Cholinergic Atrial Fibrillation in a Computer Model of a Two-Dimensional Sheet of Canine Atrial Cells With Realistic Ionic Properties

James Kneller, Renqiang Zou, Edward J. Vigmond, Zhiguo Wang, L. Joshua Leon, Stanley Nattel

Abstract—Classical concepts of atrial fibrillation (AF) have been rooted in Moe’s multiple-wavelet hypothesis and simple cellular-automaton computer model. Recent experimental work has raised questions about the multiple-wavelet mechanism, suggesting a discrete “driver region” underlying AF. We reexplored the theoretical basis for AF with a 2-dimensional computer model of a 5×10-cm sheet of atrial cells with realistic ionic and coupling properties. Vagal actions were formulated based on patch-clamp studies of acetylcholine (ACh) effects. In control, a single extrastimulus resulted in a highly meandering unstable spiral wave. Simulated electrograms showed fibrillatory activity, with a dominant frequency (DF, 6.5 Hz) that correlated with the mean rate. Uniform ACh reduced core meander of the spiral wave by 70% (as measured by the standard deviation of spiral-wave tip position) and accelerated the DF to 17.0 Hz. Simulated vagally induced refractoriness heterogeneity caused wavefront breakup as accelerated reentrant activity in regions of short refractoriness impinged on regions unable to respond in a 1:1 fashion because of longer refractoriness. In 7 simulations spanning the range of conditions giving sustained AF, 5 were maintained by single dominant spiral waves. On average, 3.0±1.3 wavelets were present (range, 1 to 7). Most wavelets were short-lived and did not contribute to AF maintenance. In contrast to predictions of the multiple-wavelet hypothesis, but in agreement with recent experimental evidence, our model indicates that AF can result from relatively stable primary spiral-wave generators and is significantly organized. Our results suggest that vagal AF may arise from ACh-induced stabilization of the primary spiral-wave generator and disorganization of the heterogeneous tissue response. The full text of this article is available at http://www.circresaha.org.

Key Words: atrial fibrillation ■ mathematical model ■ reentry ■ vagus nerve
atrial tissue with realistic ionic, coupling, and propagation properties; (2) to incorporate a representation of ACh’s ionic actions based on experimental observations to reproduce cholinergic effects on the AP; (3) to study the impact of varying intensity and distribution of ACh effects on reentrant arrhythmias in the model; and (4) to compare the determinants and properties of AF in the model with those in experimental studies.

Materials and Methods

Model Description and Implementation

The RNC model of the canine atrial AP was implemented. The cell membrane is modeled electrically as a capacitor connected in parallel with variable resistors and batteries representing the ionic channels and ionic fluxes. The total ionic current (I_{tot}) is given by the following equation:

\[ I_{tot} = I_{Na} + I_{K} + I_{Ca} + I_{Cl} + I_{p} + I_{Ca,SR} + I_{Cl,SR} + I_{Ca,JSR} + I_{Cl,JSR} \]

I_{tot} includes contributions from the fast sodium current (I_{Na}), the inward-rectifier K\(^+\) current (I_{K}), transient outward current (I_{To}), ultrarapid (I_{Na,r}), rapid (I_{K}), and slow (I_{K}) delayed-rectifier K\(^+\) current (I_{K}). Also included are the L-type Ca\(^{2+}\) current (I_{Ca}), a sarcoplasmic reticulum Ca\(^{2+}\) pump current (I_{sarc}), a Na\(^{+}\)/Ca\(^{2+}\) exchanger current (I_{Na,Ca}), a Ca\(^{2+}\)-activated Cl\(^-\) current (I_{Cl}), and background Na\(^{+}\) (I_{Na}) and Ca\(^{2+}\) (I_{Ca}) currents. A detailed tabulation of currents and their representation is provided in Ramirez et al. An expression for the ACh-activated potassium current (I_{ACh,K}) was formulated (described below) and included in Equation 1 to account for ACh effects.

