Targeting Atrio-Ventricular Differences in Ion Channel Properties for Terminating Acute Atrial Fibrillation in Pigs

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ABSTRACT

Aims. The goal was to terminate atrial fibrillation (AF) by targeting atrio-ventricular differences in ionic properties.

Methods. Optical mapping was used to record electrical activity during carbachol (0.25-0.5 µM)-induced AF in pig hearts. The atrial specific current, \( I_{Kur} \), was blocked with 100 µM 4-aminopyridine (4-AP) or 0.5 µM DPO-1. Hearts in AF and ventricular fibrillation (VF) were also subjected to increasing levels of extracellular K\(^+\) ([K\(^+\)]\(_o\): 6-12 mM), compared to controls (4 mM). We hypothesized that due to the more negative steady-state half inactivation voltage for the atrial Na\(^+\) current, \( I_{Na} \), compared to the ventricle, AF would terminate before VF in hyperkalemia. Mathematical models were used to interpret experimental findings.

Results. \( I_{Kur} \) block did not terminate AF in a majority of the experiments (6/9 with 4-AP; ¾ with DPO-1). AF terminated in mild hyperkalemia ([K\(^+\)]\(_o\) <= 10.0 mM; N=8). In contrast, only 2/5 VF episodes terminated at the maximum ([K\(^+\)]\(_o\): 12 mM [K\(^+\)]\(_o\)). \( I_{Kur} \) block did not terminate a simulated rotor in cholinergic AF because its contribution to repolarization was dwarfed by the large magnitude of the acetylcholine-activated K\(^+\) current (\( I_{K_ACh} \)). Simulations showed that the lesser availability of the atrial Na\(^+\) current at depolarized potentials, and a smaller atrial tissue size compared to the ventricle, could partly explain the earlier termination of AF compared to VF during hyperkalemia.

Conclusions. \( I_{Kur} \) is an ineffective antiarrhythmic drug target in cholinergic AF. Manipulating Na\(^+\) current “availability” might represent a viable antiarrhythmic strategy in AF.

KEYWORDS: Atrial fibrillation, \( I_{Kur} \), hyperkalemia, \( I_{Na} \) availability
INTRODUCTION

Atrial fibrillation (AF) is the most common sustained arrhythmia, and enhances the risk of stroke and heart failure.\textsuperscript{1} Although non-pharmacological options such as ablation and surgery are increasingly used, antiarrhythmic drugs constitute the main treatment option.\textsuperscript{1} However, their effectiveness has been unsatisfactory, and in some cases harmful due to side effects.\textsuperscript{2} An additional problem that is associated with drugs is that in some cases they precipitate fatal ventricular arrhythmias.\textsuperscript{2} As a result, extensive research has been directed towards finding drug targets that are exclusively present in the atria and not the ventricles.\textsuperscript{3}

It is now clear that distinct differences exist between the atria and the ventricles in terms of the ion channel currents and their underlying transcripts in many species including humans.\textsuperscript{4} A K\textsuperscript{+} current that is important in human atrial repolarization, but absent in the ventricle, i.e. the ultra-rapid delayed rectifier current, $I_{\text{Kur}}$, is currently a target for development of newer antiarrhythmic drugs to treat AF.\textsuperscript{2,3} However the results thus far have been highly controversial, with studies suggesting that blockade of $I_{\text{Kur}}$ can either be useful in terminating AF,\textsuperscript{5} or actually be pro-arrhythmic.\textsuperscript{6} Furthermore, recent studies have also identified important differences between the biophysical characteristic of the Na\textsuperscript{+} channel ($I_{\text{Na}}$) between the atria and ventricles, with the atrium displaying a more negative steady-state half inactivation membrane voltage properties.\textsuperscript{7} This study has opened up another interesting avenue for exploiting atrio-ventricular differences and targeting AF.\textsuperscript{8}

Our objective was to study acute, carbachol-induced AF in isolated pig hearts, and probe for the usefulness of both the above mentioned ion-channel based anti-arrhythmic approaches. In experiments, we studied the effectiveness of $I_{\text{Kur}}$ block in AF via selective blockers of this current: 4-aminopyridine (4-AP) and DPO-1. We also postulated that the inherent differences in atrio-ventricular Na\textsuperscript{+} channel steady-state inactivation properties could be exploited by
manipulating the concentration of extracellular K+ ions ([K+]o). We hypothesized that for mild increases in [K+]o (hyperkalemia), the atrial I_{Na} would be more depressed than its ventricular counterpart as a result of its more negative half-inactivation value, resulting in the termination of acute AF, but not ventricular fibrillation (VF). To interpret our experimental results, we also utilized concomitant computer simulations. We reformulated the equations for I_{Kur} that now take into account its rate-dependent attenuation in magnitude.9-11 The updated ionic model of an atrial cell12 was incorporated in a 2D sheet to simulate spiral waves that underlie functional reentry (rotors) and study the ionic mechanisms as in a previous study.13

