REFRACTORINESS IN HUMAN ATRIA: TIME AND VOLTAGE DEPENDENCE OF SODIUM CHANNEL AVAILABILITY

Skibsbye et al. Refractoriness in human atria

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ABSTRACT

Background: Refractoriness of cardiac cells limits maximum frequency of electrical activity and protects the heart from tonic contractions. Short refractory periods support major arrhythmogenic substrates and augmentation of refractoriness is therefore seen as a main mechanism of antiarrhythmic drugs. Cardiomyocyte excitability depends on availability of sodium channels, which involves both time- and voltage-dependent recovery from inactivation. This study therefore aims to characterize how sodium channel inactivation affects refractoriness in human atria.

Methods and Results: Steady-state activation and inactivation parameters of sodium channels measured *in vitro* in isolated human atrial cardiomyocytes were used to parameterise a mathematical human atrial cell model. Action potential data were acquired from human atrial trabeculae of patients in either sinus rhythm or chronic atrial fibrillation. The *ex vivo* measurements of action potential duration, effective refractory period and resting membrane potential were well-replicated in simulations using this new *in silico* model. Notably, the voltage threshold potential at which refractoriness was observed was not different between sinus rhythm and chronic atrial fibrillation tissues and was neither affected by changes in frequency (1 vs. 3 Hz).

Conclusions: Our results suggest a preferentially voltage-dependent, rather than time-dependent, effect with respect to refractoriness at physiologically relevant rates in human atria. However, as the resting membrane potential is hyperpolarized in chronic atrial fibrillation, the voltage-dependence of excitability dominates, profoundly increasing the risk for arrhythmia re-initiation and maintenance in fibrillating atria. Our results thereby

highlight resting membrane potential as a potential target in pharmacological management of chronic atrial fibrillation.

INTRODUCTION

Functionally, refractoriness in cardiac cells assists the coordination of electrical activity in the heart, preventing arrhythmias and tonic contractions. Short refractory periods, together with anatomical obstacles and slow conduction velocity, comprise the main determinants for the occurrence of re-entrant arrhythmias. Augmentation of refractoriness has therefore long been posited as the major mechanism for antiarrhythmic drug action [1].

The effective refractory period (ERP) depends on 1) the availability of sodium channels, 2) the balance between de- and repolarizing currents at the cellular level, and 3) the degree of electrical coupling between adjacent myocytes at the tissue level. Absolute refractoriness, on the other hand, refers to the period during which an additional electrical stimulus, regardless of current amplitude, will not lead to a second action potential. This property of the cell is defined by kinetics of the sodium channel and, in particular, recovery from inactivation, which is a time- and voltage-dependent process [2,3]. Recent work in human atrial muscle [4] substantiated earlier findings made in other species [5,6], revealing the maximum upstroke velocity (dV/dt_{max}), which is a surrogate measure for sodium channel availability in tissue, to be very sensitive to membrane voltage. More importantly, the recent findings [4] highlighted the importance of voltage-dependent modulation of excitability in physiologically relevant range: small changes in resting membrane potential (RMP) (phase 4) just preceding the action potential upstroke (phase 0) cause large changes in the dV/dt_{max} value. RMP is 3-5 mV more negative in tissue from chronic atrial fibrillation (cAF) patients vs. patients in sinus rhythm [4,7,8]. This could imply that voltage-dependent parameters of excitability, like increased availability of sodium channels, may facilitate arrhythmias.

During consecutive activity, availability of sodium channels can be reduced because of incomplete recovery from inactivation from the proceeding action potential, causing build-up of channels in the inactivated state. Indeed, time dependence of sodium channel availability has been suggested as an important factor in the understanding of atrial conduction of electrical impulses, particularly at high frequencies, including atrial flutter [9] and fibrillation [10]. These findings highlight the fact that recovery of sodium channels from inactivation follows both time- and voltage-dependent mechanisms.

In this study, we investigate 1) the relationship between dV/dt_{max} and RMP, and 2) voltage and time-dependent characteristics of refractoriness in human atrial muscle. These *ex vivo* data are further complemented with sodium current measurements made in isolated human atrial myocytes to parameterize an *in silico* model of human atrial electrophysiology. By combining *ex vivo* data and computer simulations, we characterise the importance of both time- and voltage-dependent mechanisms of sodium channel availability, which define refractoriness of the atrial action potential.

METHODS

Electrophysiological recordings in human intact cardiac atrial muscle tissue and isolated cardiomyocytes

All work with human samples conforms to the Declaration of Helsinki. The study was approved by the ethics committee of Dresden University of Technology (No. EK790799). Each patient gave written, informed consent. Right atrial appendages (RAAs) were obtained from a total of (n = 43) patients in sinus rhythm (SR) and (n = 16) patients with cAF undergoing coronary artery bypass surgery and/or valve replacement. Action potentials were recorded with standard intracellular microelectrodes in atrial trabeculae as described in [11] and ERP was determined as previously described in [12]. In (n=6) trabeculae experiments increasing concentrations of Ba^{2+} (0.3 µM - 100 µM) were added to the bath solution to decrease RMP, while measuring the concomitant concentration dependent decrease in dV/dt_{max}.

I_{Na} recordings were performed in cardiomyocytes isolated from RAA from (n = 20) patients in SR, as described previously [7]. In brief, borosilicate glass microelectrode pipettes were used to record I_{Na} in whole-cell configuration at room temperature (24±1°C). In order to block remaining L-type Ca currents, 20 µM nifedipine were used in all experiments. Drugs were applied using a fast perfusion system.

Measurement protocols and solutions are described in detail in the Supplementary Methods section.

Computational modelling

The starting point for the computational work is our *in silico* human atrial cell model [13], which recapitulates the human atrial action potential and which we recently extended to include a chronic/persistent atrial fibrillation (cAF) model variant [14]. Briefly, in the present study, we have 1) reformulated the sodium current (I_{Na}), 2) adjusted the transient outward potassium current (I_{to}) and L-type calcium current (I_{CaL}) to obtain a more spike and dome-like AP morphology in 1D tissue strand simulations, 3) extended the model to include the small conductance calcium-activated potassium current (I_{KCa}), and 4) updated the cAF model variant [14] to include disease-related remodelling confirmed recently. Full details of new model components, as well as, all the modifications and additions, including simulation protocols, are described in detail in the Supplementary Methods section.

RESULTS

Steady-state activation and inactivation of I_{Na}

To study sodium channel availability in a physiological setting (including physiological Na^+ concentrations and temperature, 37°C), we measured maximum upstroke velocity (dV/dt_{max}) in human atrial trabeculae, while the resting membrane potential was modulated with I_{K1} block by varying concentrations of barium, up to 1 mM (Figure 1A). The *ex vivo* data clearly illustrate that depolarizing RMP results in reduced upstroke velocity, which may be explained by increased voltage-dependent inactivation of the sodium channels.

