Human Atrial Action Potential and Ca$^{2+}$ Model
Sinus Rhythm and Chronic Atrial Fibrillation

Eleonora Grandi,* Sandeep V. Pandit,* Niels Voigt,* Antony J. Workman, Dobromir Dobrev, José Jalife,† Donald M. Bers†

Rationale: Understanding atrial fibrillation (AF) requires integrated understanding of ionic currents and Ca$^{2+}$ transport in remodeled human atrium, but appropriate models are limited.

Objective: To study AF, we developed a new human atrial action potential (AP) model, derived from atrial experimental results and our human ventricular myocyte model.

Methods and Results: Atria versus ventricles have lower I$_{K1}$, resulting in more depolarized resting membrane potential (~7 mV). We used higher I$_{to,fast}$ density in atrium, removed I$_{to,slow}$, and included an atrial-specific I$_{kur}$. I$_{NCX}$ and I$_{Na,K}$ densities were reduced in atrial versus ventricular myocytes according to experimental results. SERCA function was altered to reproduce human atrial myocyte Ca$^{2+}$ transients. To simulate chronic AF, we reduced I$_{Ca,L}$, I$_{to}$, I$_{kur}$ and SERCA, and increased I$_{K1}$, I$_{Ks}$ and I$_{NCX}$. We also investigated the link between Kv1.5 channelopathy, [Ca$^{2+}$]$_{i}$, and AF. The sinus rhythm model showed a typical human atrial AP morphology. Consistent with experiments, the model showed shorter APs and reduced AP duration shortening at increasing pacing frequencies in AF or when I$_{Ca,L}$ was partially blocked, suggesting a crucial role of Ca$^{2+}$ and Na$^{+}$ in this effect. This also explained blunted Ca$^{2+}$ transient and rate-adaptation of [Ca$^{2+}$]$_{i}$ and [Na$^{+}$]$_{i}$ in chronic AF. Moreover, increasing [Na$^{+}$]$_{i}$ and altered I$_{Na,K}$ and I$_{NCX}$ causes rate-dependent atrial AP shortening. Blocking I$_{kur}$ to mimic Kv1.5 loss-of-function increased [Ca$^{2+}$]$_{i}$ and caused early afterdepolarizations under adrenergic stress, as observed experimentally.

Conclusions: Our study provides a novel tool and insights into ionic bases of atrioventricular AP differences, and shows how Na$^{+}$ and Ca$^{2+}$ homeostases critically mediate abnormal repolarization in AF. (Circ Res. 2011;109:1055-1066.)

Key Words: computer model ■ action potential ■ Ca$^{2+}$ cycling ■ atrial fibrillation

At present, mechanisms leading to perpetuation of AF are still undetermined. Growing experimental evidence points to abnormal intracellular Ca$^{2+}$ handling as a key mediator in AF pathophysiology,6,7 but the mechanism through which Ca$^{2+}$- related abnormalities can lead to the occurrence and maintenance of AF are poorly understood. Models of human atrial myocytes have been developed and used to gain mechanistic insights into human atrial cell physiology and pathophysiology8–10; however, none of these included detailed descriptions of Ca$^{2+}$ (or Na$^{+}$) regulatory processes. A recent simulation study incorporated and studied the subcellular nature of Ca$^{2+}$ homeostasis and its relation to human atrial action potentials11; however, the role of Ca$^{2+}$ in mediating AF was not investigated.
We recently developed a model of the human ventricular myocyte AP and Ca^{2+} transient (CaT),^{12} a major advance over prior human ventricular models in robustly describing excitation-contraction coupling, and the model was extensively validated against a broad range of experimental data.

The aims of the present study were 2-fold: (1) to derive a new human atrial cell model with detailed Ca^{2+} handling, by implementing experimentally documented structural and ionic differences in atrial versus ventricular cells^{13} and starting from our recently published model of human ventricular myocytes^{12}; (2) to study how Ca^{2+} homeostasis is involved in abnormal APs seen in chronic AF (cAF) and AF related to Kv1.5 channelopathy and adrenergic stress. Importantly, we utilized new experimental data addressing the poorly understood molecular basis of impaired atrial Ca^{2+} signaling in cAF to constrain our model parameters.

We validated our model by testing its ability to recapitulate a wide range of physiological behaviors observed in experiments. We next investigated the mechanisms of APD and CaT rate-adaptation in sinus rhythm and cAF, and assessed the effects of blocking the atrial-specific ultrarapid K^{+} current (I_{Kur}) in the absence and presence of β-adrenergic activation, to understand arrhythmogenesis in AF related to Kv1.5 channelopathy and adrenergic stress. Finally, right-to-left gradients in repolarizing currents were also included in the model, since in a number of instances the driving source of the AF (reentry or foci) is located in the left atrium.^{2}

**Methods**

Cellular [Ca^{2+}] and electrophysiological methods are described in the Online Supplement available at http://circres.ahajournals.org and were used to tune our model and for validation. The Table shows key changes made in our new human atrial model versus our ventricular myocyte model,^{12} to account for ionic remodeling in cAF, and to simulate the effects of β-adrenergic and cholinergic stimulation. Further details are in Online Supplement, including formulation of I_{Kur} block by AVE0118.

Model differential equations were implemented in Matlab (Mathworks Inc, Natick, MA) and solved numerically using a variable order solver (ode15s). APDs were obtained after pacing digital cells at indicated frequencies at steady-state. APD was measured as the interval between AP upstroke and 90% repolarization level (APD_{90}).

