence that slow reactivation of I_{TO1} was responsible for the marked shortening of APD_{50} observed at low rates in atrial cells but not in AV nodal cells. However, I_{TO} was not involved in the rate response of either late repolarisation or refractoriness at low or high rates in either cell type. Whilst I_{TO} has been reported in some studies of AV nodal cells,[21,39] the present data are consistent with a lower I_{TO} density in rabbit AV nodal than atrial cells.[21,40] In a study on distinct AV nodal cells types from the rabbit,[11] I_{TO} was found to exist in only 42% of mid nodal or nodal-His cells.

A limitation of the present study is the unknown site of origin within the AV node of the cells studied, since there is evidence of a differing contribution of distinct regions of the AV node to refractoriness, e.g.: the ERP at slow basic rate originates in the proximal node, and fatigue effects are observed particularly in the central node.[9] *Moreover, it is acknowledged that electrotonic interaction between different cell types within the AV node may limit the extrapolation of data from single AV nodal cells to the intact node. In addition, at the fastest stimulation rates, the study is restricted to data from cells with relatively short control ERP values, in both the AV node and atrium, and the data on refractoriness must be interpreted in the context of the intrinsic electrophysiological heterogeneity of both tissues.*[10,41] *Another limitation of the study is that the only ionic mechanism examined was the 4-AP-sensitive component of* I_{TO}. *Given that the higher rate stimulus intervals investigated were shorter than the recovery time for* I_{TO1} *it will be necessary to extend the study to include additional currents, such as the* Ca^{2+}*-activated* Cl^- *current,* I_{TO2}.[18] *It should also be noted that 4-AP at 1mM*

cannot be considered to block I_{TO1} solely, since block of the ultrarapid component of the delayed rectifier, I_{Kur}, has been demonstrated in human atrium by 4-AP at 50 μM, with substantial prolongation of APD without changing the action potential morphology.[42]

The mechanisms underlying APD- and ERP-shortening during high frequency stimulation in atrium or ventricle are presently unclear,[15] *but may include rate-dependent changes in ion currents, as well as in concentrations of extracellular ions. The delayed rectifier K^+ current, I_K, may contribute to rate-dependent APD-shortening, since accumulation of I_K activation at fast rates has been shown to shorten APD.*[43] *However, this property of I_K relates to slow deactivation kinetics of the slow component of the current (I_{Ks}), and rabbit myocardium may contain only the rapid component (I_{Kr}) of the delayed rectifier K^+ current.*[44] *In rabbit heart I_{Kr} deactivation in AV nodal myocytes was relatively fast compared to ventricular cells,*[45] *thus reducing the potential for APD-shortening at fast rates in AV nodal cells. Other currents which may be involved include I_{TO2}, which has fast reactivation kinetics in the rabbit atrium, and has been shown to be responsible for a small degree of APD_{90}-shortening at fast rates.*[18] *The slow reactivation kinetics of I_{CaL}*[10] *may also contribute to shortening of APD and ERP at faster rates. In addition, in AV nodal cells, because of the absence of the rapidly reactivating I_{Na},*[46] *the kinetics of I_{CaL} may contribute to post-repolarisation refractoriness, Wenckebach periodicity and the lack of ERP-shortening. In atrial cells in-vivo, the inward rectifier K^+ current, I_{K1} may also contribute indirectly to high rate-related shortening of late repolarisation and ERP, due to its dependence on $[K^+]_o$, which is known to increase during high rate stimulation.*[15] *Thus, the ionic mechanisms underlying differences between AV nodal and atrial cells' functional responses to fast rate stimulation are likely to be multiple and complex.*

In conclusion, a lack of shortening of refractoriness at high rates in single AV nodal cells may contribute to the properties of the AV node that control the ventricular response to high atrial rates. I_{TO} contributed to the rate relation of APD_{50} in rabbit atrial, but not AV nodal, cells and did not contribute to the rate relation of refractoriness in either cell type.

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Table 1. Effects of rate change on action potential parameters of left atrial and AV nodal isolated myocytes. Values are means±S.E.; bpm=beats/min; APD_{50} and APD_{90}=action potential duration at 50 and 90% repolarisation (ms), respectively; MDP=maximum diastolic potential (mV); APA=action potential amplitude (mV); V_{max}=maximum rate of rise of action potential upstroke (V/s). In some cells, it was not possible to measure V_{max} due to interference by the stimulus artifact (and this accounts for the absence of data from AV nodal cells at 480bpm). The 300bpm data is pooled from that obtained in all rate intervention (75, 400, 480 and 600bpm) control experiments, but for statistical analyses, intervention data were compared only with the appropriate corresponding 300bpm control groups (ie: obtained from same cells); *=$P<0.05$ *vs* corresponding control group (paired students *t*-test, *n*=7-10 atrial and 5-6 AV nodal cells/rate); §=recordings not made as no 1:1 activation during conditioning train.