The cellular model constantly monitors intracellular concentrations of Na\(^+\), K\(^+\), Ca\(^{2+}\), and Cl\(^-\). Sarcoplasmic reticulum (SR) Ca\(^{2+}\) handling is described using a 2-compartment model composed of network (NSR) and junctional (JSR) components subserving Ca\(^{2+}\) uptake and release, respectively. Ca\(^{2+}\) release from the JSR is induced by Ca\(^{2+}\) influx into the myoplasm, with close coupling between sarcomembran I_{Ca} channels and subcellular I_{Ca} channels. A formulation of a Na\(^+\)/Cl\(^-\) cotransporter and the background Cl\(^-\) current (I_{Cl}) were added to account for Cl\(^-\) flux. 

Potential Maps

For display purposes, propagating wavefronts over the entire computational substrate were visualized by constructing potential maps on a 25×50 pixel display grid, after verifying that observed activation patterns were not distorted by this approach. The transmembrane potential was sampled at the center of each 12×12 segment square on the computational grid after each millisecond. The visual display of static potential maps in Figures 4, 5, 6, 8, and 10 through 12 was enhanced in a postprocessing step using Image Magick software (Dupont). Corresponding movie supplements re
tained the original sampling resolution and can be found in the online data supplement available at http://www.circresaha.org.

Simulated Pseudounipolar Electrogram

A unipolar electrogram for a sheet of cells under conditions of uniform intracellular anisotropic resistivity (assuming the sheet was immersed in a bath of infinite size) was simulated as previously described. The extracellular potential \( \Phi_e \) is given by the following equation:

\[ \Phi_e(P) = \frac{\rho_e}{4\pi} \sum_{i=1}^{M} \sum_{j=1}^{N} \frac{I_{ACh,K}}{r_{ij}} \]

where \( M \) and \( N \) are the total numbers of segments in the longitudinal and transverse directions, respectively, and \( r_{ij} \) is the distance from the observation point \( P \) to the center of the volume element \( V_i \) at node \( k,j \). The value of \( \rho_e=100 \ \Omega \cdot \text{cm} \) is close to that of 0.9% saline solution.

Signal Analysis

To compare the frequency content of experimental and model electrograms, spectral analysis of signals was performed with fast Fourier transforms.

Two-Dimensional Simulation Protocols

Model effective refractory period (ERP) was measured by simulating the experimental protocols of Wang et al. A square electrode, \( \approx 0.8 \) mm per side, was used to best compare with the 2-pronged hook electrodes in experiments (each =0.5 mm per side). All stimuli were 180 \( \mu \)A/cm\(^2\) in strength and 1 ms in duration. CV was calculated in the longitudinal direction between electrodes positioned 0.5 cm apart, and the proximal electrode was a sufficient distance from the stimulus site such that the wavefront was planar. Proximal segments were stimulated from rest at cycle lengths of 200 and 400 ms, and a single premature stimulus (S\(_2\)) was delivered after every 15 basic (S\(_1\)) stimuli. ERP was defined as the longest S\(_1\)-S\(_2\) interval that was measured at the ERP of the preceding concentration and reduced such that ERP measurements were accurate within 0.5 ms. CV was measured from S\(_1\) pulse trains at each concentration. The wavelength (ERP×CV), thought classically to represent the minimum path length for reentry and to determine the size of functional reentry circuits, was computed as previously defined. Reentry was initiated using a cross-shock protocol. An S\(_1\) stimulus was applied along the uppermost cable, creating a wavefront propagating uniformly in the transverse direction. Before the recovery front reached the half-way point, an S\(_2\) stimulus was applied to 1/4 of the sheet, establishing a single phase singularity and initiating reentry.
Fourier transformations (FFTs).\textsuperscript{3–5} Content in the 0 to 60-Hz band was analyzed. Because it was possible to scale model results uniformly, FFTs of electrograms recorded at various observation points during the same simulation were normalized to the largest peak to facilitate comparison of the relative power of spectral peaks between sites. Activity was sampled at 1000 Hz (1 ms) for 5000 frames (~5 seconds), providing a spectral resolution of 0.2 Hz.