Our results indicate that I_{Kur} is not a viable antiarrhythmic target for stopping acute, cholinergic, high frequency AF in pigs. In contrast, mild hyperkalemia effectively terminates AF, but not VF in this model.
METHODS

Experiments

This investigation conformed to US NIH Guidelines for the Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 1996). Male pigs (20-25 Kg) were anesthetized initially by a combination of xylazine (2.2 mg/Kg) and telazol (6 mg/Kg), followed by thiopental (10 mg/Kg) or propofol (6 mg/Kg). Hearts were excised and Langendorff-perfused with Tyrode’s solution whose composition in mM was: NaCl, 130; KCl, 4.0; MgCl2, 1; CaCl2, 1.8; NaHCO3, 24; NaH2PO4, 1.2; glucose, 5.6; albumin 0.04 g/L. The Tyrode’s solution was gassed with 95%O2-5%CO2, filtered using a Liliput hollow fiber oxygenator (COBE Cardiovascular, Arvada, CO) and perfused at a constant flow rate of 150-200 ml/minute, at 36 ± 1.0 °C. After an initial perfusion period of 15 minutes, blebbistatin (5-10 µM) was added to the perfusate to abolish motion of the heart. AF was then induced via electrical burst pacing (50-100 ms cycle length, ≈10 second duration) or a battery in the presence of Carbachol (0.25-0.5 µM) with 100% success, and the frequency of excitation was mapped for 10 or 20 minutes (control), before adding 4-AP (100 µM) to selectively block Ikur (for another 10 minutes). The perfusate containing the carbachol and/or 4-AP was never re-cycled. Movies of di-4-ANNEPS (10 µM) fluorescence during AF were obtained from the left atrium (LA) and the right atrium (RA) using a Little Joe CCD camera (Scimeasure; 80 by 80 pixels, 500-1000 frames per second, area of the field of view ≈ 3 cm²). Additionally 2 electrograms were simultaneously recorded in the LA and RA to ascertain the presence of either a normal sinus rhythm or AF. Dominant frequency (DF) maps during AF (in control; or in presence of carbachol or carbachol+4-AP) were constructed as in previous studies,14 and the maximum DF (DFmax) was quantified as a function of time. Dominant frequency analyses allows for the quantification of how fast the functional reentrant activity is at each point on the heart surface in time domain during fibrillation, and DFmax measures the fastest fibrillatory activity. In an additional series of experiments, we investigated the effect of
another selective blocker of I_{Kur}, DPO-1.\textsuperscript{15} We first studied the effect of DPO-1 on the atrial and ventricular action potential durations (APD) during pacing and also its effect on carbachol-induced AF, and VF dynamics.

A second set of experiments studying the effect of hyperkalemia (6 or 8 mM of K\textsuperscript{+} ([K\textsuperscript{+}]_o; control 4mM) on carbachol-induced AF was carried out after completing the 4-AP study, by perfusing hearts for 30 min with normal, non-recycled carbachol-free Tyrode’s solution (N=4) to washout all drugs (carbachol, 4-AP).

In a third series of experiments (N=5), carbachol-induced AF and VF was simultaneously mapped in the LA and the left ventricle (LV). The hearts were subsequently perfused with increasing values of [K\textsuperscript{+}]_o, either 6, 8, 10, or 12 mM for approximately 10 minutes at each concentration (compared to 4 mM in controls).

All data are shown as mean ± SEM. For comparing APDs, repeated two-way ANOVA with Bonferroni post-test was used. For comparing dominant frequencies for AF/VF in control and drug conditions, unpaired student’s t-test was used; p < 0.05 was considered statistically significant.

**Simulations**

To investigate the effects of blocking I_{Kur} and changing [K\textsuperscript{+}]_o on reentry dynamics, we used a modified human atrial cell ionic model\textsuperscript{12} incorporated in a 2D sheet to simulate a rotor, as in a previous study.\textsuperscript{13} Research in the last two decades has firmly established that transient or stable spiral waves, also called rotors, sustain cardiac fibrillation, and represent a useful way to study the underlying ionic properties\textsuperscript{16} The specific model modifications and simulations are discussed in detail in the Results section.
RESULTS