Rate (bpm)	75	300	400	480	600
Left atrial					
APD_{50}	29±6*	53±4	42±5*	42±5*	25±4*
APD_{90}	76±7*	90±4	73±6*	71±5*	51±5*
MDP	-69±2	-66±1	-67±1	-67±1	-63±3*
APA	110±8*	105±4	99±10	97±4*	83±9*
V_{max}	146±21*	96±14	68±27	79±17	82±19
AV nodal					
APD_{50}	41±3*	53±4	48±5*	33±4*	§
APD_{90}	70±5*	86±5	78±4*	71±6	§
MDP	-57±1	-61±2	-63±1	-66±4	§
APA	78±2	92±5	84±4	97±11	§
V_{max}	9±2	9±2	5±2	-	§

Figure legends

Fig 1. Comparison of rate-dependent activation between atrial and AV nodal isolated cells.
Action potentials recorded during repetitive stimulation at progressively increasing rates (indicated above each trace) in a single left atrial and AV nodal cell. Recordings show 1:1 activation of the atrial cell during repetitive stimulation at up to 600 bpm, but activation failure (2:1) in the AV nodal cell, within 1 s of stimulation at 480 and 520 bpm. Dotted lines=0 mV. Calibration bars apply to all traces.

Fig 2. Rate-dependent activation delay and failure (Wenckebach periodicity) in a single AV nodal cell.
Action potential recordings from a single AV nodal cell, showing activation delay and failure following an increase in stimulation rate from 300 to 400 bpm. Activation delay was evident 2 beats after stimulation onset, and beat-to-beat delay increased progressively until activation failure occurred, which settled into a Wenckebach pattern with stimulus to response ratio of 3:2. Dotted lines=0 mV. Calibration bars apply to both traces.

Fig 3. Effects of rate change on action potentials and refractoriness in a single left atrial myocyte.
(A) Superimposed action potentials elicited by the 7th and 8th S_1 current pulses of an 8-beat conditioning train delivered at rates indicated, followed by increasingly premature test pulses. ERP is indicated by solid bars. Dotted lines=0 mV. Calibration bars apply to all traces. (B) Line graph showing effects of a progressively increasing stimulation rate on ERP and APD in the same atrial cell as (A)*, with reversal of changes to ERP and* APD_{90}*, but not* APD_{50}*, upon returning to 300 bpm control rate ("Recovery").*

Fig 4. Rate-dependency of ERP in atrial isolated myocytes.
*Histogram showing effects on ERP of both an increase and decrease in stimulation rate from 300 bpm, in groups of 7-10 left atrial myocytes, obtained from 4-7 rabbits, respectively. Values are means±S.E.. * indicates significant difference between intervention rate value and corresponding control (P<0.05). For each rate shown, open column (C) indicates the ERP at 300 bpm (control); filled column, ERP at the intervention rate; open column (R), ERP on return to 300 bpm (recovery).*

Fig 5. Effect of rate change on action potentials and refractoriness in a single AV nodal cell.

Action potentials evoked by the 7th and 8th S_1 current pulses of conditioning trains, followed by increasingly premature test pulses, in a representative AV nodal cell. Traces show effects on APD and ERP of rate change from 300 to 75, then back to 300 bpm. ERP is indicated at each rate by solid bars. Dotted lines=0 mV. Calibration bars apply to all traces.

Fig 6. Rate-dependency of ERP in AV nodal isolated *cells*.

*Histogram to show effects on ERP of both an increase and decrease in stimulation rate from 300 bpm, in groups of 5-6 AV nodal cells, obtained from 4-6 rabbits, respectively. Values are means±S.E.. * indicates significant difference between intervention rate value and corresponding control (P<0.05). For each rate shown, open column (C) indicates ERP at 300 bpm (control); filled column, at intervention rate; open column (R), on return to 300 bpm (recovery). ERP could not be recorded at 600 bpm due to activation failure.*

Fig 7. Rate-dependent effect of 4-aminopyridine on APD in atrial and AV nodal isolated myocytes.

(A) Superimposed action potentials from a single left atrial and AV nodal cells stimulated at rates indicated, in the absence (C), presence (4-AP) of 1 mM 4-AP. Calibration bars apply to all traces. (B) Histograms of atrial mean (±S.E.) APD_{90} and APD_{50} at different rates (n=7 or 9 cells/rate) in the absence (open bars) and presence (hatched bars) of 1 mM 4-AP. * indicates significant difference between 4-AP and control data.