**Experimental Techniques for \( I_{K(ACH)} \)**

**Characterization**

Single canine atrial myocytes were isolated as previously described.\textsuperscript{24} The right atrium from adult mongrel dogs (20 to 26 kg) of either sex was dissected and mounted via the right coronary artery to a Sylgard perfusion system. Preparations were then perfused with \( \text{Ca}^{2+}- \) containing Tyrode’s solution at 37°C and then with \( \text{Ca}^{2+} \)-free Tyrode’s solution for 20 minutes at 12 mL/min, followed by the same solution containing collagenase (110 U/mL CLS II; Worthington Biochemical) and 0.1% BSA. Dispersed cells were stored in KB medium at 4°C.

The Tyrode’s solution contained (in mmol/L) NaCl 136, KCl 1, CaCl\(_2\) 1, Na\(\text{H}_2\)PO\(_4\) 0.33, glucose 10, and 2-hydroxyethylpiperazone-N\(_2\)-2-ethanesulfonic acid (HEPES) 5; pH 7.4 (NaOH). The KB medium contained (in mmol/L) KCl 20, KH\(\text{HCO}_3\) 1.2, MgSO\(_4\) 1.2, KH\(_2\)PO\(_4\) 1.2, NaHCO\(_3\) 25, and glucose 5.5. Arterial leaks were ligated and the tissue perfused at 10 mL/min for 5 seconds, providing a spectral resolution of 0.2 Hz.

**Experimental Determination of Cholinergic Effects on the AP**

APs were recorded from intact atria using standard microelectrode techniques as described previously.\textsuperscript{25} Adult mongrel dogs (n=7, 20 to 32 kg) were anesthetized with pentobarbital (30 mg/kg IV). Their hearts were removed, and the right atrium was dissected and perfused via the right coronary artery with Krebs’ solution (37°C, pH 7.35 to 7.40, 95%O\(_2\)/5% CO\(_2\)) containing (in mmol/L) NaCl 120, KCl 3.8, CaCl\(_2\) 1.2, MgSO\(_4\) 1.2, KH\(\text{PO}_4\) 1.2, NaHCO\(_3\) 25, and glucose 5.5. Arterial leaks were ligated and the tissue perfused at 10 to 12 mL/min, approximating normal canine atrial flow.\textsuperscript{26} Preparations were stimulated endocardially with square-wave pulses (4 ms), 1.5 to 2 times diastolic-threshold current. Glass microelectrodes were filled with 3 mol/L KCl (12 to 20 MΩ) and coupled to an Axoclamp 2B amplifier.

Endocardial APs were recorded after pacing to steady state at 0.1, 0.2, 1, 2, 4, and 6 Hz before and during perfusion with carbamylcholine (Sigma). Carbamylcholine was used to obtain reproducible dose-response relations as ACh is rapidly broken down by tissue cholinesterases. Carbamylcholine concentrations of 0.1 and 1 μmol/L were used, as the AP was maximally reduced at 1 μmol/L. The stimulus site was at least 1 cm from the impaled cell,\textsuperscript{27} and an interval of constant resting potential between the stimulus artifact and the AP was confirmed.\textsuperscript{28} Recovery of control AP morphology was confirmed on washout.

**Terminology**

The following terminology was used:

**AF**: defined by rapid and irregular electrograms with varying morphologies. Potential maps were required to demonstrate propagation along lines of block and ongoing wavefront breakup, which changed on a cycle-to-cycle basis.\textsuperscript{3,5}

**Wavebreak**: a discontinuity that allows contact between a wavefront and its own repolarization tail. Wavebreak occurs after blockade along a wavefront while remaining segments continue to propagate.\textsuperscript{4}

**Phase Singularity (PS)**: a point around which any arbitrary neighborhood contains all phases of the waveform. It is often the point where the depolarizing front and repolarizing tail of a wavefront meet.\textsuperscript{29} PSs arise from wavebreaks and are located at the instantaneous center of rotational activity.\textsuperscript{4}

**Rotor**: a spiral wave of excitation rotating around a PS for 1 or more cycles.\textsuperscript{4}

**Chirality**: the sense of rotation, clockwise or counterclockwise, around a PS.\textsuperscript{4}

**Core**: area circumscribed by the trajectory of a PS. A small region of excitable but unexcited tissue around which a rotor rotates.\textsuperscript{30}

**Wavelet**: segments of excitation wavefronts, regardless of size, flanked by 2 PSs or a PS and a boundary.\textsuperscript{4}

All data are presented as mean±SD.