This fundamental feature of sodium channel availability is central in recapitulating refractoriness *in silico*. As Figure 1A shows, the existing mathematical formulations of I_{Na} that have been employed in human atrial cell models [15–17] recapitulate the *ex vivo* data rather poorly. To set the parameter values in our new I_{Na} formulation, we used two data sets, published by Christ et al. [18] and Schneider et al. [3]. To our knowledge, these two are the only *in vitro* data sets for which the steady-state properties of native human atrial I_{Na} in isolated cardiomyocytes have been measured using solutions that do not include Fluoride (F^-), which is known to shift voltage dependence. The original Christ et al. [18] *in vitro* data on steady-state activation and inactivation as well as the I_{Na} I-V curve measured in isolated atrial cardiomyocytes is shown in Figure 1B and C. One limitation of these two *in vitro* data sets, however, is that the measurements were performed at room temperature (24°C), rather than physiological temperature (37°C). It has been reported previously that per 10°C increment increase in temperature, the steady-state inactivation curve shifts right by 4.7 mV [19] and 11 mV [20], while the steady-state voltage-dependent activation curve shifts right by 4.3 mV [19] and 5 mV [20]. By accounting for these temperature-dependent shifts,

adjusting the data for the time-dependent drifts in the range of 2-4 mV [3,18], and correcting the data for the shift due to junction potential, we obtain the steady-state activation and inactivation data shown in Figure 1D. Parameters for the new *in silico* model were obtained by first calculating an inverse-SEM weighted average of the two *in vitro* data sets, and shifting the steady-state activation. Additionally, we shifted the curves to the left by 5 mV to obtain a better overall fit with our *ex vivo* data (Figure 3). To validate the parameterization at the cellular level, we simulated the I_{Na} I-V relation in a standard voltage clamp protocol at two temperatures: 24°C and 37°C. As Figure 1E shows, the *in silico* results are nicely in line with the *in vitro* findings.

Taken together, our analysis suggested that in human atrial cardiomyocytes, at physiological temperatures, the $V_{½}$ of inactivation is ≈ -72 mV (Figure 1D), which is close to the RMP reported in human atrial tissue ≈ -74 mV [8]. Figures 1A and E demonstrate that *in silico* results correspond with the *ex vivo* measurements and *in vitro* data by Christ et al. [18]. As the comparisons shown in Figure 1A and supplementary Figure S1 reveal, the new I_{Na} formulation replicates the *in vitro* and *ex vivo* results more accurately than to those used in previously published human atrial cell models [15,21,22].