**Results**

The baseline alterations to our ventricular cell model resulted in a typical Type-3 human atrial AP morphology^{15} (Figure 1A, right panel). The higher density of K^{+} currents that are active in AP phase 1 (early repolarization, I_{to}+I_{Kur}) confers the AP a triangular shape lacking a plateau phase. We have investigated the impact on AP shape of varying I_{to} and I_{Kur} densities, and quantified the changes in the plateau potential, which gets more depolarized as the degree of K^{+} channel blockade increases (Online Figure I, Online Supplement). AP waveform also feeds back onto ion channel gating determining notable differences in atrial currents. For example, although atrial I_{to} is almost twice as large in voltage clamp experiments (see Online Figure II), in current clamp conditions it is comparable to ventricular I_{to} (Figure 1G, right versus left panel). Maximal velocity of AP upstroke was comparable to that measured in experiments of ~140 V/s (versus 250 V/s in cAF)^{10} and was smaller than in the ventricular cell model (372 V/s in the epicardial cell model paced at 1 Hz)^{12}. In fact, I_{to} is remarkably reduced in atrial versus ventricular cells (Figure 1C, right versus left panel) during the AP, due to more inactivated channels (because of slower recovery from inactivation at more depolarized atrial resting membrane potential). Although I_{Kr} or I_{Ks} were not modified versus ventricular myocytes, the spiky AP reduced net I_{Kr} and I_{Ks} (Figure 1E and 1F). It is noteworthy that the reduction of I_{Ncx} from the ventricular model resulted in larger I_{Ncx} in the atrial model (Figure 1K). This is presumably because of the short early repolarization in atrium and the slightly larger CaT, both favoring inward I_{Ncx}. I_{Cal} is similar in atria and ventricle in voltage-clamp conditions, but the AP shape causes I_{Cal} to be much larger in atrial versus ventricular myocyte model (Figure 1D). I_{K1} is smaller in
atria, consistent with its lower maximal conductance. INaK is decreased (not as much as its pump rate because the higher [Na\(^{+}\)]\(_{i}\), 9.1 in atrium versus 8.2 mmol/L at 1 Hz pacing rate, activates the pump more).

A typical Ca\(^{2+}\) transient is shown in Figure 1B (right panel): at 1-Hz pacing rate, diastolic [Ca\(^{2+}\)]\(_{i}\) is 207 nmol/L and peaks at 462 nmol/L. Simultaneous ICaL and [Ca\(^{2+}\)]\(_{i}\) measurements in human atrial myocytes at physiological temperature are shown in Figure 2D through 2G and compared with simulated traces (Figure 2A and 2B; 0.5 Hz).

Simulated CaT amplitude and rate of CaT decay matched the experimental data (Figure 2B gray line versus E, and Figure 2H and 2I), as did peak ICaL (−6.47 A/F versus −6.78±0.36 in experiments, Figure 2A gray line versus D). When cAF was simulated, by accounting for ion channel remodeling as illustrated in the methods, ICaL was greatly diminished (Figure 2A, black versus gray lines), as shown in experiments (Figure 2D versus F).19 The reduced ICaL could explain the reduced sarcoplasmic reticulum (SR) Ca\(^{2+}\) release and CaT amplitude (Figure 2B, 2E, 2G, and 2H), even if SR Ca\(^{2+}\) content were unaltered. However, the reduced ICaL and SERCA function (rate of twitch [Ca\(^{2+}\)]\(_{i}\) decline; Figure 2I) and the elevated SR Ca\(^{2+}\) leak and NCX function (greater I\(_{\text{NCX}}\) for a given [Ca\(^{2+}\)]\(_{i}\); Figure 3A through 3G) all tend to lower SR Ca\(^{2+}\) content in cAF, which is apparent in the model (but not significantly so in the experiments; Figure 3H).

We next tested the response of our model to changes in pacing frequency. Simulated human atrial cell APs under baseline conditions (Figure 4D) shorten with faster pacing rates (Figure 4J, black circles) as shown in atrial myocytes from patients in normal sinus rhythm (Figure 4A and 4M, black circles).19 To illustrate the effect of a reduction in ICaL Van Wagoner et al19 recorded APs from the same myocytes at various cycle lengths in the presence of the ICaL blocker nifedipine (10 \(\mu\)mol/L), showing little rate-dependent change in APD (Figure 4C and 4M, gray open circles). Li and Nattel20 obtained analogous results. Similarly, simulated APs after 50% ICaL block (Figure 4F) exhibited impaired AP rate-adaptation (Figure 4J, gray open circles). Myocytes from chronic AF patients (Figure 4B) are characterized by shorter APD\(_{90}\) values,16,19,21–23 with less variation as a function of cycle length than control (sinus rhythm) myocytes (Figure 4M, squares).4,16,19,22,23 Analogously, our cAF model predicts shorter APs than sinus rhythm (solid versus dashed line in Figure 4D, inset), and

### Table. Main Changes to our Human Ventricular Model to Generate the Human Atrial Model, Simulate cAF, and \(\beta\)-Adrenergic Stimulation