Fig 1

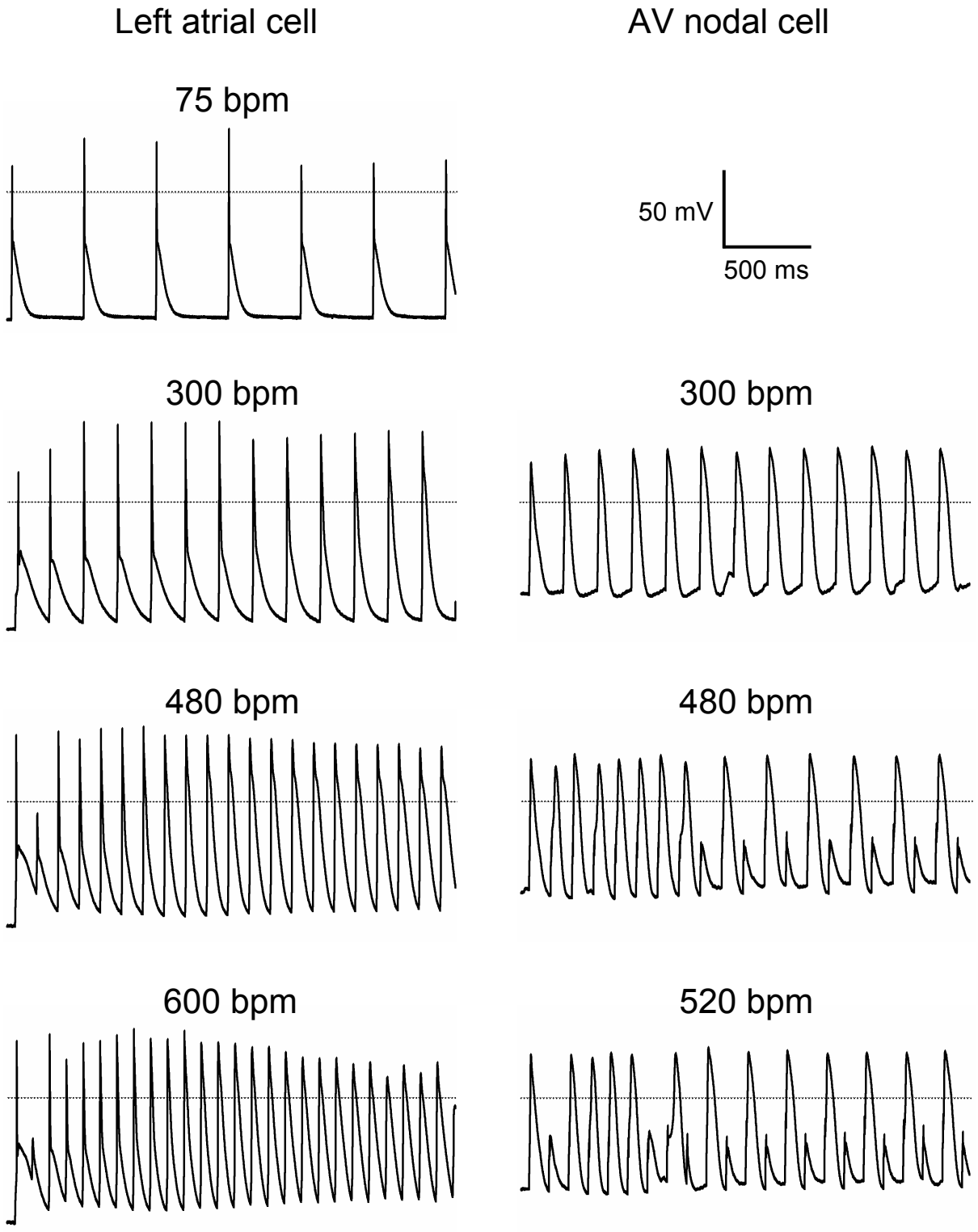

Left atrial cell
AV nodal cell
75 bpm
50 mV
500 ms
300 bpm
300 bpm
480 bpm
480 bpm
600 bpm
520 bpm