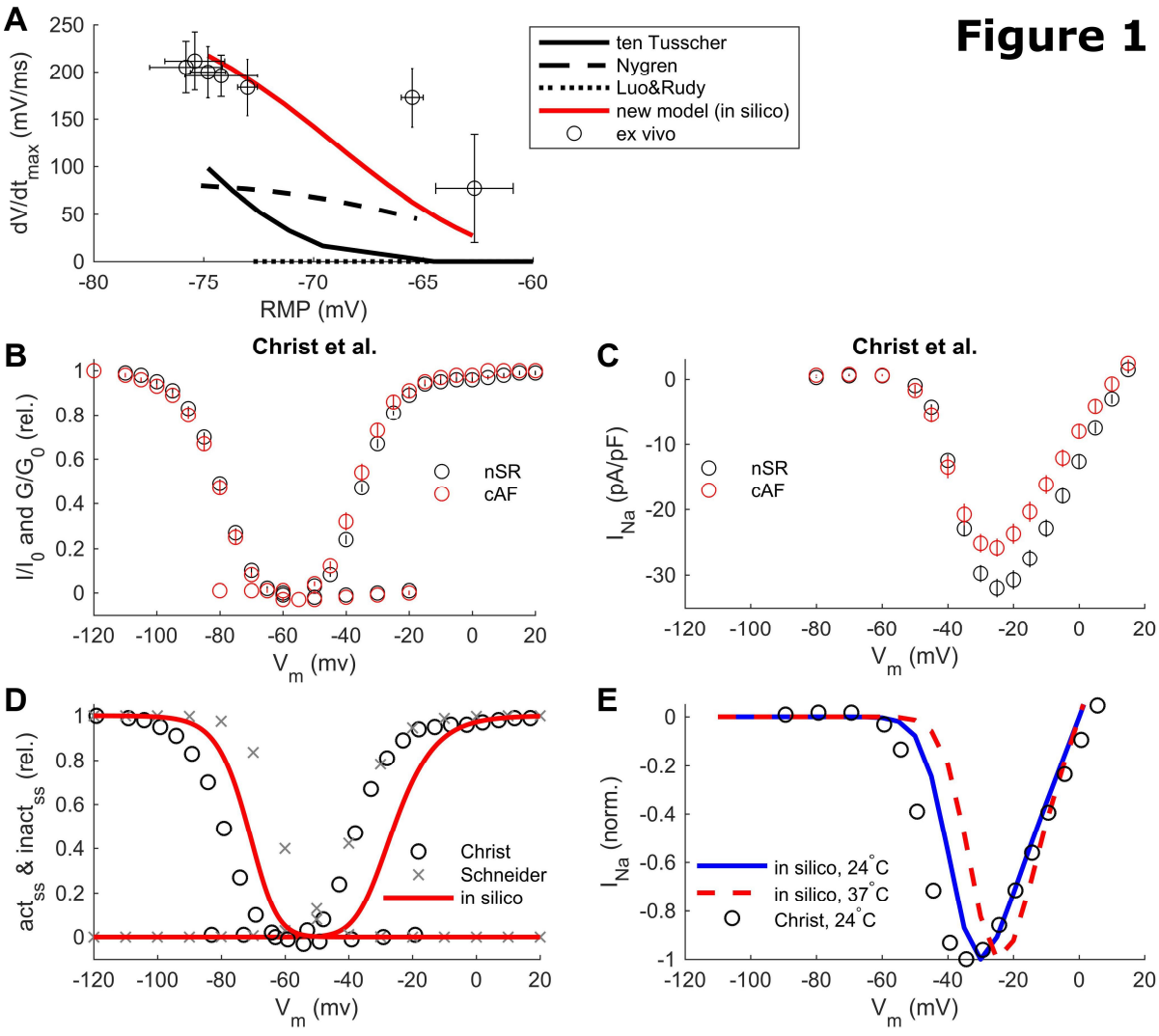

Figure 1

Figure 1. Steady-state properties of I_{Na}. A) *Ex vivo* data showing maximum upstroke velocity (dV/dt_{max}) as a function of RMP, measured in human atrial trabeculae muscle from (n = 6) patients in sinus rhythm. RMP was depolarized by adding increasing concentrations of Ba^{2+} (0.3, 1.0, 3.0, 10, 30 and 100 µM) to the bath solution resulting in a concomitant decrease in dV/dt_{max}. The red line represents *in silico* data simulated with the new I_{Na} model and black lines with previously published models in a 1D tissue strand. B&C) Original *in vitro* I_{Na} data, without corrections, on steady-state activation and inactivation, as well as, I-V curve in normal sinus rhythm (nSR) and chronic AF (cAF) isolated atrial cardiomyocytes. D) Steady-state activation and inactivation data *in vitro* Christ et al. [18] and Schneider et al. [3], as well as the ones incorporated into the new model. The data have been corrected for junction potential, time-dependent shift, and temperature-dependent effects, as explained in detail in the Supplementary Methods section. E) Simulated I-V curves at 24°C and 37°C, as well as *in vitro* data, measured in isolated cardiomyocytes from patients (n = 20) in SR, at 24°C (n = 58/20;

cells/patient for steady-state Inactivation) and (n = 61/20; cells/patient for steady-state activation) from Christ *et al*.

Kinetics of I_{Na} recovery

Recovery of sodium channels from inactivation is a nonlinear, inter-reliant voltage- and time-dependent process. Recovery from inactivation of sodium channels is an important property of excitability, and therefore one of the main mechanisms underlying cardiac refractoriness. Specifically, in the voltage range relevant for the cardiac AP, the voltage dependence of recovery time constants defines the availability of sodium channels following previous activation. According to *in vitro* data from Schneider et al. [3] and Sakakibara et al. [2], recovery becomes increasingly rapid at more negative potentials (Figure 2A and B). As the previously published I_{Na} formulations [15–17] did not recapitulate these findings, we reparameterised the time constants. As the precise speed of recovery is notably temperature-dependent [19], absolute magnitudes have not been employed when incorporating these results into the model. Instead, in the *in vitro* data the focus was on the slope of voltage-dependent recovery time constant, i.e. how steeply the recovery time constant decreases as the potential becomes increasingly hyperpolarized.

To test whether the I_{Na} kinetics that emerge from the new ion current formulation aligned with what has been reported *in vitro*, we simulated a double pulse voltage clamp recovery protocol [3]. Employing the new model, we found that *in silico* recovery kinetics matched well with *in vitro* findings obtained via the same protocol by Schneider et al. [3] (Figures 2C and D). To extrapolate recovery kinetics to a more physiological context, we also simulated the same protocol at 37°C with less negative holding potentials (Figure 2E). The simulation

results suggested that, near the atrial RMP, it takes about 90, 60 and 40 ms for the channel to recover at the holding potentials of -70, -75 and -80 mV, respectively. The effect of faster recovery is striking when considering that at a RMP of -75 mV the time to half I_{Na} recovery is only ≈10 ms. In this respect, the new I_{Na} formulation differs substantially from those previously published, for which the channel recovery time is 2 to 10 times longer (Figure S2 C-E, Supplementary Data).

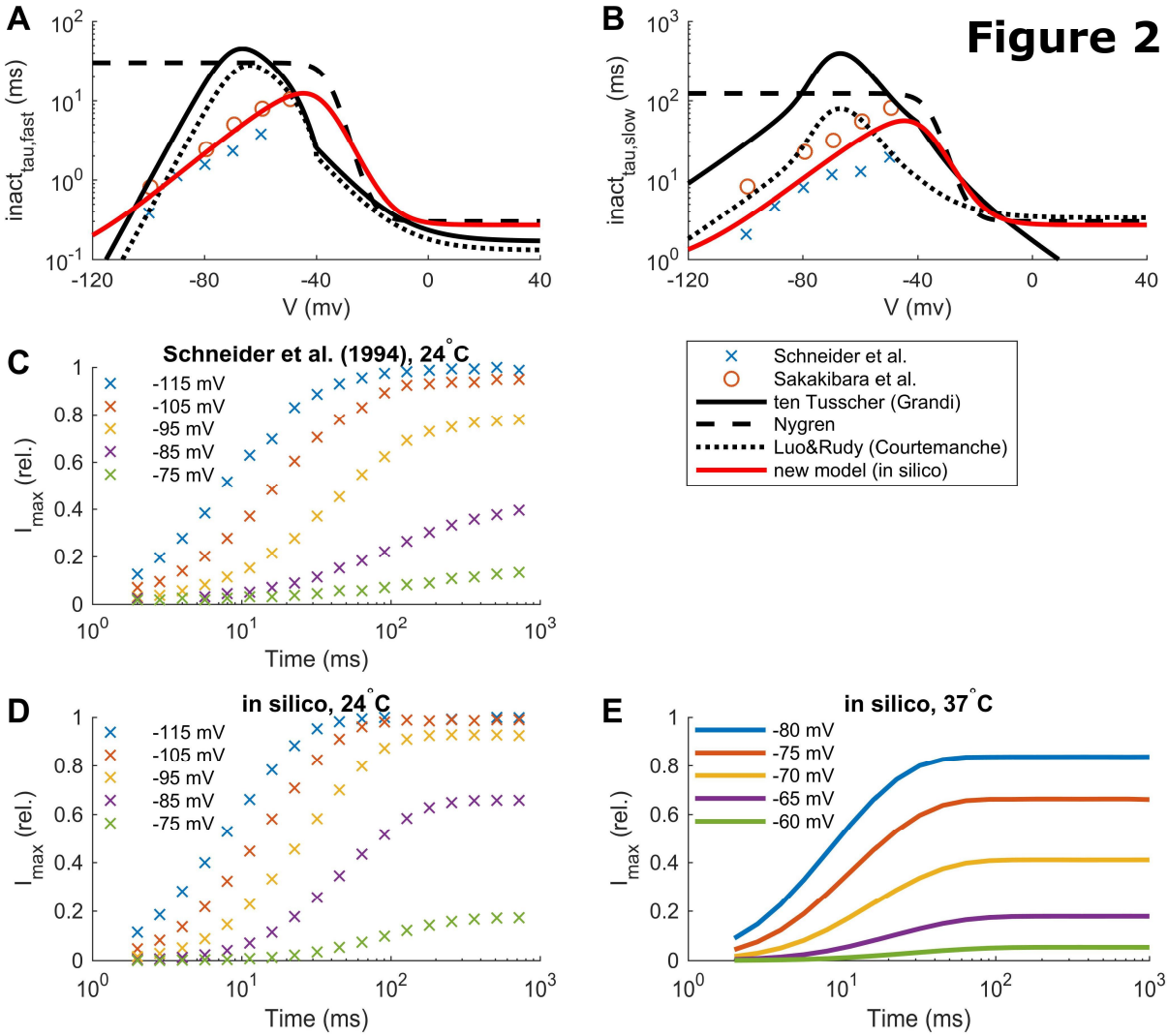

Figure 2. Time-dependent properties of I_{Na}. (A and B) Voltage dependence of fast and slow inactivation and recovery. Previously used formulations in human atrial cell models are shown for comparison. (C-E) I_{Na} recovery in voltage clamp *in silico* and *in vitro* in isolated cells. Lines represent normalised peak I_{Na} elicited by