Experimental: We induced acute AF in 9 hearts in the presence of carbachol. We recorded the activation frequencies for 10 minutes in control conditions, before perfusing 100 µM 4-AP for another 10 minutes (In 3 hearts we ascertained that AF was sustained continuously, and thus stable for 20 minutes, i.e. for the duration of most of our 4-AP experiments). Fig. 1A depicts a representative DF map during acute AF in the pig heart. Similar to previous studies in sheep \(^{17}\) we found that the DF\textsubscript{max} was larger in the LA compared to the RA; this was the case in a majority of the DF maps constructed (≈80%). The 1-sec single pixel recordings from LA and RA shown below the maps also demonstrate the faster activation of the electrical excitation in the LA. The variation of DF\textsubscript{max} with time in control conditions, and in presence of the drug, 100 µM 4-AP is shown in Fig. 1B for 6 experiments (each set of symbols represents one experiment) in which AF did not terminate. Statistical analyses of all the movies in these 6 hearts showed that there was a very small but significant increase in the DF\textsubscript{max} after addition of the drug compared to control frequencies (Control: 20.97 ± 0.26 Hz n =79; N=6; Drug 4-AP: 21.98± 0.35 Hz; n =65; N=6; p<0.05). In the remaining 3 experiments, AF did terminate in 2/3 after addition of 4-AP; however in 1 experiment, sustained AF could be re-induced even in the presence of 4-AP.

We also tested the effects of DPO-1 on pig cardiac electrophysiology. First, the effect of DPO-1 on action potential durations at 50% and 75% repolarization (APD\textsubscript{50}, APD\textsubscript{75}) was measured at a pacing cycle length of 300 ms (in absence of carbachol). Representative atrial (Fig. 2A) and ventricular action potentials (Fig. 2B) are shown in control (solid) and in the presence of 0.5 µM DPO-1 (dashed). The average effects of DPO-1 (0.5, 1.0 and 5.0 µM) on APD\textsubscript{50,75} in left atrial (Fig. 2C), and left ventricular (Fig. 2D) tissue are shown. The results show that at 0.5 µM concentration, the pig atrial APD is significantly prolonged (Control: APD\textsubscript{75} = 114.95± 6.125 ms; DPO-1: APD\textsubscript{75} = 136.27 ± 5.24 ms; N=3, p<0.05). An additional increase in concentration (1.0
and 5.0 μM) did not increase the atrial APD any further (APD75: 141.53 ± 10.33 ms and 143.86 ± 8.87 ms respectively). The ventricular APDs were not significantly altered by DPO-1 (Control: APD75 = 203.12 ± 1.95 ms; DPO-1, 0.5 μM: APD75 = 209.43 ± 3.15 ms; DPO-1, 1.0 μM: APD75 = 205.09 ± 2.7 ms; N=3, p=NS). We then tested the effect of 0.5 μM DPO-1 on carbachol-induced AF (Fig. 2E) and VF (Fig. 2F); AF was not terminated in ¾ experiments, whereas, VF did not terminate in 4/4 experiments. In the 3 experiments where AF did not terminate, DPO-1 (0.5 μM) decreased the Dfmax slightly, but significantly (Control: 23.06 ± 0.72 Hz, n =62; N=3; Drug DPO-1: 20.61 ± 0.69 Hz; n =32; N=3; p<0.05). There was no difference in VF frequencies (Control: 14.11 ± 0.22 Hz, n =40; Drug DPO-1: 13.68 ± 0.12 Hz; n =81; N=4; p=NS). In additional experiments, we subjected hearts in AF/VF to higher concentrations of DPO-1, i.e. 1.0 and 5.0 μM (Fig. 2G and Fig. 2H). At the highest concentration of DPO-1 (5.0 μM), AF terminated in 4/4 experiments, whereas VF did not. Our analyses show no effect of DPO-1 on atrial conduction velocities at all concentrations (data not shown). Therefore these results suggest that at higher concentrations, DPO-1 also blocks the acetylcholine-dependent K⁺ channel, Ik,ACh (activated by carbachol).

In a second series of experiments, hearts in AF were subjected to mild hyperkalemic conditions, with [K⁺]o values increased to either 6 or 8 mM, compared to 4 mM (controls). In 4/5 experiments, AF terminated spontaneously, and in 2 experiments, AF converted to an atrial tachycardia before termination (data not shown).