<table>
<thead>
<tr>
<th></th>
<th>Atrial Versus Ventricular</th>
<th>cAF Versus Sinus Rhythm</th>
<th>(\beta)-Adrenergic Stimulation</th>
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<tbody>
<tr>
<td>Ionic currents</td>
<td></td>
<td></td>
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<tr>
<td>(I_{\text{Na}})</td>
<td>Unchanged</td>
<td>−10% peak density(^{66})</td>
<td>Unchanged</td>
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<tr>
<td>(I_{\text{Na,L}})</td>
<td>None</td>
<td>Added late component(^{66})</td>
<td>Unchanged</td>
</tr>
<tr>
<td>(I_{\text{k}})</td>
<td>Unchanged</td>
<td>Increased 2-fold(^{23})</td>
<td>Enhanced maximal conductance (3-fold) and leftward shift in IV relationship (by 40 mV)(^{57})</td>
</tr>
<tr>
<td>(I_{\text{k}})</td>
<td>Unchanged</td>
<td>Unchanged</td>
<td>Unchanged</td>
</tr>
<tr>
<td>(I_{\text{K}})</td>
<td>Added</td>
<td>−55% in the RA</td>
<td>Enhanced maximal conductance (3-fold)(^{18})</td>
</tr>
<tr>
<td>(I_{\text{k1}})</td>
<td>85% reduction(^{43})</td>
<td>Upregulated +100%(^{2,21})</td>
<td>Unchanged</td>
</tr>
<tr>
<td>(I_{\text{to}})</td>
<td>No (I_{\text{to,slow}})</td>
<td>(I_{\text{to,fast}}) activation and inactivation negatively shifted and slower inactivation; Larger amplitude(^{59})</td>
<td>Unchanged</td>
</tr>
</tbody>
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<tr>
<th>Ca and Na handling</th>
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<tbody>
<tr>
<td>(I_{\text{CaL}})</td>
<td>Matched amplitude and kinetics at 37°C from our data and Ref 20</td>
<td>Current density is reduced by 50% in cAF(^{17,18}) (and present data)</td>
<td>Increased fraction of available channels (+50%), and channel availability shifted leftward (by 3 mV)(^{57})</td>
</tr>
<tr>
<td>(I_{\text{NCX}})</td>
<td>Atrium &lt; ventricle (−30%)(^{60})</td>
<td>Upregulated in cAF (+40%)(^{17,25,61,62})</td>
<td>Unchanged</td>
</tr>
<tr>
<td>SERCA</td>
<td>No changes in maximal pump rate</td>
<td>Reduced maximal pump rate(^{17})</td>
<td>Forward mode (k_{\text{off}}) reduced by 50%(^{57})</td>
</tr>
<tr>
<td>RyR</td>
<td>Unchanged</td>
<td>Increased sensitivity for luminal Ca(^{2+}) (2-fold)(^{17,61})</td>
<td>Sensitivity to [Ca(^{2+})](_{\text{SR}}) enhanced 2-fold(^{17})</td>
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<tr>
<td>SR Ca(^{2+}) leak</td>
<td>Unchanged</td>
<td>Increased by 25%</td>
<td>Unchanged</td>
</tr>
<tr>
<td>TnI</td>
<td>Unchanged</td>
<td>Unchanged</td>
<td>Affinity for Ca(^{2+}) decreased(^{17})</td>
</tr>
<tr>
<td>(I_{\text{INKA}})</td>
<td>Atrium &lt; ventricle (−30%)(^{60})</td>
<td>Unchanged(^{64})</td>
<td>Affinity for [Na(^{+})] increased by 25%(^{65})</td>
</tr>
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reduced adaptation to changes in pacing frequency (Figure 4J, squares). At 4-Hz pacing, AP duration alternates (Figure 4E, and so does $[\text{Ca}^{2+}]_i$ in Figure 4H). The sinus rhythm model exhibits this behavior at higher frequency (Figure 4O at 6-Hz pacing rate). The model predicts a positive dependency of CaT amplitude on the pacing rate in sinus rhythm (Figure 4G and 4K, black circles), which is in agreement with intracellular $[\text{Na}^+]_i$ measurements by aequorin light signals (Figure 4N, gray circles and dashed line) and twitch force measurements (Figure 4N, open circles and solid line). Positive dependency is impaired when $I_{\text{CaL}}$ is partially inhibited (Figure 4F and 4K, gray open circles) and in cAF (Figure 4E and 4K, squares). Similarly, atrial myocytes from patients with cAF show impaired contractility (Figure 4N, squares). Our model also predicts the increase of intracellular $[\text{Na}^+]_i$ with increasing pacing frequency, as shown in Figure 4L (and Online Figure III, A), which is more limited in cAF and with inhibition of $I_{\text{CaL}}$ compared with sinus rhythm (squares and gray open circles versus black circles).

Figure 5 and Online Figure III show that $[\text{Na}^+]_i$ is critical for APD rate-adaptation. Time courses of APD and $[\text{Na}^+]_i$ changes subsequent to an increase in pacing frequency from 0.5 to 1 Hz (Online Figure III, C) suggest that non steady-state measurements (before $[\text{Na}^+]_i$ slowly reaches steady-state) may give rise to highly variable experimental APD adaptation curves. Moreover, if $[\text{Na}^+]_i$ is clamped in the model, the APD rate adaptation is nearly abolished (Figure 5A). Simulation of partial block of NKA causes a biphasic APD response (Figure 5D): first, APD prolongation by acute NKA current block, then as $[\text{Na}^+]_i$ rises it increases outward NKA causing APD shortening. Importantly, we validated these model predictions in isolated human atrial myocytes challenged with strophanthidin (10 $\mu$mol/L). Acute NKA inhibition was confirmed by abrupt and relatively sustained depolarization of resting membrane potential (Figure 5C). Figure 5C shows a typical time course of APD from a representative cell and pooled data (n=10). Strophanthidin application produces an initial marked increase and subsequent decrease in APD (Figure 5C, right). Similar behavior has been described in guinea pig ventricular myocytes, human atrial fibers, and rabbit atrial myocytes (not shown).

Blockade of the atrial specific current $I_{\text{Kur}}$ has been proposed to improve atrial contractility without increasing the risk of ventricular arrhythmias. In fact, in human atrial myocardium, block of $I_{\text{Kur}}$ results in a prolongation and elevation of the AP plateau, which elicit a positive inotropic effect. Thus, we assessed the impact of $I_{\text{Kur}}$ block (modeled as shown in Online Methods and Online Figure IV) on APD and CaT (Figure 6 and Online Figure V). Moderate blockade of $I_{\text{Kur}}$ (by 25% to 50%) increases CaT amplitude (Figure 6B) with little effects on APD (Figure 6A) both in sinus rhythm and cAF models, in agreement with experimental results (Figure 6A and 6B, insets). Enhancement of CaT amplitude is greatly increased when $I_{\text{Kur}}$ is more fully (75% to 100%) blocked (Figure 6B), paralleled by AP prolongation (Figure 6A) in agreement with $I_{\text{Kur}}$ inhibitor AVE0118 on contractile force of atrial trabeculae from patients in sinus rhythm and in AF (black symbols and axis). To study AF associated with Kv1.5 mutation during $\beta$-adrenergic activation, we also investigated the effect of adrenergic stimulation on atrial AP (Figure 6D) by incorporating steady-state effects of PKA-dependent phosphorylation
on $I_{\text{Ca},i}$, $I_{\text{Ks}}$, $I_{\text{Kur}}$, PLN-SERCA2a, RyR2, troponin Ca$^{2+}$ affinity, and Na/K-ATPase (see Online Supplement). In our simulations, administration of isoproterenol (ISO) causes the CaT amplitude to increase (by ~65%, not shown) without major changes in the duration of repolarization (Figure 6D, solid black versus blue lines), in agreement with data from human atrial preparations.30 When simulating the block of $I_{\text{Kur}}$ (a current which is enhanced during adrenergic activation) in the presence of ISO, early after-depolarizations (EADs) occurred (Figure 6D, green dashed line). These results are in agreement with data from Olson et al14 (Figure 6D, inset) showing that 4-AP (50 μmol/L) prolonged APD in human atrial myocytes and caused EADs and triggered activity upon ISO (1 μmol/L) challenge. Notably, simulation of $I_{\text{Ks}}$ (also increased by ISO) blockade (50%) did not affect atrial AP markedly (Figure 6D, red line almost completely overlaps black line).