the second test pulse, P2, to -20 mV after recovery at varying conditioning potentials. For each potential, the conditioning pulse duration (length of interval between the pulses P1 and P2) was increased logarithmically from 2 to 1024 ms. C) I_{Na} recovery *in silico* at 24°C, normalised to -135 mV. D) I_{Na} recovery i*n vitro* at 24°C, as reported by Schneider et al. [3], normalised to -135 mV. E) I_{Na} recovery in the digital cell at 37°C, normalised to -120 mV.

Voltage and time dependence of effective refractoriness in the human atria

To characterise the effect of sodium channel availability and inactivation on action potential refractoriness, intact human atrial muscle *ex vivo* data were included and compared to *in silico* 1D tissue strand simulations (Figure 3A). AP recordings in atrial trabeculae from SR patients revealed spike and dome morphology. Following S1 stimulation, fast depolarization to positive potentials was immediately followed by repolarization to a potential around -20 mV, after which a plateau phase was preceded by slow repolarization to the resting membrane potential (RMP) as described previously [4]. A new AP could be elicited in phase 3 of the action potential by a second current impulse (S2).

These experiments were included to analyse how time- and voltage-dependent mechanisms each contribute to refractoriness. Comparison of Figure 3B and Figure 3E shows the trajectories AP_{amp} and dV/dt_{max} at 1 Hz and 3 Hz frequencies differ substantially, as action potential durations are different and refractoriness essentially is function of APD_{90} (Table 1). However, the voltage thresholds at which a new AP cannot be initiated are not different (Figure 3C). This is around -65 mV at both fast and slow pacing frequencies. These results thereby clearly suggest that within the time-scale of the cardiac AP, at physiological pacing frequencies, the voltage dependence of refractoriness dominates over time dependence. That is, both AP amplitude and maximum upstroke velocity (dV/dt_{max}) dropped along the

same trajectory as a function of voltage for the second (S2) initiated AP, independent of the pacing frequency (1 vs. 3 Hz). Comparison of the *ex vivo* and *in silico* results shows that the emergent properties of the computational model, which is based on the new I_{Na} formulation, to a large extent replicated the behaviour observed *ex vivo*.

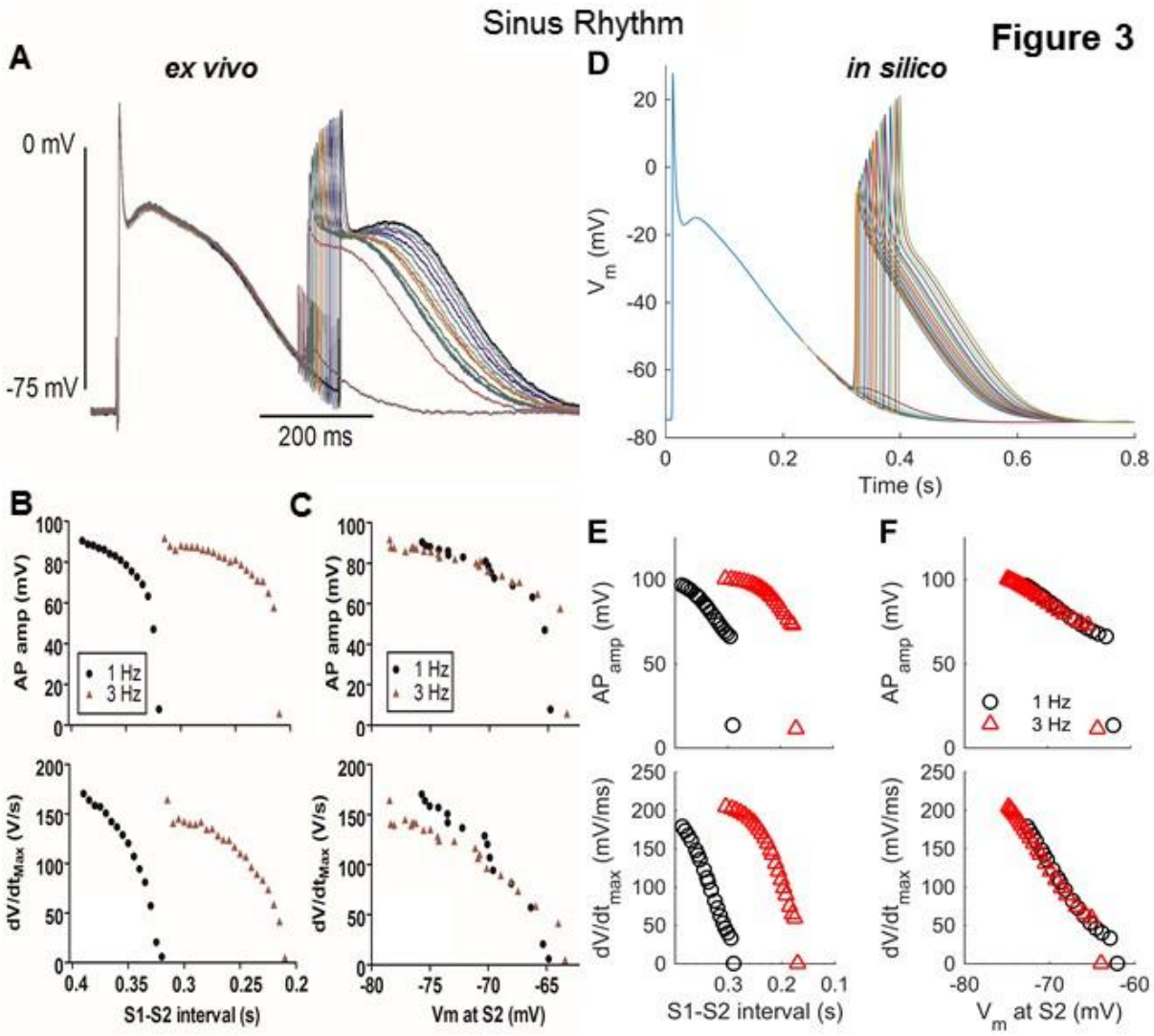

Figure 3. Effective refractory period *ex vivo* in intact human atrial muscle from patients in sinus rhythm (n = 17) and *in silico* in 1D tissue strand, in normal sinus rhythm. A and D) Original data at 1 Hz pacing. Action potential amplitude and maximum upstroke velocity as a function of S1-S2 interval *ex vivo* (B) and *in silico* (E). Action potential amplitude and maximum upstroke velocity as a function of membrane voltage at S2 *ex vivo* (C) and *in silico* (F).