To study the effect of hyperkalemia on cardiac fibrillation more systematically, in our last series of experiments, both AF and VF were induced and mapped simultaneously. The pig hearts were perfused with Tyrode’s solution containing varying [K⁺]o, viz. 4,6,8,10 and 12 mM. Fig 3A presents representative DF maps, along with Dfmax values in LA and LV, at 4 and 6 mM [K⁺]o.
VF was relatively stable at each individual \([K^+]_o\), and the \(DF_{\text{max}}\) decreased gradually with increasing \([K^+]_o\). The \(DF_{\text{max}}\) in AF was relatively stable at 4 and 6 mM \([K^+]_o\). However, it decreased rapidly during the 10 minute perfusion of 8 mM \([K^+]_o\), as illustrated by DF maps in the initial, middle, and final periods at that \([K^+]_o\). In addition, AF became more organized, converting to atrial tachycardia (ATach) in the final stage, and subsequently terminating during transitioning from 8 to 10 mM \([K^+]_o\). Composite data from all 5 experiments in which AF and VF were mapped simultaneously at varying \([K^+]_o\) are quantified in Fig. 3B. The rate of decrease in \(DF_{\text{max}}\) as a function of \([K^+]_o\) is seen to be different between AF and VF, with a much steeper slope in the former. Overall, when the hyperkalemia experiments were pooled together, the value of \([K^+]_o\) for AF termination was always \(\leq 10\) mM (N=8). In comparison, VF only terminated in 2/5 cases at 12 mM \([K^+]_o\); in 3/5 cases, VF did not terminate even at 12 mM \([K^+]_o\).

**Numerical:** For simulations, \(I_{Kur}\) inactivation in the human atrial model\textsuperscript{16} was re-formulated to include a weighted inactivation of fast and slow gating variables as follows:

\[
I_{Kur} = G_{Kur} u_a^f (u_{if} + bu_{ls})(V - E_K),
\]

(1)

where \(G_{Kur}\) [ns/pF] is the maximal channel conductivity, \(u_a\), \(u_{if}\) and \(u_{ls}\) are activation, and fast and slow inactivation gating variables, respectively, \(a\) and \(b\) are weighting coefficients, \(V\) [mV] is the transmembrane voltage, and \(E_K\) is the potassium reversal potential. The separation of inactivation into fast and slow components was done to reproduce the biexponential recovery of \(I_{Kur}\) that was observed in porcine atrial cells.\textsuperscript{15} The following set of equations and parameters were used for the calculation of (1):

\[
G_{Kur} = 0.005 + \frac{0.05}{1+\exp\left(-\frac{V-E_K}{10}\right)}
\]

\[
u_a = \frac{e^{\frac{V-E_K}{\nu_a}}}{1+e^{\frac{V-E_K}{\nu_a}}}, \quad a \in \{a, if, ls\}
\]

\[
u_a^{cc} = \frac{1}{1+\exp\left(-\frac{V-E_K}{\nu_a}\right)}
\]
\[\alpha_{u_2} = 0.65 \frac{1}{\exp\left(\frac{-V_{ma}}{n}\right) + \exp\left(-\frac{V_{ma}}{n}\right)}\]

\[\beta_{u_2} = 0.65 \frac{1}{0.5 + \exp\left(\frac{V_{ma}}{1}\right)}\]

\[\tau_{u_2} = \frac{1}{n \alpha_{u_2} + \beta_{u_2}}\]

\[u_{i_2} = u_{i_2}^{\infty} = \frac{1}{1 + \exp\left(-\frac{V_{ma}}{5}\right)}\]

\[\tau_{ui} = 400 + 1068\exp\left(-\left(\frac{V}{50}\right)^2\right)\]

\[\tau_{u_i} = 2000 + 60000\exp\left(-\left(\frac{V+20}{50}\right)^2\right)\]  \hspace{1cm} (2)

and \((a, b) = (0.25, 0.75)\).