Fig 2

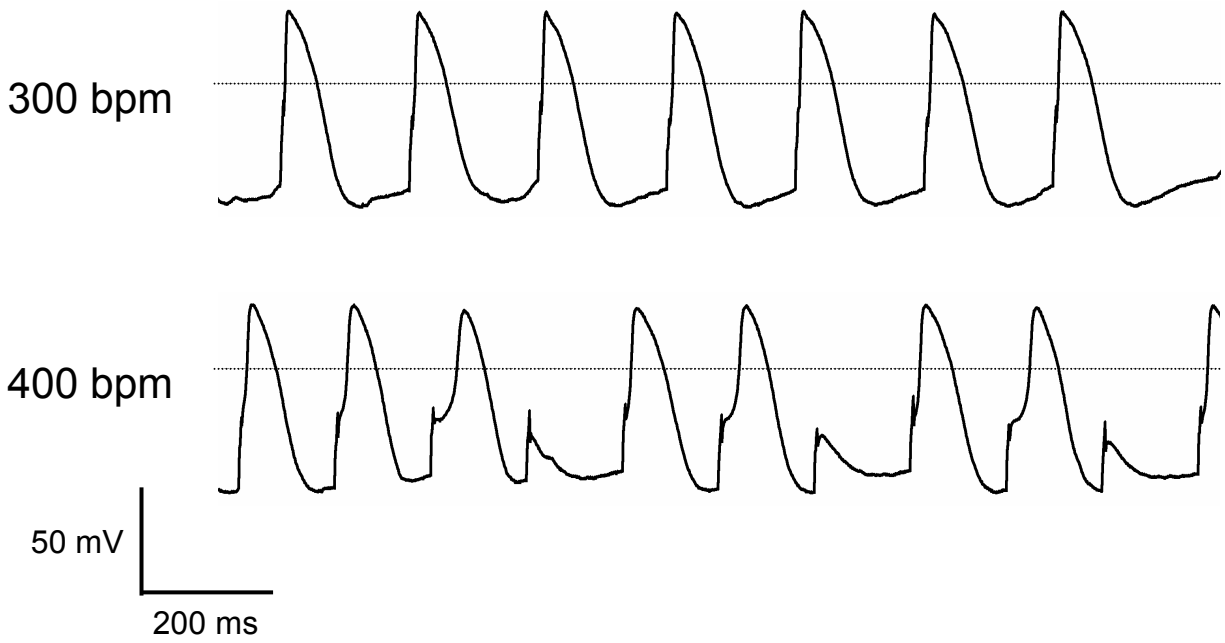


Fig 3A

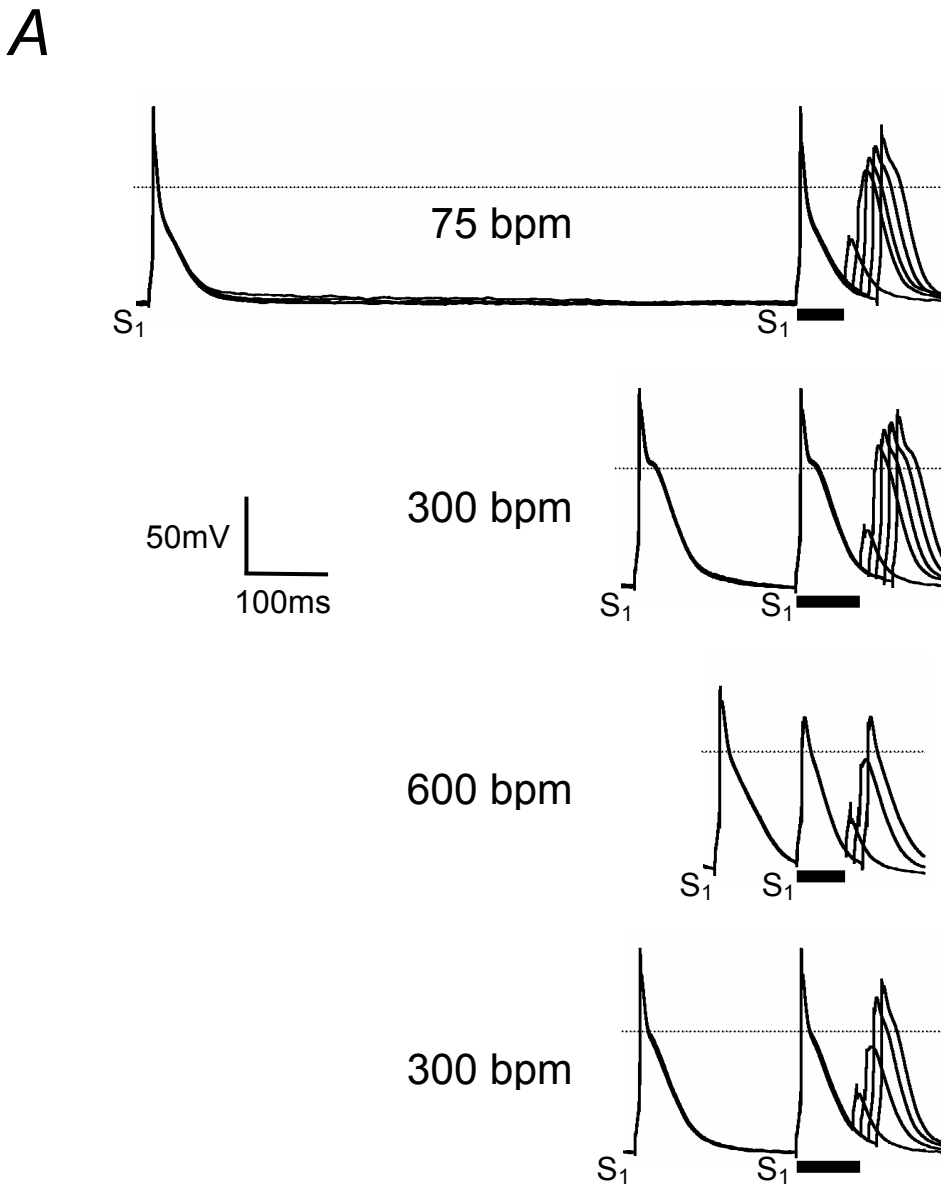


Fig 3B

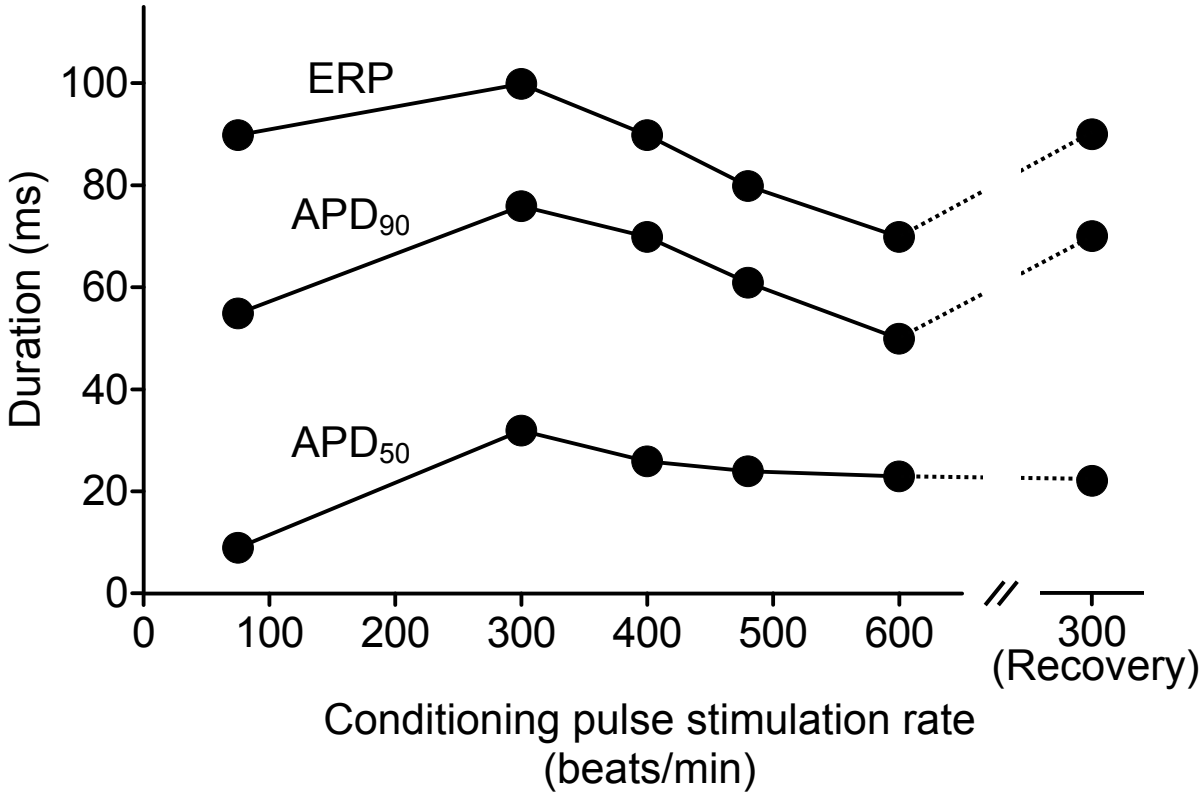

ERP
APD90
APD50
Duration (ms)
100
80
60
40
20
0
0
100
200
300
400
500
600
300
(Recovery)
Conditioning pulse stimulation rate
(beats/min)