To study the voltage and time dependence of refractoriness in the pathophysiological context of atrial electrical remodelling, we incorporated *ex vivo* results obtained from chronic AF patient atrial muscle and compared these to the *in silico* AF model. As expected, AP morphology recorded in cAF tissue (Figure 4) was considerably changed as compared to SR tissue (Figure 3). RMP was hyperpolarized and a more triangular shape AP with shorter APD was observed as opposed to a characteristic spike and dome morphology. Under the same analysis, results from cAF patients in Figure 4 corroborate the findings for healthy atria (Figure 3). If AP amplitude and dV/dt_{max} are shown as a function of the S1-S2 interval, the trajectories differ substantially (Figure 4B and G). Whereas, these variables follow the same trajectory as a function of voltage at S2, independent of the pacing frequency (1 vs. 3 Hz) (Figure 4 C and H). Interestingly, when comparing the threshold voltages (Figure 4 E and J), at which an S2 stimulus could not initiate a second AP, the values do not differ significantly neither between SR and cAF, nor at slow and fast frequencies (1 Hz vs. 3 Hz). However, refractory time periods (Figure 4 D and I) differ significantly (1 Hz vs. 3 Hz in SR, and 1 Hz vs. 3 Hz in cAF). This finding further confirms the pivotal role of voltage-dependent inactivation, which, at repolarized potentials around -65 mV (Table 1), dominates refractoriness.

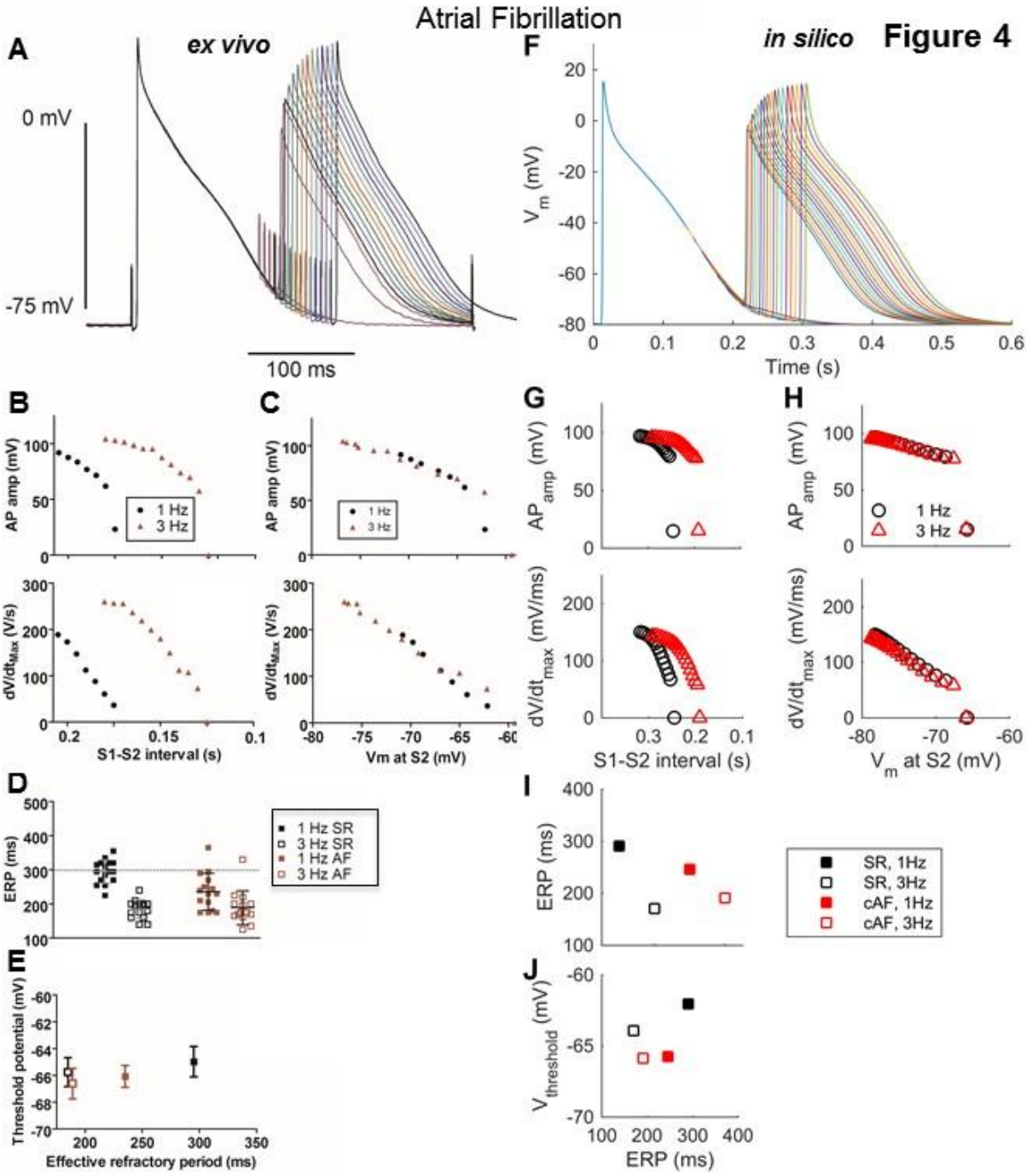

Figure 4. Effective refractory period in chronic atrial fibrillation. Measured *ex vivo* in intact human atrial muscle from patients in chronic AF (n = 16) and *in silico* in 1D tissue strand. A and F) Original data at 3 Hz pacing. Action potential amplitude and maximum upstroke velocity as a function of S1-S2 interval *ex vivo* (B) and *in silico* (G). Action potential amplitude and maximum upstroke velocity as a function of membrane voltage at S2 *ex vivo* (C) and *in silico* (H). Refractory periods recorded in the four *ex vivo* (D) and *in silico* (I). Threshold membrane voltage as a function of effective refractory period (ERP) *ex vivo* (E) and *in silico* (J).