Figure 4A shows the fast and slow inactivation rate constants as a function of voltage. The dashed line represents the calculated values per eq. (2), and the filled circles show experimental data. The rate dependence of the normalized \(I_{Kur}\) is shown in Fig. 4B, using a pacing protocol adapted from Feng et al. (their figure 5). We employed 50-ms long pacing pulses rather than the 100- or 200-ms that were originally used to account for the shorter action potential duration in swine atrial myocytes compared to human cells. An exponential decay in the relative current (i.e. current magnitude following the test pulse divided by the maximal current magnitude at the lowest frequency of 0.1Hz) is demonstrated, with the current magnitude decreasing to about 10% at 19Hz compared to its 0.1Hz-pacing value. Additional modifications that were employed to adapt the human atrial ionic model to the swine kinetics were the inclusion of a more detailed calcium handling, the modification of \(I_{K1}\) conductance by \([K^+]_o\), and the replacement of the transient outward current, \(I_{to}\), by a calcium-activated \(I_{to2}\) that was observed in pigs. The resulting characteristic action potential of the adapted ionic kinetics model is shown in Fig. 4C for steady state 1Hz pacing in normal (continuous) and blocked \(I_{Kur}\) (dotted) conditions, exhibiting resemblance to measured swine action potentials in cellular experiments.
A simulated reentry was initiated via cross-field stimulation, where two appropriately timed line stimuli (S1 and S2) were given from adjacent edges of a 2D sheet, such that S2 was perpendicular to S1, and the coupling interval between the two stimuli allowed for the interaction between the wavefront of S2 and the recovering tail of S1 to generate a rotor. The simulations were run for 5 seconds. The leftmost panels in figure 5A show a snapshot of a rotor (at a particular time) in terms of the absolute membrane voltage in controls, and when $I_{Kur}$ was blocked. The DF maps for these rotors are shown in the 2 right panels of figure 5A; the rotor frequencies were similar in both conditions (19.5 Hz). To understand whether $I_{Kur}$ blockade had no effect on rotation frequency due to its slow recovery kinetics, we performed an additional numerical experiment, in which $I_{Kur}$ fast and slow inactivation time constants ($\tau_{\text{fast}}$ and $\tau_{\text{slow}}$) were made very similar; however this also did not affect the rotor frequency when $I_{Kur}$ was blocked during carbachol-induced AF (data not shown). We then compared the magnitude of $I_{Kur}$ during reentry to the largest active $K^+$ conductance, i.e. $I_{K,ACh}$. Figure 5B shows the distribution of $I_{Kur}$ (left panel) and $I_{K,ACh}$ (right panel) underlying the rotor. $I_{K,ACh}$ is $\approx 7$ times larger in magnitude than $I_{Kur}$ in the rotors. These simulations suggest that $I_{K,ACh}$ masks any effect of $I_{Kur}$ changes, and thus has minimal effect on rotor frequency in carbachol-induced AF. However, to test whether $I_{Kur}$ block is effective in terminating reentry initiated in the absence of an active $I_{K,ACh}$, we conducted additional simulations of reentry in a 4x4cm tissue (Fig. 5C). A stable reentry (with a large core size of $\sim 2x2\text{cm}$) and a DF=4.4Hz was observed (Top 2 left panels; Fig. 5C). At 2.5 sec post stimulation, $I_{Kur}$ was blocked, resulting in the annihilation of the rotor at the boundary at 3177ms (i.e. after 677ms). Two snapshots of rotors during $I_{Kur}$ block (at $t=3117\text{ms}$) and annihilation (at $t=3177\text{ms}$) are shown (Bottom 2 left panels; Fig. 5C). The right panel depicts the spiral wave trajectory before and after $I_{Kur}$ block (Fig. 5C, right panel). Thus, in absence of $I_{K,ACh}$ at a reentry frequency of approx. 5 Hz, block of $I_{Kur}$ terminates reentry.
We conducted additional simulations to study reentry dynamics during hyperkalemia. We hypothesized that increased levels of $[K^+]_o$ may terminate reentry activity via reduced sodium availability.\textsuperscript{14} The left panel in Fig. 6A shows the voltage dependence of the steady state sodium inactivation (i.e., the product of the \( h \) and \( j \) gates). We incorporated a left-shifted inactivation curve in the atrium compared to the ventricle, as was shown experimentally in dogs.\textsuperscript{7} (Data are currently unavailable pertaining to \( I_{Na} \) differences between pig atrial versus ventricular cells). As $[K^+]_o$ increases, the resting membrane potential will be depolarized according to Nernst equation. Due to the differences in inactivation curves between the atrium and the ventricle, the availability of sodium channels (\( hj \)) will therefore be significantly decreased in the atrium as $[K^+]_o$ increases, compared to the ventricle, as shown in Fig. 6A, right panel. In Fig. 6B, we simulated the effects of hyperkalemia on AF reentry in a 3x3 cm\(^2\) atrial tissue model. We investigated the consequences of increasing $[K^+]_o$ from 5 mM to 5.5, 6.0 and 6.2 mM every 1000ms on rotor stability (4 snapshots in Fig. 6B, left panels). We noticed that as $[K^+]_o$ was increased, the meandering of the spiral wave became more significant, increasing the core size. When $[K^+]_o$ reached a level of 6.2mM, the spiral wave drifted rapidly to the boundary and annihilated. This process is demonstrated by the time-space plot of the spiral tip location that is given in the right panel of Fig. 6B. For reference, we repeated these simulations with the sodium inactivation curve relevant to ventricular tissue, for both the same tissue size of 3x3 cm\(^2\) (Fig. 6C, upper panels) as well as for an enlarged tissue of 5x5 cm\(^2\) (Fig. 6C, bottom panels). In both simulations the tissue sustained spiral wave activity up to much higher $[K^+]_o$ levels than those with the left-shifted sodium inactivation curve of the atrium. In the 3x3 and 5x5 cm\(^2\) tissue models, simulated reentrant activity was terminated when $[K^+]_o$ was higher than 11.0 and 11.2 mM, respectively, by the rotor drifting onto the boundary.
DISCUSSION