**Results**

**I\(_{K(ACH)}\) Formulation**

Figure 1A shows \( I_{K(ACH)} \)-V relations and the model fit. The current was formulated as follows:

\[
I = \frac{a_0 + \frac{a_1}{1 + \exp \left( \frac{V + a_2}{a_3} \right)}}{(V - E_k)}
\]

Parameters were determined by nonlinear regression (10\(^{-6}\) error tolerance) at each concentration using the experimental reversal potential \( E_k \) and are listed in the Table.

The dose-response relation was determined using nonlinear regression and is given by the following:

\[
E = E_{\text{max}} \left[ \frac{1}{1 + \frac{EC_{50}}{[\text{ACh}]}^n} \right]
\]

where \( E_{\text{max}} \) is the maximal effect, \( EC_{50} \) is the concentration for half-maximal effect, and \( n \) is the binding order. The dose-response characteristics were best reproduced with \( E_{\text{max}} = 10, EC_{50} = 9.13652, \) and \( n = 0.47781 \) (Figure 1B).

Expressed in pA/pF, \( I_{K(ACH)} \) is given by the product of Equations 3 and 4 (as indicated in Equation 5):

\[
I_{K(ACH)} = \frac{10}{1 + \frac{9.13652}{[\text{ACh}]}^{4.13652}} \left[ 0.0517 + \frac{0.4516}{1 + \exp \left( \frac{V + 59.53}{17.18} \right)} \right] (V - E_k)
\]

with the best-fit parameters at 1 μmol/L from the Table.

Figure 1C shows model \( I_{K(ACH)} \) with accompanying APs at 1 and 6 Hz in control and with 0.003 μmol/L and 0.03 μmol/L ACh.
Experiment and Model AP Comparisons

Figure 2 compares experimental (left) and model (right) results. Mean AP durations (APDs) to 90% repolarization (APD90) are shown in Figure 2A. Representative APs are shown in Figure 2B. ACh concentrations of 0.003 and 0.03 μmol/L had effects in the model similar to carbamylcholine (CBC) concentrations (0.1 and 1 μmol/L) producing moderate and strong APD abbreviation in tissue preparations and were therefore used in model simulations. Consequently, all model simulations were conducted within the range of ACh concentration over which the \( I_{K(ACh)} \)relation was in closest agreement with experiments (Figure 1B). Overall APD90 was reduced at maximum cholinergic stimulation by 81.7% and 73.8% in experiments and the model, respectively. APD rate adaptation (the difference in APD90 from 0.1 to 6 Hz) was substantial under control conditions (144 ms in experiments and 175 ms in the model; Figure 2C), but nearly abolished at maximal \([ACh]\) (14 ms in experiments and 19 ms in the model; Figure 2D). Resting membrane potential was slightly hyperpolarized by \( I_{K(ACh)} \). No significant ACh-dependent changes in AP amplitude and upstroke velocity (Vmax) were discernible.

Experiment and Model Distributed Properties

Figure 3B compares in vivo measurements of canine atrial ERP at increasing levels of vagal stimulation in the 2-dimensional model as \([ACh]\) was increased.21 In the absence of ACh, the model ERP agreed well with experimental controls (166 versus 160 ms, respectively). Consistent with AP findings, ERP matched experimental data at maximal vagal stimulation (63 versus 60 ms, respectively). Like

\[ \text{Equation 3 Coefficients for Each [ACh]} \]