To reflect parasympathetic effects, we also included an $I_{\text{KACH}}$ model (fitted with human data) and demonstrate a dose-dependent reduction in human atrial AP and CaT in response to the parasympathetic transmitter acetylcholine (Online Figure VI). The APD shortening is consistent with experiments in human atria. We did not integrate crosstalk between β-adrenergic and acetylcholine or CaMKII pathways, or develop compartmentalized dynamic G-protein–coupled receptor models as done recently for animal myocyte models,31,32 but those would be logical extensions of our model.

There are limited data available concerning intra-atrial heterogeneities in repolarizing currents in human atrial myocytes. Caballero et al found a gradient of $I_{\text{Kur}}$ (a current which is enhanced during adrenergic activation) in the presence of ISO, early after-depolarizations (EADs) occurred (Figure 6D, green dashed line). These results are in agreement with data from Olson et al14 (Figure 6D, inset) showing that 4-AP (50 μmol/L) prolonged APD in human atrial myocytes and caused EADs and triggered activity upon ISO (1 μmol/L) challenge. Notably, simulation of $I_{\text{Ks}}$ (also increased by ISO) blockade (50%) did not affect atrial AP markedly (Figure 6D, red line almost completely overlaps black line).

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left-to-right gradients and may contribute to the perpetuation of arrhythmia. To account for variability in AP morphology between and within atria, we also varied $I_{Kur}$ and $I_{CaL}$ and $I_{Kr}$ by 50% and 400%, respectively, to produce a Type-1 AP, that is, similar to the manipulation attempted in an earlier modeling study by Nygren et al.\textsuperscript{9} This results in a larger $I_{Kr}$ and $I_{kr}/I_{to}$ ratio, more depolarized plateau potential, and steeper phase-3 repolarization (Figure 7B, left) as reported by the Nattel group\textsuperscript{36} (Figure 7B, right). Importantly, as in experiments,\textsuperscript{28,37} we show that when $I_{Kur}$ is blocked Type-3 AP prolongs (7C, left), whereas Type-1 APD is almost unaltered (7C, right).

**Discussion**

We developed a new mathematical model of human atrial myocyte with detailed electrophysiology and $Ca^{2+}$ handling, including ionic and $Ca^{2+}$ handling remodeling in cAF. This places our present understanding of atrial myocyte function in a useful quantitative framework to understand how changes in ion channel and $Ca^{2+}$ handling influence function.

**Atrial Versus Ventricular Cell Models**

Understanding atrioventricular ionic differences is important, has been investigated in simulations and experiments,\textsuperscript{38,39} and may lead to safer therapy as a result of targeting atrial-specific ion channels for AF.\textsuperscript{1,40} We used the Grandi-Pasqualini-Bers model of the human ventricular AP and $CaT$ as a framework for model development. As a result, the 2 models have a common format and similar aspects that may be convenient for integrating into whole heart models. Similarities include the $Ca^{2+}$ handling processes, which are also based on the Shannon-Bers model of the rabbit ventricular myocyte.\textsuperscript{41} However, appropriate changes to many model parameters were introduced to recapitulate experimental findings in atrial samples from patients in sinus rhythm and cAF. Specific amalgams of ion channel expression and function confer differential AP characteristics for various cardiac regions.\textsuperscript{32,43} For example, it is well known that atrial $I_{K1}$ density is smaller than ventricular $I_{K1}$, explaining the slightly less negative atrial diastolic membrane potential (by $\approx 5$–$10$ mV), reduced Na$^{+}$ channel availability and slower phase-3 repolarization.\textsuperscript{43} Again, in humans, $I_{Kur}$ is present in atria but not in ventricles,\textsuperscript{44} and in human atrium, $I_{to}$ is encoded entirely by Kv4.3 (responsible for $I_{to,fast}$),\textsuperscript{45} whereas both fast and slow $I_{to}$ components are detected in human ventricle. We also simulated different AP morphologies and included right-to-left gradients in $I_{to}$ and $I_{Kur}$ as reported recently in human myocytes from the RA and the LA from patients in sinus rhythm or with cAF. This new set of models accounting for tissue-specific ion current differences will be useful for understanding regional electrophysiology, $Ca^{2+}$ handling and arrhythmia mechanisms.

**Novelties of the Model Compared With Previous Models**

Computational cell modeling has been widely used to understand how individual ionic/molecular components (often studied in isolation) interact in the integrated environment of the cardiac myocyte. For human atrial myocyte models, the Courtemanche\textsuperscript{10} and Nygren\textsuperscript{9} models, which focused primarily on ion channels generating the atrial AP, have been useful to investigate physiological\textsuperscript{46,47} and pathophysiological\textsuperscript{48,49} mechanisms of the human atrium. However, those models have vastly different properties, especially in their rate-dependent behavior.\textsuperscript{50} Recently, Maleckar et al\textsuperscript{8} incorporated new experimental K$^{+}$ current data into the Nygren model, including formulations of $I_{Kur}$ and $I_{to}$ that we have also adopted here. They also studied the early and late phase of atrial repolarization and improved the rate-dependent properties of the AP model.

However, no previous model focused on $Ca^{2+}$ handling properties of human atrial myocytes, and it is increasingly clear that $Ca^{2+}$-handling and electrophysiology are intimately
Figure 4. APs recorded at different cycle lengths in a control human atrial myocyte (A), in a cell from cAF patient (B), and in the same control myocyte exposed to Ca^2+ channel block (10 μmol/L nifedipine, C). Simulated steady-state AP and CaT traces are shown for pacing frequencies 0.5, 1, 2, 3, and 4 Hz, for sinus rhythm (sr, D and G), cAF (E and H), and sr with 50% I_{CaL} block (F and I). Simulated APD_{90} (J) decreases at increasing pacing frequency in sr, but rate-adaptation is impaired in cAF or
linked with respect to arrhythmias.\textsuperscript{7} Cherry et al\textsuperscript{50} showed CaT differences between the 2 above models, with a more gradual longer lasting transient in the Courtemanche compared with a much sharper CaT in the Nygren/Maleckar models. Our human model uses the Ca\textsuperscript{2+} handling framework developed by Shannon et al for rabbit ventricular myocytes,\textsuperscript{41} which was the first to introduce both a junctional cleft (where ryanodine receptor, RyR, and most ICaL function) and also a subsarcolemmal Ca\textsuperscript{2+} compartment, where Ca\textsuperscript{2+}-dependent currents (eg, IC\textsubscript{Na} and IC\textsubscript{Ca}) sense different local [Ca\textsuperscript{2+}]\textsubscript{i} compared with bulk [Ca\textsuperscript{2+}].\textsuperscript{51} We have characterized Ca\textsuperscript{2+} handling properties in atrial myocytes from patients in sinus rhythm and with cAF, and modified the Ca\textsuperscript{2+} handling parameters in our model accordingly. This recapitulates experimental data including simultaneous measurements of IC\textsubscript{CAL} and CaT, caffeine-induced CaT amplitude (ie, SR content) and decay time (ie, SERCA and NCX function) and SR Ca\textsuperscript{2+} leak at physiological temperature. Our human atrial model provides an accurate representation of Ca\textsuperscript{2+} homeostasis in human atrial myocytes.