The main new findings from this study are: (1.) $I_{Kur}$ block is not able to terminate carbachol-induced AF in isolated pig hearts. Simulations suggest that this ineffectiveness is mainly due to the smaller contribution of $I_{Kur}$ to the net repolarizing current in the presence of a large $I_{K,ACh}$. (2.) Elevated $[K^+]_o$ terminates AF more readily and effectively than VF. Numerical simulations indicate that this may be partly due to the steady-state inactivation property differences of $I_{Na}$ between the atrium and the ventricle, and larger tissue size of the ventricle.

Is $I_{Kur}$ an effective antiarrhythmic target in AF?

$I_{Kur}$ is an attractive target for antiarrhythmic drug development, primarily due to its presence in the atrium, and not ventricle in humans.\textsuperscript{2,3} However, some doubts exist regarding the efficacy of blocking $I_{Kur}$ to terminate AF due to its frequency-dependent reduction in magnitude.\textsuperscript{9-11} Furthermore, $I_{Kur}$ may be reduced in chronic AF conditions in humans.\textsuperscript{13} Despite this, in a chronic AF model in goats, AVE0118, a relatively selective blocker of $I_{Kur}$, was successful in cardioverting AF.\textsuperscript{5} $I_{Kur}$ block also terminated reentry under simulated chronic AF conditions.\textsuperscript{13} In contrast, in a canine model of cholinergic AF, blocking $I_{Kur}$ with 4-AP did not terminate AF.\textsuperscript{6} Instead, $I_{Kur}$ blockade was pro-arrhythmic.\textsuperscript{6} Our experimental results in pigs are consistent with this study.\textsuperscript{6} Furthermore, our simulation results mimic the experimental findings. Therefore, our working hypothesis is that, at least in acute or paroxysmal models of AF, at frequencies > 10 Hz, $I_{Kur}$ is unlikely to be a useful drug target. However, our current (Fig. 5C) and previous simulation results\textsuperscript{13} also suggest that $I_{Kur}$ block can terminate reentry at slower reentry frequencies of 4-6 Hz. Additional experiments in chronic AF with specific $I_{Kur}$ blockade (4-AP, DPO-1) will be required to test this hypothesis, since AVE0118 is not a very specific blocker of $I_{Kur}$, blocking additionally $I_{to}$ and $I_{K,ACh}$.\textsuperscript{20}
Hyperkalemia, preferential termination of AF, and its implications.

Our study also demonstrates that hyperkalemia superimposed upon fibrillation (AF or VF) tends to be antiarrhythmic. Much smaller changes in $[K^+]_o$ are needed to terminate AF as compared to VF. This potentially relates to the differences in the biophysical properties of $I_{Na}$ availability between the atrium and the ventricle, and also partially explains the well known, but poorly understood sensitivity of the atrium to hyperkalemia. An interesting aspect to note in this regard is that at $\approx 8$ mM $[K^+]_o$ where AF seems to terminate, the normal ventricular excitability is expected to be increased, from the well known biphasic changes in conduction velocity and excitability as a function of $[K^+]_o$. This has been attributed to the decreased difference between the resting membrane potential and the threshold membrane potential. Previous in vivo experiments in dogs showed that both atrial and ventricular tissue demonstrated the biphasic response. However, the threshold value for $[K^+]_o$ at which the intraventricular conduction time started to reduce with respect to its own controls was much higher (9.3 mM), compared to a similar threshold for intraatrial conduction times (7.5 mM). Simultaneous ECG recordings in that study suggested that the QRS and P wave durations mirrored the changes in the intraventricular and intraatrial conduction times respectively. Thus it is possible to envision a scenario that in a heart with normal ventricular function, small increases in $[K^+]_o$ may not be pro-arrhythmic and thus be safe for terminating AF. However, whether this will also apply in cases of abnormal ventricular function, in hearts with an infarct or even pump failure remains unknown. The use of hyperkalemia to terminate AF in our experiments demonstrates a “proof-of-principle” experiment that exploits the inherent differences in the $Na^+$ channel availability between the atria and the ventricles to attempt and terminate AF. It raises the intriguing question as to whether it might be possible to design a drug that acts by depolarizing the membrane potential or affects $I_{Na}$ channel availability for facilitating the termination of AF, but without compromising safety in the ventricle (i.e. being pro-arrhythmic).
Limitations