<table>
<thead>
<tr>
<th>[ACh], μmol/L</th>
<th>( a_0 )</th>
<th>( a_1 )</th>
<th>( a_2 )</th>
<th>( a_3 )</th>
<th>( a_4 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02</td>
<td>1.3384 ( \times 10^{-2} )</td>
<td>2.2663 ( \times 10^{-2} )</td>
<td>8.0253 ( \times 10^{-1} )</td>
<td>5.0714 ( \times 10^0 )</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>2.5400 ( \times 10^{-2} )</td>
<td>1.4080 ( \times 10^{-1} )</td>
<td>6.5730 ( \times 10^1 )</td>
<td>1.1690 ( \times 10^0 )</td>
<td></td>
</tr>
<tr>
<td>1*</td>
<td>5.1700 ( \times 10^{-1} )</td>
<td>4.5160 ( \times 10^{-1} )</td>
<td>5.9530 ( \times 10^1 )</td>
<td>1.7180 ( \times 10^1 )</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>9.6176 ( \times 10^0 )</td>
<td>5.6040 ( \times 10^1 )</td>
<td>6.3561 ( \times 10^1 )</td>
<td>2.3897 ( \times 10^1 )</td>
<td></td>
</tr>
</tbody>
</table>

*1 μmol/L parameters used in Equation 5.
The experimental data, model CV changes due to ACh were minimal (Figure 3C).

Associated wavelength reductions agreed quantitatively with experimental findings and paralleled ERP changes because CV was relatively constant (Figure 3D).

ACh accelerates the rate of atrial reentry. To evaluate this effect in the 2-dimensional model, reentry was initiated under control conditions (no ACh) and then with increasing [ACh]. The reentry period was determined from activation times in the upper left corner of the grid. In the absence of ACh, model reentry periods (Figure 3A) agreed with experimental controls.

The mean rate increased 2.7-fold across concentrations, whereas the SD decreased by 95%, indicating that ACh had accelerated and organized reentry.

Reentry in the Absence of ACh

Figure 4A shows a representative example at a single time point of a single reentry circuit under control conditions. Animation of activity in Figure 4A is also provided as an online-only movie (see online Movie 1). The rotor was unstable, propagating along a hypermeandering and irregular trajectory. The mean reentry cycle length was 155±16 ms (range, 132 to 195 ms), in agreement with previous measurements in isolated canine right atria (162±20 ms; range, 130 to 220 ms). The cycle-to-cycle variability in position was quantified by plotting the trajectory of the rotor tip in 10 ms intervals (Figure 4B). The standard deviation of the longitudinal position between cycles was 0.95 cm. The core area was calculated by dividing rotor-tip points into cycles (n=10) and constructing polygons traced by the tip for each cycle. The mean core area per cycle was thus determined to be 2.78±0.42 cm². Sustained activity was rare in the absence of ACh. In this example, the wave encountered the no-flux boundary at the edge of the tissue substrate and terminated after ~3 seconds of spontaneously sustained activity.

Electrograms were recorded within the region of reentry (Figure 4C) and at a more remote area (Figure 4D). In agreement with previous findings that a single meandering spiral is sufficient to produce AF-like electrograms, electrogram complexes at both sites were rapid, irregular, and polymorphous. Activations at the distal site were irregular because of the nonuniform rate of reentry (Figure 4D). As observed experimentally, signal polymorphism (variability in size and shape of electrograms) was greater in proximity to the spiral-wave core (Figure 4C). When the core drifted away from the recording site, electrical activity exhibited large-amplitude complexes. Return of the core again decreased signal amplitude. Large complexes were indicative of local activation, whereas deflections <10% of these were considered to be passive depolarizations (red arrows). FFTs of electrograms revealed narrow-banded frequency content containing discrete peaks, with a dominant frequency (DF) at 6.5 Hz that correlated with the mean cycle length (Figures 4C and 4D). Higher-frequency peaks at decreasing power represent...
harmonics of the DFs (blue arrows). The notches that broaden each harmonic were attributed to meandering of the spiral wave pivoting point around an instantaneous center that drifts, with possible modulation by the Doppler phenomenon.\textsuperscript{37,38} Comparison of Figures 4C and 4D indicates that spectral power was reduced in proximity to the spiral core.