Recently, the Tavi group proposed a model describing heterogeneous subcellular Ca\textsuperscript{2+} dynamics for human atrial cells presumed to lack t-tubules.\textsuperscript{11} They produced a biphasic rise of [Ca\textsuperscript{2+}]\textsubscript{i}, as seen at 22°C in human atrial myocytes.\textsuperscript{52} In their model the biphasic [Ca\textsuperscript{2+}] rise resulted from delay between peripheral and central SR Ca\textsuperscript{2+} release. An extensive t-tubular network has been reported in atrial myocytes from large mammals.\textsuperscript{53} Because we did not observe biphasic [Ca\textsuperscript{2+}]\textsubscript{i} rise in our human atrial myocytes at 37°C (time to peak \textapprox 60 ms) and quantitative data on t-tubule organization in human atrial myocytes are lacking, we did not assume slowly propagating Ca\textsuperscript{2+} release toward the cell center.

### Rate-Dependent APD Adaptation

Using our human ventricular myocyte model, we found that the increase in [Na\textsuperscript{+}], at fast pacing rates feeds back to shorten APD through outward (repolarizing) shifts in Na\textsuperscript{+}/K\textsuperscript{+} pump (NKA) and NCX currents.\textsuperscript{12} Our human atrial model (Figure 5) and that of the Tavi group\textsuperscript{11} exhibit analogous behavior. The model showed negligible APD-rate adaptation when [Na\textsuperscript{+}] was clamped to a certain value (Figure 5A). Notably, we confirmed experimentally in human atrial myocytes the prediction of our model that acutely blocking NKA causes AP prolongation followed by APD shortening (Figure 5C and 5D), thus supporting the involvement of [Na\textsuperscript{+}]\textsubscript{i} in APD (through shift in NKA current, Figure 5B) and rate-dependent APD adaptation in human atrial cells. Furthermore, we show that IC\textsubscript{CAL} block has a similar effect on normal (sinus rhythm) and cAF human atrial action potentials (Figure 4), and in fact similar reductions in APD and APD rate-dependence occur in atrial myocytes isolated from patients with chronic AF. If IC\textsubscript{CAL} is blocked, APD is shorter (less depolarizing current), but also the CaT is greatly diminished, causing less extrusion of Ca\textsuperscript{2+} and less Na\textsuperscript{+} entry via NCX.
addition, the positive inotropy observed in normal atrial myocytes is lost in cAF, also limiting NCX-dependent Na\(^+\)/H\(^+\) accumulation at fast rates (as in Figure 4I). Thus, our model recapitulated experimental results and points to \([\text{Na}^+]_i\) and \(I_{\text{CaL}}\) as critical components of the normal rate-dependent modulation of atrial APD. Although direct effects of \([\text{Na}^+]_i\) on APD are compelling and logical, additional experimental validation of these effects would be valuable. We have discussed previously the role of delayed-rectifier \(K^+\) currents in APD rate adaptation,\(^{12}\) and showed in Online Figure VII that \(I_{\text{Kr}}\) block has little effect on APD. Here we ruled out an important role of the atrial-predominant \(I_{\text{Kur}}\) (see Online Figure VIII).

Role of \([\text{Ca}^{2+}]_i\) in Mediating AF in the Presence of \(I_{\text{Kur}}\) Channelopathies

Atrial contractility is decreased in cAF, largely due to electrical remodeling that is associated with downregulation of \(I_{\text{CaL}}\),\(^{28,29}\) which reduces CaT amplitude. Our simulation demonstrated that block of \(I_{\text{Kur}}\) enhances CaT amplitude of human atrial myocytes, both in patients in sinus rhythm or AF (Figure 6), thus pointing to \(I_{\text{Kur}}\) as an atrial-specific target to counteract hypocontractility associated to cAF. Indeed, experiments have shown that \(I_{\text{Kur}}\) blockers in ventricle did not appreciably alter APD or CaT.\(^{29}\)

We hypothesize that \(I_{\text{Kur}}\) in the atrium may serve the same function as \(I_{\text{Ks}}\) in the ventricle, that is opposing AP prolongation expected from larger inward \(I_{\text{CaL}}\) and \(I_{\text{NCX}}\) during \(\beta\)-adrenergic stress.\(^{54}\) Indeed, our simulations showed that block of \(I_{\text{Kur}}\) (to mimic \(Kv1.5\) mutation that leads to nonfunctional current, and AF) in the presence of adrenergic challenge causes EADs (Figure 6D). That agrees with experimental data,\(^{14}\) where \(I_{\text{Kur}}\) inhibition led to EADs in human atrial myocytes challenged with ISO. On the other hand, \(I_{\text{Ks}}\) block did not appreciably affect APD. Administration of ISO also led to cellular arrhythmic depolarizations when stimulating our model at low pacing frequency (not shown), in accordance with experimental work.\(^{14,55}\)

Conclusions

We developed a new computational framework to study the contribution of individual ionic pathway differences between atrial and ventricular cells to AP phenotype differences in the human atrium versus ventricle. It also established that \([\text{Ca}^{2+}]_i\) and \([\text{Na}^+]_i\) handling processes are major contributors to atrial
APD and its rate-related behavior in both normal and cAF conditions, and identified the role of $I_{Kur}$ in helping prevent EADs in the presence of adrenergic stress. This model (available at https://somapp.ucdmc.ucdavis.edu/Pharmacology/bers/) will also be useful for integrating into multicellular models of the human heart.

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**Disclosures**

None.

**References**


What is Known?

- Atrial cells exhibit electrophysiological characteristics that differ from those of ventricular cells due to structural differences and specific combinations of ion channel/transporter expression and function.
- During chronic atrial fibrillation (AF), electrical and structural remodeling contributes to the development of the AF substrate, and abnormalities in intracellular Ca\(^{2+}\) cycling have emerged as key mediators in AF pathophysiology.
- Detailed models of myocyte Ca\(^{2+}\) cycling have typically focused on ventricular rather than atrial myocytes, in part because of limited appropriate experimental data (especially from human atrial myocytes).