In our Langendorff-perfused experiments, we have used the motion uncoupler Blebbistatin; the use of this drug may adversely affect ion channels in the atrium. In our experiments, the heart was not superfused with Tyrode’s solution. This will inscribe some temperature gradients across the surface of the heart. Our results suggest that DPO-1 in addition to blocking $I_{Kur}$ at lower concentrations (0.5 µM), may also block $I_{K,ACH}$ (> 1 µM). Additional patch clamp experiments will be necessary to systematically determine the $I_{K,ACH}$ blocking properties of DPO-1, including its EC$_{50}$. We have not yet directly investigated the ionic and molecular differences in $I_{Na}$ between atrium and the ventricle in the pig, including the steady-state inactivation properties in isolated cells. In our computer simulations, we have only used a modified human atrial model (not a detailed pig atrial or ventricular ionic model), and also studied the dynamics of a single rotor, without considering the multiple wavelet theory, or the complex 3D structure of the atria.\(^1\) Additionally, to mimic differences between AF and VF seen experimentally, we have only changed the properties of the Na$^+$ current, and tissue size. Clearly, many other differences in ion channels, including the inward rectifier K$^+$ current, $I_{K1}$, exist between the atrium and the ventricle,\(^23\) and will need to be more accurately modeled to quantitatively reproduce experimental data.
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CONFLICT OF INTEREST: None Declared.
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FIGURE LEGENDS

Figure 1. Experiments in acute AF and in presence of \( I_{Kur} \) blockade. (A.) Representative dominant frequency (DF) maps, and single pixel recordings during acute AF in the left atrium (LA) and the right atrium (RA). (B.) Plot of the maximum dominant frequency (DF\(_{\text{max}}\)) in acute AF experiments in Pig hearts versus time. Time before the introduction of the drug to selectively block \( I_{Kur} \) (100 \( \mu \)M 4-AP) is assigned negative values.

Figure 2. Experiments in acute AF and VF and in presence of \( I_{Kur} \) blockade (via DPO-1). (A.) Representative pig optical atrial action potential in control (solid) and in presence of 0.5 \( \mu \)M DPO-1 (dashed) at 300 ms pacing. (B.) Representative optical pig ventricular action potential in control (solid) and in presence of 0.5 \( \mu \)M DPO-1 (dashed) at 300 ms pacing. (C.) Average atrial APDs at 50\% (APD\(_{50}\)) and 75\% (APD\(_{75}\)) repolarization in control and in presence of 0.5, 1.0, and 5.0 \( \mu \)M DPO-1. (D.) Average ventricular APDs at 50\% (APD\(_{50}\)) and 75\% (APD\(_{75}\)) repolarization in control and in presence of 0.5, and 1.0 \( \mu \)M DPO-1. (E.) Plot of DF\(_{\text{max}}\) in acute AF experiments in control and in presence of 0.5 \( \mu \)M DPO-1. (F.) Plot of DF\(_{\text{max}}\) during VF in control and in presence of 0.5 \( \mu \)M DPO-1. (G.) Plot of DF\(_{\text{max}}\) in acute AF experiments in control and in presence of 1.0 and 5.0 \( \mu \)M DPO-1. (H.) Plot of DF\(_{\text{max}}\) during VF in control and in the presence of 1.0 and 5.0 \( \mu \)M DPO-1.

Figure 3. Experiments in acute AF and VF simultaneously during hyperkalemia (6-12 mM \([K^+]_o\)). (A.) Representative plots of the DF maps in acute AF (in the left atrium, LA) and VF (in the left ventricle, LV) in the same heart, that was perfused at different concentrations of \([K^+]_o\) at 4, 6, 8, 10 and 12 Mm, for 10 minutes at each concentration. The DF\(_{\text{max}}\) of AF changed substantially during perfusion of 8 mM \([K^+]_o\), hence 3 maps at the initial, middle, and final stages of the 10 minute perfusion period are shown. AF converted to atrial tachycardia (ATach) in the final
stages, and subsequently terminated between transition from 8 to 10 mM [K⁺]₀. In this experiment, VF did not terminate even at 12 mM [K⁺]₀. (B.) Comparison of average DF max values during AF and VF at different concentrations of [K⁺]₀.

**Figure 4.** New numerical formulation of I_{Kur}. (A.) Plots of the model time constants for the fast and slow time constants of the inactivation for I_{Kur} in the human atrial mathematical model (Courtemanche), adjusted according to data for inactivation in the pig atrium. (B.) This plot shows the frequency dependence of I_{Kur}; this current is reduced in magnitude in response to pulses at higher frequencies. (C.) Shows the plot of the model action potential at 1 Hz in control conditions (to mimic the pig atrial action potential), and the plot of the action potential when I_{Kur} was completely blocked at 1 Hz.