To further analyse the contribution of sodium channel availability to refractoriness, the novel model was employed in several theoretical scenarios (Figure 5). Simulations were performed in the AP clamp mode to elucidate the influence of voltage during an excitation cycle. Traces for sodium channel availability were obtained in three different scenarios. Firstly (panels A-F in Figure 5), we used an AP shape derived from pacing to steady-state at 1 Hz. Secondly, an associated, contrived AP shape representative of pacing at "2 Hz" that was obtained by simply dividing the time vector by two (effectively shrinking the time-axis by a factor of two). We then compared sodium channel availability at 1 Hz, "2 Hz" and a third case, wherein sodium availability was calculated directly from that of the 1 Hz trace by modifying the corresponding time vector (dividing by two). As shown in Figure 5F, the traces of the simulated "2 Hz" AP clamp and that derived by simply halving the time course of the calculated availability at 1 Hz (panels D and E) nearly overlap. This suggests that time dependence of sodium channel recovery from inactivation play a minor role when the kinetics of cardiac AP at normal beating frequencies are considered.

Furthermore, the cAF cell model variant was employed to explore sodium channel availability during fast pacing (Figure 5 panels G-H). The AP of the cAF model was used as a voltage clamp to create a hypopolarised version, wherein the RMP was depolarized by 4 mV, corresponding to the difference in RMP observed in SR vs. cAF tissue [8]. As shown in Figure 5H, this slight depolarization had a strong effect on channel availability (dashed trace). However, even with rapid pacing (4Hz, green trace) availability reached almost identical levels (as compared to 1 Hz, blue trace) during the repolarization phase. Sodium availability only began to decrease at 5 Hz pacing (red traces), recovering to about 78 % of that reached at 1 Hz pacing and dV/dt_{max} was reduced by 22 % (data not shown). At 6 Hz, the capture was lost and APs could not be triggered. This confirms that time-dependent

recovery from inactivation does play a larger role in sodium channel availability at pathologically high pacing frequencies. Furthermore, these results are consistent with previous human atrial *ex vivo* studies, where AF remodelled tissue could follow pacing frequencies until ~6 Hz before starting to loose capture concomitant to an abrupt drop in dV/dt_{max}[7].

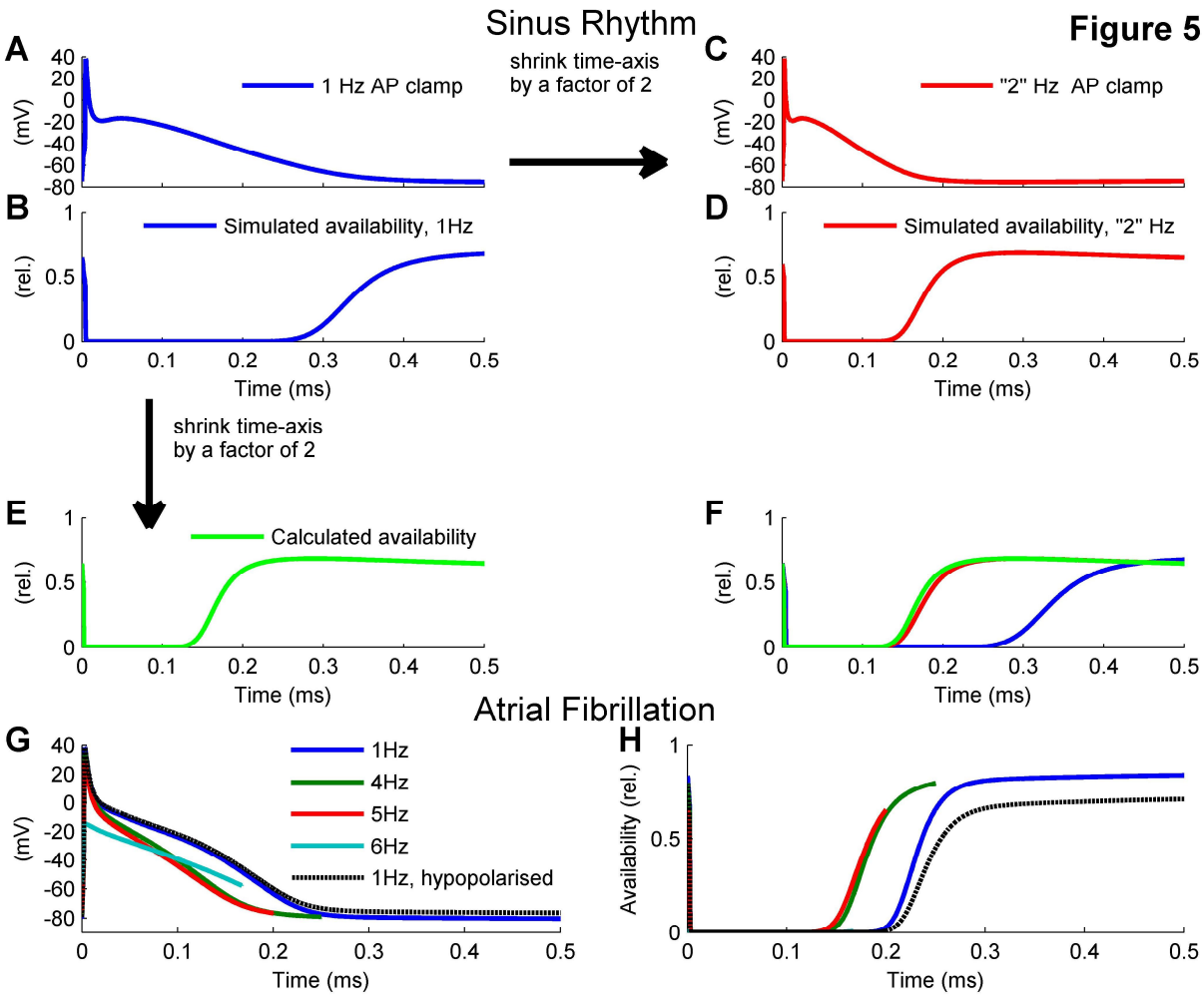

Figure 5. Absolute refractoriness in a single *in silico* cell. AP clamp (A) and simulated availability (B) at 1 Hz pacing. AP clamp (C) obtained by shrinking the time-axis by a factor of two, and corresponding simulated availability (D) at 2 Hz. E) Availability trace obtained from panel (C) by shrinking the time-axis by a factor of two. F) Comparison of the availability in the above three cases. Simulated APs (G) at different pacing frequencies and the corresponding availability traces (H), using the cAF model variant.

We also investigated the role of RMP in 2D spiral wave simulations. Results shown in Supplementary Video S1 indicate that a more hyperpolarized RMP does not necessarily indicate greater excitability in tissue nor potentiality for breakup, but does seem to have a role in arrhythmia entrenchment/spiral wave stability. It appears that compared to control the meander of the spiral wave was increased when RMP was depolarized close nSR value (nSR RMP model variant).