What New Information Does This Article Contribute?

- Based on recent data from human atrial cells, we have developed a new mathematical model of the human atrial myocyte that accounts for the electrophysiological and Ca\(^{2+}\) handling properties of atrial cells in both normal and chronic AF conditions.
- Simulations indicate that heart rate-dependent action potential duration (APD) shortening in healthy atrial cells involves the accumulation of intracellular [Na\(^+\)] at high frequencies that causes outward shifts in Na\(^+\)/Ca\(^{2+}\) exchange and Na\(^+\)/K\(^+\) pump currents, whereas ionic and Ca\(^{2+}\) handling remodeling lead to reduced Na\(^+\) accumulation in chronic AF, which causes a blunted APD rate-dependent response.
- Our modeling suggests that IKur is a key component of the adrenergic response of human atrial cells, as its loss (such as in Kv1.5 channelopathy) results in predisposition to early afterdepolarizations in the presence of isoproterenol and may help explain the bouts of stress mediated AF observed in these patients.

It is increasingly clear that Ca\(^{2+}\)-handling and electrophysiology are intimately linked to the development and perpetuation of AF. Thus, understanding AF requires an integrated quantitative understanding of ionic currents and Ca\(^{2+}\) transport in healthy and remodeled human atrium. However, no previous model focused on Ca\(^{2+}\) transport in human atrial myocytes in chronic AF. We developed a new human atrial myocyte model that incorporates the latest experimental data and modern concepts relating to intracellular Ca\(^{2+}\) homeostasis and related electrophysiology, including ionic and Ca\(^{2+}\) handling remodeling seen in chronic AF. Our simulation showed that IKur block enhances the amplitude of the Ca\(^{2+}\) transient of human atrial myocytes, representing an atrial-specific target to counteract hypocontractility associated to AF. This current is also predicted to oppose APD prolongation expected from larger inward I\(_{\text{calc}}\) and I\(_{\text{ncx}}\) currents in atrial fibrillation effects of ranolazine treatment. Our model provides novel insights into the mechanism of APD rate-dependent adaptation, by showing that accumulation of [Na\(^+\)]\(_{\text{cyt}}\) at fast heart rates feeds back to shorten APD via outward shifts in Na\(^+\)/Ca\(^{2+}\) exchange and Na\(^+\)/K\(^+\) pump currents. This human atrial model provides a useful tool to investigate atrioventricular differences with respect to arrhythmogenesis and therapeutic approaches.
Human Atrial Action Potential and Ca\textsuperscript{2+} Model: Sinus Rhythm and Chronic Atrial Fibrillation
Eleonora Grandi, Sandeep V. Pandit, Niels Voigt, Antony J. Workman, Dobromir Dobrev, José Jalife and Donald M. Bers

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Supplementary materials

Human Atrial Action Potential and Ca\(^{2+}\) Model:
Sinus Rhythm and Chronic Atrial Fibrillation

Eleonora Grandi, Ph.D.,\(^1,*\) Sandeep V. Pandit, Ph.D.,\(^2,*\) Niels Voigt, M.D.,\(^3,*\)
Antony J. Workman, Ph.D,\(^4\) Dobromir Dobrev, M.D.,\(^3\)
Jose Jalife, M.D.,\(^2,**\) and Donald M Bers, Ph.D,\(^1,**\)

\(^1\)Department of Pharmacology, University of California at Davis, Davis, CA, USA
\(^2\)Center for Arrhythmia Research, University of Michigan, Ann Arbor, MI, USA
\(^3\)Division of Experimental Cardiology, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany
\(^4\)Institute of Cardiovascular and Medical Sciences, University of Glasgow, UK

* E.G., S.V.P., and N.V. contributed equally to this work
** J.J. and D.M.B. share senior authorship

Methods

Electrophysiology
Right atrial appendages from patients with sinus rhythm or chronic atrial fibrillation (cAF) were used for electrophysiology experiments approved by the ethics committees of the Medical Faculty Mannheim, University of Heidelberg (#2011-216N-MA) and University of Glasgow (#99MC002).

Atrial myocytes were isolated using enzymatic dissociation as described in detail by Voigt et al.\(^1\)

All cellular [Ca\(^{2+}\)]\(_i\) and electrophysiological measurements were performed at 37 °C. For detecting [Ca\(^{2+}\)]\(_i\), cells were loaded with Fluo-3AM (Invitrogen, Carlsbad, USA; 10 \(\mu\)mol/L, 10 min loading and 30 min de-esterification) [Ca\(^{2+}\)]\(_i\) measured with an inverted epifluorescence microscope assuming a K\(_d\) of 864 nmol/L as previously described.\(^2, 3\)

Simultaneous voltage-clamp experiments were performed using whole-cell ruptured patch. Electrode resistances were between 2 and 5 M\(\Omega\) when filled with electrode solution containing (in mmol/L): EGTA 0.02, Fluo-3 0.1 [Invitrogen], GTP-Tris 0.1, HEPES 10, K-aspartate 92, KCl 48, Mg-ATP 1, Na\(_2\)-ATP 4; pH=7.2). Myocytes were superfused at 37°C with a bath solution containing (mmol/L): 4-aminopyridine, 5 mmol/L, BaCl\(_2\) 0.1, CaCl\(_2\) 2, glucose 10, HEPES 10, KCl 4, MgCl\(_2\) 1, NaCl 140, probenecid 2; pH=7.4. Caffeine (10 mmol/L) was applied to the bath solution for depletion of SR Ca\(^{2+}\). All simultaneous [Ca\(^{2+}\)]\(_i\), and membrane current recordings were conducted at the Division of Experimental Cardiology, Medical Faculty Mannheim, University of Heidelberg.