Fig 4

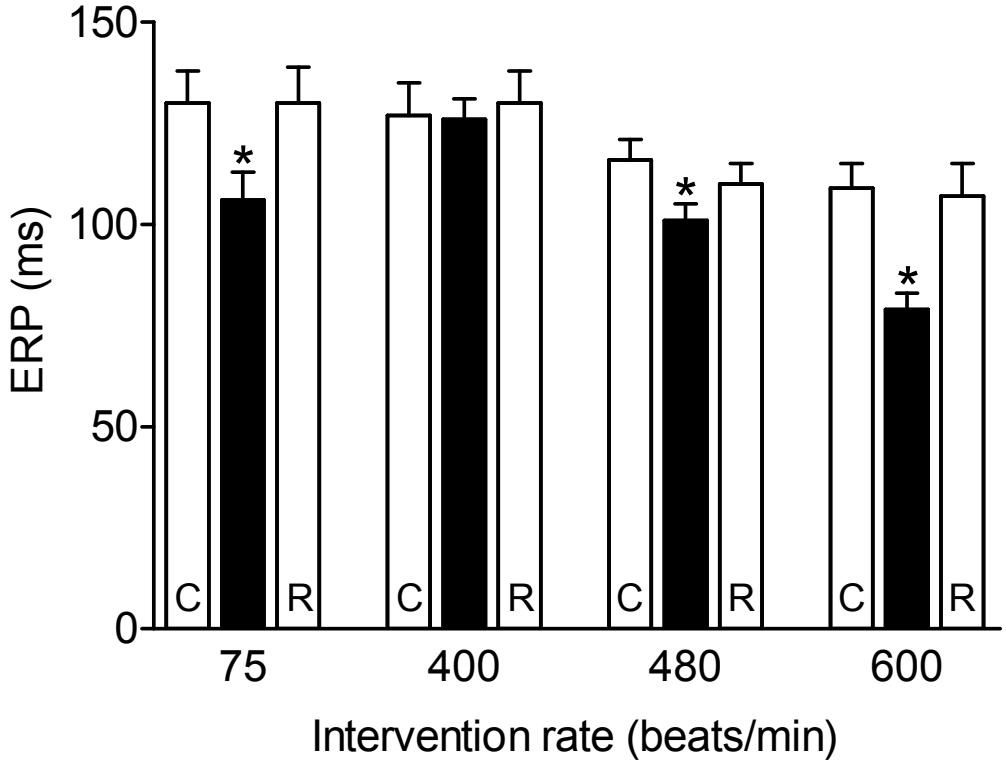

150
100
50
0
ERP (ms)
C
R
C
R
C
R
C
R
*
*
*
75
400
480
600
Intervention rate (beats/min)