DISCUSSION

Diastolic membrane potential critically affects steady-state I_{Na} availability in a voltage range relevant for human atria

Since sodium channel availability is a key factor in determining myocardial refractoriness, we aimed at establishing an updated *in silico* model replicating atrial I_{Na} availability obtained from analyses of human atrial cardiomyocytes. In the present study, analysis of I_{Na} properties was based on previously unpublished *ex vivo* data, as well as previously published *in vitro* data of our own [18] and from others [3]. Importantly, the strong temperature dependence of the steady-state activation and inactivation properties of the cardiac sodium channel was incorporated in the model [19,20]. Our results suggest that in human atrial cardiomyocytes, at physiological temperatures, the $V_{½}$ of inactivation is ≈ -72 mV: a value close to the RMP reported in human atrial tissue ≈ -74 mV [8]. This implies that even small changes in RMP will have strong influence on I_{Na} availability in atrial cells, and thus on upstroke and conduction velocities in atrial tissue, as changes in the resting potential will shift channel availability on the steep part of the inactivation curve (see Figure 1B). Here the abrupt drop in dV/dt_{max} and I_{Na} availability fall within a range of -62 to -66 mV, nicely recapitulating the findings of AP threshold voltage potential recorded in our experiments of refractoriness (Figures 3 and 4). These data, suggesting a more negatively shifted I_{Na} inactivation curve, are in line with recent findings reported in canine ventricular muscle where it was established that dV/dt_{max} is very sensitive to changes in membrane voltage close to RMP [5]. Furthermore, our results highlight the potential significance of this dominant voltage sensitivity of I_{Na} in atrial fibrillation, where RMP is 3-5 mV more negative

in tissue from cAF patients vs. patients in sinus rhythm, increasing sodium channel availability [4,7,8].

How does the presented *in silico* model compare with previous approaches?

There are three pedigrees of human atrial *in silico* cell models developed by: 1) Nygren et al. [15], 2) Courtemanche et al. [21], and 3) Grandi et al. [22]. Each of these models, and their descendants, employ a different I_{Na} formulation: Nygren et al. [15], Luo&Rudy [16], and ten Tusscher et al. [17], respectively. The steady-state properties of the ten Tusscher I_{Na} differ from the other models in that both the activation and inactivation curves are shifted to more negative potentials, which may stem from the fact that these properties of the model were fitted to *in vitro* data obtained from HEK cells rather than from native cardiac myocytes (Figure S1C and D). The steady-state properties of the new model, particularly voltage dependence of inactivation, are instead closer to those of Luo&Rudy [16] and Nygren et al. [15]. On the other hand, the kinetic properties of I_{Na} in these models differ substantially (Figure 2, S2 and S5) from the new formulation employed here. The most prominent difference is that, in this new model, recovery (or release) from inactivation is 2-10 times faster than in the previously published models. This reflects the importance of temperature dependence, which was not taken into account in previous models.

What emerges from these computational details is none of previous I_{Na} formulations captures the steep decline of sodium channel availability and dV/dt_{max} with depolarized membrane potentials. On the other hand, in the Luo&Rudy [16] and Tusscher et al. [17] I_{Na} formulations the voltage dependence of recovery kinetics does exist. There are, however, differences in the precise properties, which is likely related to temperature-dependent factors and species-specific differences. For example, the Luo&Rudy [16] model was

originally fitted to guinea pig *in vitro* ventricular data, even though it was later adopted to a human atrial cell model by Courtemanche et al. [21].

Voltage dependence of refractoriness dominates over time dependence within a physiological time-scale

When first analysing the *ex vivo* data on ERP in human atrial tissue, it was surprising to find that the values of threshold voltage (Figure 4 E and J, Table 1) at which an S2 stimulus could not initiate a second AP did not differ significantly between SR and cAF nor between pacing rates (1 Hz vs. 3 Hz). However, ERPs (Figure 4 D and I) differ significantly between the same groups when viewed independently. These results are contradictory when compared to the standard paradigm of cardiac refractoriness mechanisms, in which both the time and voltage dependence of sodium channel recovery play a large role [6]. This finding of voltage-dependent dominance in atrial refractoriness is also supported by results obtained by varying Ba^{2+} concentrations (Figure 1). It should, however, be noted that the results obtained with Ba^{2+} are completely void of any time-dependence in channel recovery.

To analyse the contribution of voltage- and time-dependent mechanisms to refractoriness we further employed the new *in silico* model in AP simulations. Comparing the time-course of sodium channel availability in three different AP simulation scenarios (Figure 5 A-F) provided further support for the concept that, *within the time-scale of the cardiac AP and physiological heart rates*, *the voltage-dependent mechanism dominates over time dependence of channel recovery*. This is an important basic physiological finding that has implications also for pathological situations, which we discuss in detail below.

How does refractoriness change in atrial fibrillation?

The properties of I_{Na} appear not to be consistently changed in the electrical remodelling related to cAF. Firstly, the steady-state activation curve is not shifted in cAF [18,23], and the activation and inactivation time constants also remain unchanged [23]. Secondly, both a leftward [18] and rightward [23] shifts of the steady-state inactivation curve have been observed in cAF. This might indicate that these small shifts are related to associated clinical conditions and not to cAF-related remodelling *per se*. On the other hand, a slight decrease in I_{Na} conductance (18-20 %) has been consistently reported in several studies [7,18,24]. Taking together, considering these previous reports, along with the present analysis of *ex vivo* refractoriness showing almost identical threshold potentials in both SR and cAF tissue, it is likely the negative shift of RMP reported in cAF is more relevant for changes in upstroke and conduction velocity than possible changes in biophysical properties of sodium channels.

At above normal physiological frequencies, e.g. during atrial tachycardia, flutter or fibrillation, time-dependent parameters of refractoriness may indeed be very relevant. Results published recently by Wettwer and colleagues [7] demonstrated that, when atrial trabeculae muscle paced at 5 Hz (for SR) and 6 Hz (for cAF), upstroke velocity decreases to such an extent that excitation failure occurs. The authors describe excitation failure at these frequencies as being a result of partial I_{Na} blockage, which is both time and frequency-dependent. For several reasons, cAF tissue is prone to follow faster frequencies: one key reason is shorter AP duration, resulting in longer diastolic intervals and more time for sodium channels to recover. Secondly, RMP is more hyperpolarized due to electrical remodelling, which likely plays an important role in both time- and voltage dependant sodium channel recovery from inactivation.