APs were recorded in current-clamp with a similar bathing solution as above (but without 4-aminopyridine or BaCl\(_2\)) and pipette solution (without Fluo-3). In a subset of cells (2 of the 10 used) the intracellular solution was slightly different (K-aspartate 110, KCl 20, MgCl\(_2\) 1, EGTA 0.15, Na\(_2\)ATP 4, Na\(_2\)GTP 0.4 and HEPES 5) as previously described.\(^4\) APs were stimulated at 30 beats/min. After reaching steady-state, strophanthidin (10 \(\mu\)mol/L) was applied and AP continuously monitored until a new steady-state was reached (typically 100 s).
Model development

Changes made from ventricular to atrial model

Atrial myocytes are smaller than ventricular myocytes and the surface:volume ratio (S/V) is reduced, due to a reduced t-tubular density in atrial vs. ventricular cell. Accordingly, we decreased S/V from 4.5 pF/μL to 3.3 pF/μL in our 33 μL atrial myocyte model. Of these, cleft, sarcoplasmic reticulum (SR) and submembrane space occupy 0.04, 2.3 and 1.3% respectively. The membrane capacitance (i.e., the surface area) was decreased from 138 to 110 pF, as measured in human atrial cells from patients in sinus rhythm.

The atrial inward rectifier K+ current I_{K1} is around 6-10 times smaller than in ventricular cells. We set I_{K1} maximal conductance to 15% of that of ventricular cells (approx. 6 fold smaller). Decreasing I_{K1} amplitude produced a depolarization shift in the atrial (vs. ventricular) resting membrane potential from -81.3 mV to -74.3 mV at 1 Hz pacing rate. In human atrium V_{1/2} of inactivation of the transient outward K+ current I_{to} is more negative (-40.5 mV) compared to human ventricle (-19.5 mV, data from Nabauer et al. corrected for CdCl2 with 10 mV negative shift). This is similar to experimental work from the Ravens group where inactivation was more negative for atrial cells. In experiments inactivation is faster in atrial cell, but recovery from inactivation is slower. We used the I_{to} formulation in the Maleckar model (with slightly increased time constants of inactivation and recovery from inactivation). I_{to} density was increased in the atrium compared to ventricles (Figure II), in agreement with experimental findings. A formulation for the atrial-specific ultrarapid delayed-rectifier K+ current I_{Kr} was included as in Maleckar et al. The slowly and rapidly activating delayed-rectifier K+ currents, I_{KS} and I_{Kr}, were left unchanged from our ventricular cell model. It has been shown that the Ca^{2+}-activated K+ (SK2) channels are more abundant in atria vs. ventricles, and their inhibition causes AP prolongation in human and mouse. However, another study showed that full blockade of these channels by 100 nmol/L apamin did not cause measurable electrophysiological changes in rat and dog atrial and ventricular multicellular preparations. Since it is not yet clear what the expression and functional impact of SK2 current is in human atria, we did not incorporate it in the model at this time.

I_{Na} was unchanged in atrial vs. ventricular model. Maximal Na+/K+ pump (NKA) rate was reduced by 30% in the atrial cell model, as suggested by reduced protein expression levels compared to ventricular myocytes, and matched data from Workman et al.

L-type Ca^{2+} current (I_{CaL}) parameters were tuned to reproduce I_{CaL} amplitude and kinetics measured at physiological temperature. I_{CaL} density also matched our voltage clamp data when simultaneously measuring I_{CaL} and [Ca^{2+}] (see Figure 2). We did not include a formulation for T-type Ca^{2+} current I_{CaT}, based on the observation from Li et al. finding no evidence for I_{CaT} in human atrial cells. Maximal Na+/Ca^{2+} exchange (NCX) current was reduced by 30% in the atrial (vs. ventricular) cell model to account for lower protein expression in atrial vs. ventricular myocytes. SR Ca^{2+} ATPase (SERCA) function was altered to reproduce [Ca^{2+}] handling properties measured in human atrial cells. Namely, k_m of SERCA was increased based on lower protein and mRNA levels of phosphohemlan in. No changes in maximal pump rate were incorporated with respect to the ventricular cell model. The ryanodine receptor (RyR) model was unaltered in the atrial cell model.

Changes made in atrial model to simulate chronic AF

Choices had to be made in changing model parameters to reproduce cAF, given sometimes-large ranges of variability in experimental measurements and variable degrees of remodeling. The rationale for adjusting the model parameters were the following (in order of importance): i) functional experiments were used over mRNA/protein expression data when the former were available; ii) we used our own data when available, or from our collaborators (we then better know the conditions); iii) parameter choices in previous modeling studies have been considered;
and iv) we tried to match experimental AP morphology and duration at 1 Hz (within the range of variability).

To simulate chronic AF, I_{Ka} maximal conductance was doubled in agreement with the reported AF-induced increase in current density (from +75 to +140%) and mRNA levels (+140%) \(^{18-20}\). I_{Io} maximal conductance was reduced to 30% to reproduce experimental findings showing 44-80% downregulation in current density \(^{18, 21}\) and 39-49% decrease in kv4.3 protein expression levels \(^{16}\). I_{Kur} maximal conductance was decreased by 50% to reproduce experimental results (no changes to -55% in current density, \(^{18, 21}\) and -54-84% in kv1.5 protein expression levels \(^{18}\)). Caballero et al. \(^{21}\) showed that I_{Ks} is enhanced in cAF. We modeled this by doubling I_{Ks} maximal conductance in cAF. Knockout of SK2 channels prolongs AP and cause AF in mouse \(^{22}\), however we did not model this current in the present study (see above).

I_{Na} maximal conductance was reduced by 10% to reproduce a slightly reduced peak I_{Na} density in cAF vs. sinus rhythm, and a late component was added as previously described (G_{Na}=0.0025 mS/µF) \(^{23, 24}\), as late I_{Na} was found significantly increased in myocytes from AF atria \(^{25}\). According to Workman et al. \(^{4}\), NKA was kept unaltered in the model of cAF.

I_{Cat} density is greatly reduced (~50%) in cAF compared to sinus rhythm (as seen in Fig. 2 and \(^{6, 18, 26}\)), whereas no changes were detected in channel activation and inactivation properties \(^{27}\). There is evidence that NCX is upregulated in cAF. Indeed, NCX protein expression is increased \(^{6, 28, 29}\) and the decay rate of caffeine-evoked Ca\(^{2+}\) transient (CaT, attributable to Ca\(^{2+}\) removal by NCX) decayed faster in human (in simulations 1.9 vs. 1.4 s\(^{-1}\)) \(^{6, 30}\) and sheep \(^{5}\) cAF vs. sinus rhythm myocytes. Accordingly, we increased NCX by 40% in the cAF model. The increased NCX activity was confirmed by plotting I_{NCX} density during CaT decline as a function of [Ca\(^{2+}\)] (experimental and simulated results in Figure 3E and F). With cAF, the curve shifted reflecting more I_{NCX} at a given [Ca\(^{2+}\)] (Figure 3E-G). SERCA pump rate was decreased, to recapitulate experimental findings showing less SERCA protein expression and slower CaT decay compared to sinus rhythm (our data in Figure 2 and \(^{6, 29}\)). Passive SR Ca\(^{2+}\) leak was increased (+25%) and RyR sensitivity to [Ca\(^{2+}\)]\textsubscript{SR} was enhanced twofold, in agreement with experimental results showing increased SR Ca\(^{2+}\) leak and increased RyR phosphorylation in AF \(^{6, 30-32}\).