Fig 5

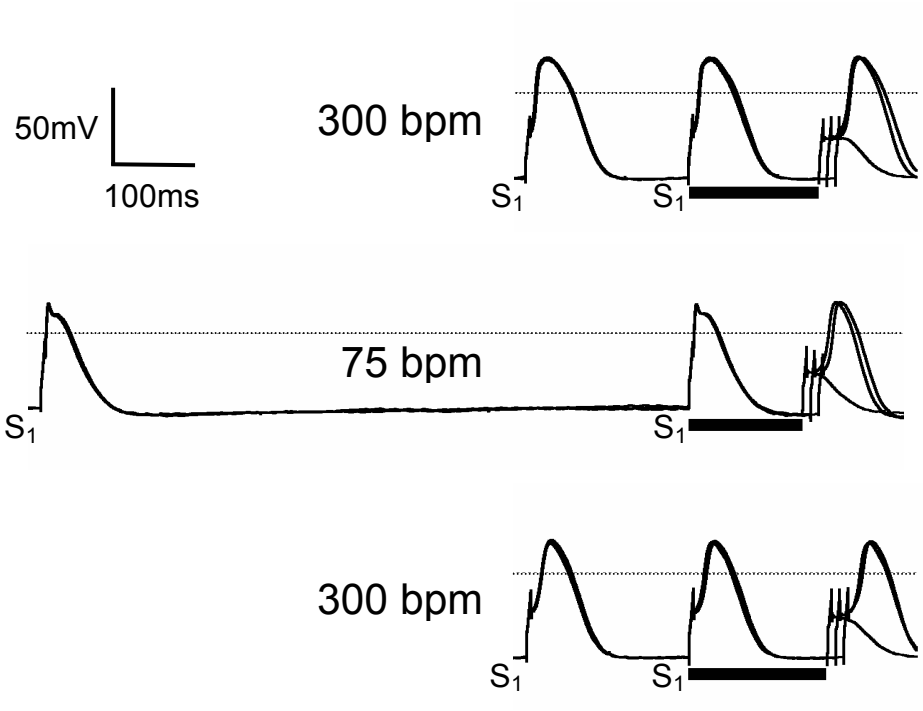


Fig 6

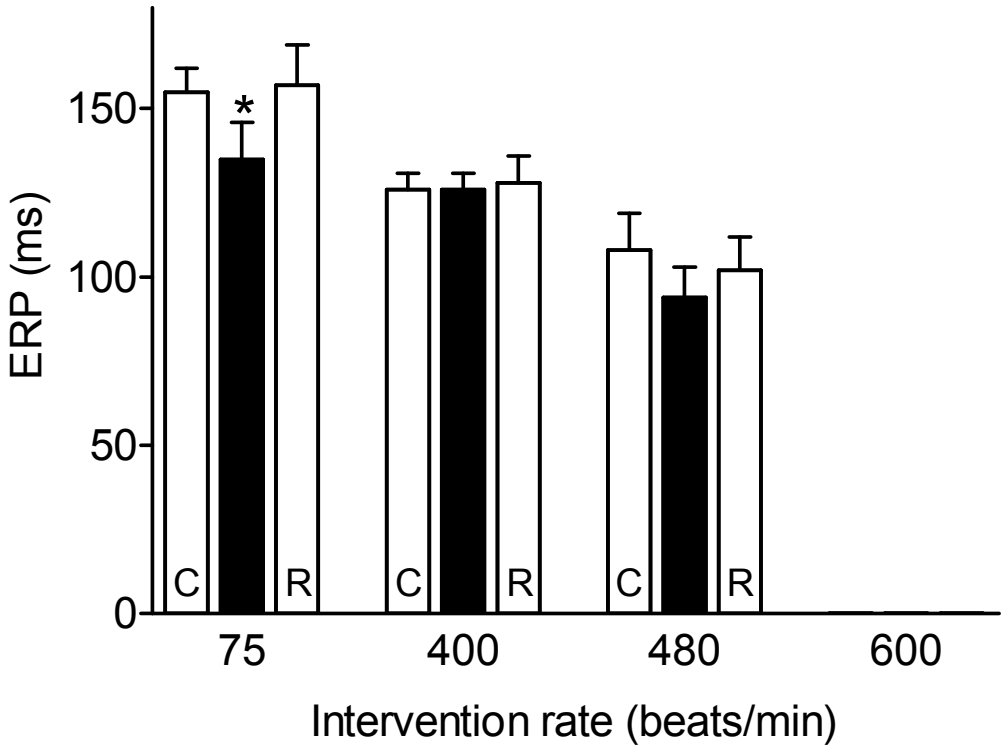

ERP (ms)
150
100
50
0
*
C
R
C
R
C
R
75
400
480
600
Intervention rate (beats/min)