To this end, the presence and degree of hyperpolarization of RMP in cAF could become an additionally important factor in the ability of atrial tissue to support repetitive firing (ectopic foci) or to sustain multiple re-entrant arrhythmias. This becomes relevant when time and voltage-dependant parameters of sodium channel availability are influenced by the hyperpolarized RMP accompanied by shortened action potential duration. Such mechanisms might be important in both the initiation and perpetuation of atrial arrhythmias, by providing a substrate for early reactivation (re-entry) in atrial tissue. From the perspective of pharmacological management of cAF, our results highlight potential new avenues: a combination drug therapy targeting RMP and APD might prove beneficial.

Refractoriness and conduction velocity in re-entry

A clear relationship between refractory period, conduction velocity and functional wavelength exists [25]. According to the classical leading-circle theory [26], these two electrophysiological parameters are considered independent factors (WL = CV x ERP). This theory might again be put up for re-evaluation, taking into account both experimental results and present understanding of antiarrhythmic mechanisms. In the review by Comtois *et al.* [27], the authors note limitations of the leading circle model in that "*it does not consider key biophysical properties like electrotonic interaction, complex properties of the medium, source-sink relations and dynamic core behaviours"*. This paradigm is especially limited when applied towards a mechanistic understanding of the antiarrhythmic effects of Na^+ channel blockers, which the theory would predict to favour re-entry by shortening the functional wavelength.

More recent models of re-entry predict that changes, which prolong or cause heterogeneous ERP, do indeed increase source-to-sink mismatch in excitation, in this way

slowing conduction velocity. As we recently reported [25], slowing of CV was mirrored by reversed changes in ERP, opposing wavelength shortening by actually prolonging the theoretical spatial wavelength. This concept could explain the relatively high efficiency of sodium channel blockers in the abolishment of re-entrant arrhythmias. Sodium channel blockers shift the steady-state inactivation curve to more negative potentials [28]. Thus, it requires more hyperpolarized potentials to reach the critical degree of sodium channel recovery to produce the next action potential. Massive reduction in sodium channel availability reduces excitation through simultaneously increasing refractoriness and decreasing conduction velocity, which, in synergy, cause re-entry to become unsustainable. To this end, time-dependence of recovery from inactivation might be an important factor in understanding the pro-arrhythmic effects of pharmacological treatment. Classical sodium channel inhibitors prefer to bind to the channel in its inactivated state, and thus channel recovery from inactivation is very much dependent on the dissociation kinetics of that particular drug [29]. In the case of I_{Na} inhibitors exhibiting slow binding kinetics, it is reported that the ½ recovery time-intervals could be increased up to a 100 fold (from 10 – 1000 ms) [29].

LIMITATIONS OF THE STUDY

There are methodological limitations that should be taken into account in consideration of the results of this study. Due to difficulties in proper voltage-clamp control close to physiological temperatures, I_{Na} recordings in isolated human atrial myocytes were performed at 24°C [18]. However, the modified activation and inactivation properties of the presented *in silico* model are a substantial improvement and of physiological relevance as compared to previous mathematical formulations, which were based on *in vitro* data

obtained either in 1) non-native cells (expression systems) [19], 2) less reliable measurement conditions (solutions containing Fluoride, F^-)[2], or 3) non-human cells (guinea-pig cardiomyocytes) [16]. We have not accounted for the electrical heterogeneity known to be present in the human atria, which should be covered in future studies. Also, the presented *in silico* model does not fully recapitulate the AP plateau characteristics observed *ex vivo*. This limitation is related to the properties fast potassium currents (I_{to} and I_{Kur}) and future work will address this in more detail.

CONCLUSIONS

Refractoriness of the atrial myocardium is a critical parameter, both in sustaining normal sinus rhythm and in understanding the development and maintenance of atrial arrhythmias. Our results from combined *in vitro*, *ex vivo* and *in silico* experimentation suggest that voltage dependent recovery from inactivation represents the dominant mechanism defining sodium channel availability at physiologically relevant rates and temperatures in healthy human atria. It is only at high activation rates that the time-dependence of channel recovery becomes a contributing factor. Hence, as the resting membrane potential is hyperpolarized to different degrees in chronic AF vs. SR tissue, this voltage-dependent dominance of excitability will almost certainly influence the risk of re-initiating and maintaining arrhythmia in fibrillating atria. Our results thus highlight RMP as a potential target in pharmacological management of chronic AF which merits further investigation.

TABLES

Table 1. *Ex vivo* vs. *in silico* AP data from SR and cAF at 1 Hz and 3 Hz (mean ± SEM). P value are statistically tested between 1 and 3 Hz *ex vivo* data using students paired T-test. *In silico* data was measured from 1D tissue strand simulations.

		ex vivo		***in silico***	
SR		**1 Hz (n=17)**	**3 Hz (n=16)**	**1 Hz**	**3 Hz**
Threshold	(mV)	-64.6 ± 1.2	-66.0 ± 1.1 (p=0.40)	-62.0	-63.9
APD_{50}	(ms)	134.3 ±12.2	95.2 ± 12.7 (p>0.05)	93.4	22.7
APD_{90}	(ms)	285.2 ±11.9	180.0 ± 12.1 (p>0.001)	297.5	165.3
ERP	(ms)	295.0 ± 8.5	184.7 ± 7.0 (p>0.0001)	290.3	170.1
RMP	(mV)	-74.6 ± 0.7	-75.9 ± 0.7(p >0.05)	-75.2	-75.5
dV/dt_{max}	(V/s)	209.3 ±14.1	223.8 ± 25.5 (p=0.21)	217.5	209.1
APA	(mV)	93.8 ± 1.9	93.0 ± 2.4 (p=0.80)	103.0	101.1
cAF		**1 Hz (n=16)**	**3 Hz (n=14)**	**1 Hz**	**3 Hz**
Threshold	(mV)	-65.8 ± 0.4	-66.6 ± 1.2 (p=0.56)	-65.7	-65.9
APD_{50}	(ms)	105.6 ± 16.1	98.3 ± 13.2 (p=0.24)	110.5	95.0
APD_{90}	(ms)	211.6 ± 9.4	176.8 ± 10.1 (>0.001)	203.1	193.4
ERP	(ms)	209.2 ± 13.9	189.5 ± 13.2 (p>0.05)	245.5	191.1
RMP	(mV)	-77.1 ± 0.8	-77.7 ± 0.9 (p=0.62)	-80.1	-80.9
dV/dt_{max}	(V/s)	224.1 ± 5.1	220.2 ± 18.6 (p=0.51)	160.9	148.0
APA	(mV)	102.4 ± 2.6	97.5 ± 2.5 (p=0.18)	98.5	96.3

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DISCLOSURES

None.

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