**Simulation of β-Adrenergic challenge**

PKA-specific regulation of various targets was modeled as following (e.g., similar to Shannon et al. \(^{33}\)): I_{Ks} maximal conductance was enhanced (3 fold) and the current-voltage relationship was left-shifted (by 40 mV); I_{Kur} maximal conductance was enhanced (3 fold) \(^{34}\), troponin I affinity for Ca\(^{2+}\) was decreased (i.e., k_{d} was increased by 50%); Ca\(^{2+}\)-sensitivity of SERCA was enhanced (i.e., the forward mode k_{w} was reduced by 50%); L-type Ca\(^{2+}\) channels were increased (+50%) and channel availability was shifted leftward (by 3 mV); RyR sensitivity for luminal Ca\(^{2+}\) was increased (2 fold); affinity of Na\(^{+}\) pump for intracellular [Na\(^{+}\)] was increased by 25% \(^{35}\).

**Simulation of acetylcholine challenge**

We investigated the effect of acetylcholine on the human atrial action potential by incorporation of a time-independent, acetylcholine-activated K\(^{+}\) current I_{KACH} (Figure VI). Voltage-dependence (Fig. VIA) and dose-dependence (Fig. VIB) were formulated based on experimental data from Koumi et al. \(^{36}\) as follows:

\[
I_{KACH} = \frac{1}{1 + \left(\frac{0.03}{[ACh]}\right)^{2.1}} \left(0.04 + \frac{0.04}{1 + \exp\left(\frac{V_{m} + 91}{12}\right)}\right) \cdot \left(V_{m} - E_{K}\right)
\]

where [ACh] is the concentration of acetylcholine (at fixed levels ranging from \(10^{-4}\) to 1 µmol/L), V\(_m\) the membrane potential, and E\(_K\) the reversal potential for K\(^{+}\).
**Simulation of I_{Kur} block**

AVE0118 concentration-dependence of I_{Kur} block was modeled to reproduce experimental data of Decher *et al.*\(^{37}\).

$$f_{AVE0118} = 1 - \frac{1}{1 + \left(\frac{5.6}{[AVE0118]}\right)^{1.23}}$$

where [AVE0118] is in µmol/L (Fig. IVA-B).

Frequency-dependent block of I_{Kur} was simulated by inclusion of a state variable y describing the changes in the fraction of unblocked channels, similarly to Tsujimae *et al.*\(^{38}\).

$$I_{Kur(AVE0118)} = I_{Kur} \cdot f_{AVE0118} \cdot y$$

where

$$\frac{dy}{dt} = \frac{y_{\infty} - y}{\tau_y} \quad y_{\infty} = \frac{1}{1 + \exp\left(\frac{V_m - 75}{10}\right)} \quad \tau_y = t_{on} + \frac{t_{rec} - t_{on}}{1 + \exp\left(\frac{V_m + 75}{10}\right)}$$

Time constants of onset of block (t_{on}=10 ms) and recovery from block (t_{rec}=700 ms) were chosen to match the fast onset of AVE0118 and its frequency-dependent effect shown by Decher *et al.*\(^{37}\) (Figure IV, C experiments vs. D simulations).

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**Figure I** - AP was simulated with various degrees of I_{to} and I_{Kur} expression (A and B) and the plateau potential (measured as the average between 20 and 80 ms from AP upstroke) was plotted against the percentage of change in current density (C).
Figure II - IV relationship of $I_{to}$ was obtained by stimulating the digital atrial (red) and ventricular (black) cells with 200ms-long voltage steps from -90 mV to various membrane potentials.

Figure III - A) Membrane potential (i), [Na$^+$]$_i$ (ii) CaT (iii) and SR Ca$^{2+}$ content (iv) changes due to rapid pacing (at time 0) from steady-state at 0.5 Hz pacing frequency. B) Simulated steady-state APD$_{90}$ at varying pacing frequencies are compared to experimental data $^{27, 39}$. C) Time course of the changes in APD$_{90}$ and [Na$^+$]$_i$ after the pacing frequency is increased from 0.5 Hz (at steady-state) to 1 Hz. APD$_{90}$ slowly adapts to the faster rate in ~5 minutes.
Figure IV — A) Modeled concentration-effect relationship for Kv1.5 block by AVE0118 (from Decher et al. 37). B) Effect of varying [AVE0118] on $I_{Kur}$ evoked by the voltage step (inset, at 0.5 Hz). Measured (C) and simulated (D) normalized current amplitudes (with respect to pulse #1) during subsequent pulses at varying pacing frequencies.
Figure V – Simulations of $I_{kur}$ block in Figure 6 are performed with the concentration-response for AVE0118 from Christ et al. $^{40}$ (shown in inset).
**Figure VI** - A) Modeled $I_{KAC}$ is compared to experimental data from Koumi et al. B) Achetylcholine concentration-response curve was fitted to the data of Koumi et al. C) Simulated AP and Ca$^{2+}$ transient (D) traces at varying [ACh]. Experimental human atrial APs are shown in inset. E and F show APD$_{90}$ and CaT amplitude changes at increasing [ACh].
Figure VII – Simulation of $I_{\text{Kr}}$ block shows little effect on APD in agreement with data from Wettwer et al. (inset)\(^4^1\).

**Figure VIII –** A) $I_{\text{Cal}}$ and $I_{\text{Kur}}$ peaks do not change appreciably at 0.5 and 2 Hz pacing rates. Thus, rate-dependence of these currents does not play a role in APD rate-adaptation. $I_{\text{Kur}}$ is evoked by square (B) or triangular (C) voltage pulses applied at 0.5 and 2 Hz. Reduced $I_{\text{Kur}}$ availability at fast rate with square voltage pulses (similar to experimental results, as shown previously\(^1^1\)) is significantly attenuated by the triangular pulse (e.g., similar to AP waveform).
References