**Figure 5.** Simulations of I_{Kur} block on rotor. (A.) Left 2 panels depict representative snapshots of a rotor in control conditions, and when I_{Kur} was blocked completely, in carbachol-induced AF; Right panels show respective DF maps. (B.) Snapshot of spatial distribution of I_{Kur} and I_{K,ACH} current in a rotor during AF. (C.) Top 2 left panels depict a DF map and a rotor snapshot when AF was induced, in absence of carbachol. The rotor tip trajectory before I_{Kur} block is plotted in blue. Bottom 2 left panels depict rotor snapshots in simulated AF (in absence of I_{K,ACH}) when I_{Kur} was blocked. The two snapshots are given at t=3117ms and just before termination of reentry at t=3177ms. Right most panel depicts rotor tip meander before and after I_{Kur} block.

**Figure 6.** Effect of Hyperkalemia on simulated rotor. (A.) The left panel depicts differences between the steady-state inactivation properties of I_{Na} in the atrium (red) and the ventricle (blue). The inactivation is measured as the product of the fast (h) and the slow (j) sodium-channel inactivation gates: hj. The right panel depicts how steady-state inactivation properties of I_{Na} vary as a function of increases in [K⁺]₀. (B.) The left panels show snapshots of the simulated
rotor (with atrial model steady-state Na\(^+\) inactivation) at different values of \([K^+]_o\). The right panel depicts the trajectory of the rotor tip at different values of \([K^+]_o\). The rotor is seen to terminate at 6.2 mM \([K^+]_o\). (C.) The top panel shows snapshots of the simulated rotor in a 3×3 cm tissue, (with ventricular model steady-state Na\(^+\) inactivation) at different values of \([K^+]_o\). The rotor is seen to terminate at 10.6 mM \([K^+]_o\). The bottom panel depicts snapshots of the simulated rotor, but now in a 5×5 cm tissue, (with ventricular \(I_{Na}\)) at different values of \([K^+]_o\). The rotor is now seen to terminate at 11.4 mM \([K^+]_o\).
Fig. 1.
Fig. 2.
A

Fig. 3.

B

[DF]_max (Hz)

\[\begin{array}{c}
\text{[K^+]_o (mM)} \\
4 \\
6 \\
8 \\
10 \\
12 \\
14 \\
\end{array}\]

\[\begin{array}{c}
\text{DF} \\
26 \\
14.2 \\
27.3 \\
13.6 \\
20.7 \\
10.7 \\
15.5 \\
11.3 \\
11.8 \\
11.2 \\
8.9 \\
10.7 \\
11.8 \\
4.7 \\
\end{array}\]

\[\begin{array}{c}
\text{AF termin.} \\
\text{AF termin.} \\
\text{AF termin.} \\
\text{AF termin.} \\
\end{array}\]

\[\begin{array}{c}
\text{No termin.} \\
\text{No termin.} \\
\text{No termin.} \\
\text{No termin.} \\
\end{array}\]

\(\text{A} \quad \text{V} \quad \text{(N=5)}\)
Fig. 4.

A

\[ \tau_f, \tau_s \]

B

Relative Current

Frequency [Hz]

C

Pacing at 1Hz

V [mV]

\[ \text{Normal } I_{Kur}, \text{ Blocked } I_{Kur} \]
Fig. 5.

A

Control

Blocked $I_{Kur}$

Control

Blocked $I_{Kur}$

DF maps

B

$I_{Kur}$ [pA]

$I_{KAc} [\text{pA}]$

C

DF map

$t=880\text{ms}$

$t=3117\text{ms}$

$4.4 \text{ Hz}$

$19.5 \text{ Hz}$

Tip trajectory

Before $I_{Kur}$ block

After $I_{Kur}$ block

$x [\text{mm}]$

$y [\text{mm}]$

$z [\text{s}]$
Fig. 6.

A

- Left-shifted model
- Standard atrial model

B

- Tip trajectory
- Time [s]
- X [mm], Y [mm], Z [mm]

C

- Tissue size: 3x3 cm
- Tissue size: 5x5 cm

- [K+]0 = 5 mM
- [K+]0 = 5.5 mM
- [K+]0 = 6 mM
- [K+]0 = 6.2 mM

- [K+]0 = 7 mM
- [K+]0 = 8 mM
- [K+]0 = 10 mM
- [K+]0 = 11.0 mM

- [K+]0 = 7 mM
- [K+]0 = 9 mM
- [K+]0 = 11 mM
- [K+]0 = 11.2 mM