Fig *7A*

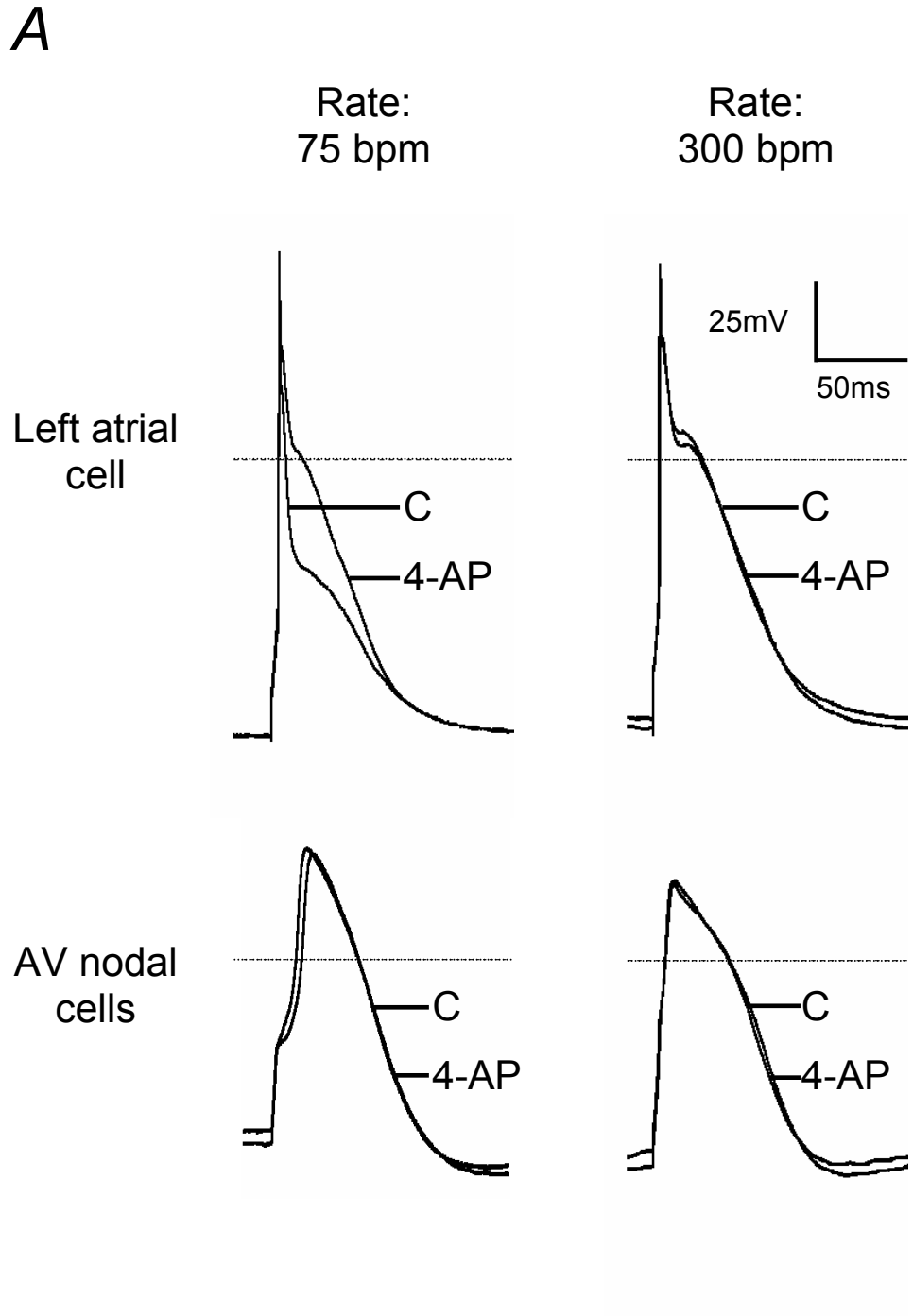
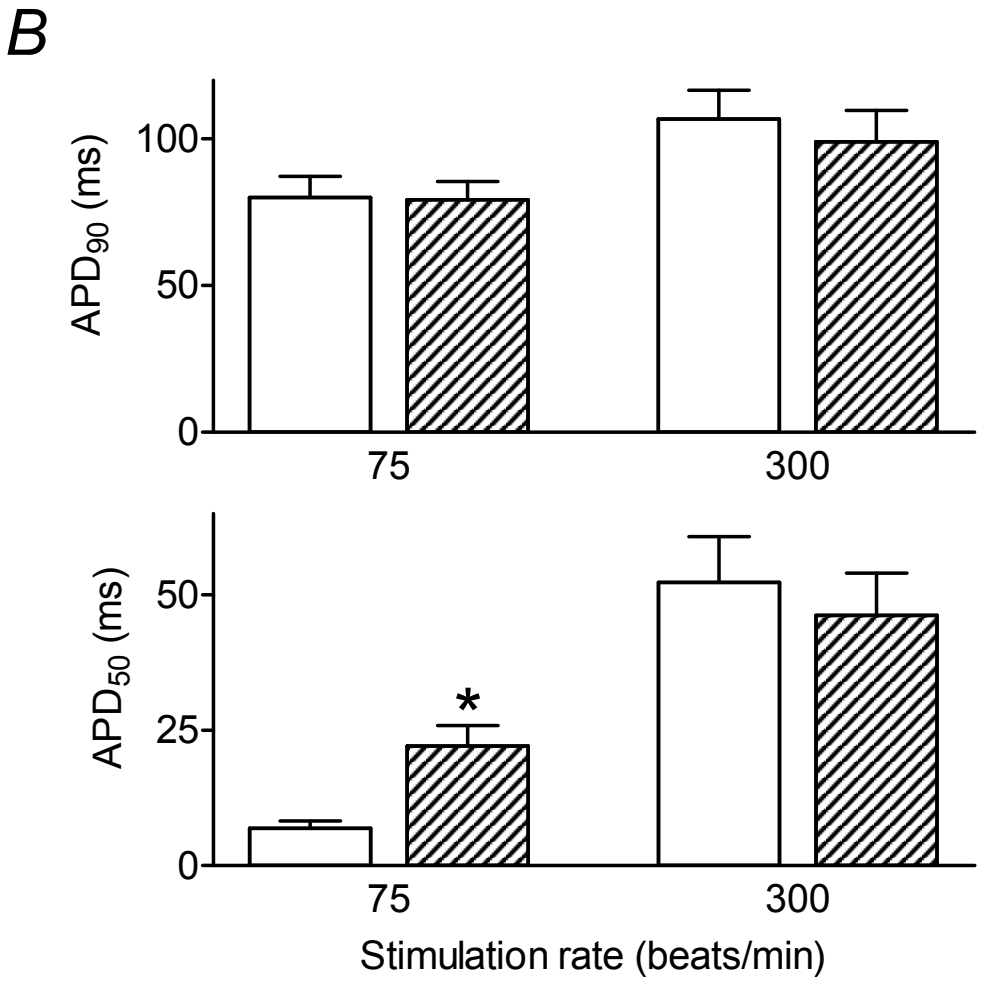

A
Rate:
75 bpm
Rate:
300 bpm
25mV
50ms
Left atrial
cell
C
4-AP
C
4-AP
AV nodal
cells
C
4-AP
C
4-AP

Fig *7B*


B
APD90 (ms)
100
50
0
75
300
APD50 (ms)
50
25
0
*
75
300
Stimulation rate (beats/min)