**Organization of Reentry by ACh**

Figure 5A shows a single reentry circuit with 0.03 μmol/L ACh distributed uniformly over the computational substrate. Animation of activity in Figure 5A is also provided as an online-only movie (see online Movie 2). Reentry was organized into a stable stationary rotor (Figure 5B). The mean cycle length was 58±0.8 ms, a 2.7-fold rate increase from control (Figure 3A). Reflecting increased organization, the standard deviation of the position was 0.28 cm, reduced by 70% from control. The area of the core was 1.25±0.097 cm\(^2\), reduced by ∼55% from control.

An electrogram within the spiral core (+, Figure 5C) and an example representative of activity at more distal locations (o, Figure 5D) are shown. Complexes were uniform and regular outside the core, characteristic of atrial flutter.\textsuperscript{39}

Within the core, meander of the spiral tip caused polymorphic activity. FFTs of electrograms reflected global organization. A dominant peak at 17.0 Hz correlated with the mean rate of reentry. Discrete peaks at higher harmonics were also seen (blue arrows). An additional peak at 28 Hz (Figure 5C) due to meandering of the spiral may be seen as a high-frequency wobble of the spiral core in the online Movies. This motion was not evident at the distal site (Figure 5D). Spectral power was markedly reduced within the core.

**APD Heterogeneity and AF**

Cholinergic effects are heterogeneously distributed in vivo.\textsuperscript{30,41} To investigate the role of dispersion of APD and refractoriness in AF, ACh concentration was varied according to a sinusoidal distribution across the computational substrate. Equation 6 gives the distribution of ACh at position \(x, y\) as follows:

\[
\text{ACh}(x, y) = b_{\text{ACh}}[1 + B \sin(2\pi C x + \phi) \cos(2\pi C y)]
\]

where \(1 \leq x \leq 600\) and \(1 \leq y \leq 300\) along the longitudinal and transverse dimensions, respectively, \(b_{\text{ACh}}\) represents baseline [ACh], \(B\) determines the relative amplitude of ACh oscillations around \(b_{\text{ACh}}\) with \(0 \leq B \leq 1\), and \(C\) controls the periodicity of the spatial distribution. \(\phi = 0\) by default but was set to \(\pi/2\) to initiate reentry when the heterogeneity was such that the \(S_2\) stimulus encountered block in the transverse direction and failed to launch a rotating circuit.

Equation 6 created APD and consequently ERP gradients (graded changes from lowest to highest ACh concentrations). To obtain an estimation of spatial APD variation, the entire substrate was stimulated from rest at 6 Hz, and AP duration to −60 mV (APD\(_{60}\)) was measured across the grid for the 10th pulse. APD\(_{60}\) was used because −60 mV is the approximate voltage at which excitability is restored and APD\(_{60}\) is therefore closely related to the ERP.

Reentry was simulated at varying [ACh] distributions (distance between lowest and highest concentrations) and a constant mean [ACh]. Figure 6 shows representative results. When [ACh] was closely distributed (Figure 6A: ∼0.67 cm), electrotonic influences limited APD variations; the spiral was distorted, but lines of block failed to form and no wavebreak resulted. Electrograms were regular, and associated FFTs confirmed a single discrete peak throughout the recording field. AF resulted when [ACh] gradients were distributed over larger distances (Figure 6B: 3.3 cm). Ongoing breakup into independent wavelets was observed. Electrograms were irregular and polymorphous. FFTs were disorganized, yet frequency content was narrow-banded with one or multiple discrete peaks, the largest of which correlated with mean frequency. Flutter-like activity was restored when the ACh distribution was sufficiently diffuse (Figure 6C; 10 cm).

The DFs were from 12.2 to 15.3 Hz, reflecting substantial ACh-induced acceleration of reentry relative to control (Figure 4), despite the absence of ACh in certain regions. In Figure 4A, stable reentry was possible at the rapid rate because electrotonic modulation shortened ERP in regions where ACh was absent. In Figure 4B, the greater dispersion of vagal effects decreased electrotonic modulation, and on-
going breakup occurred because reentry was faster in regions of high [ACh] with short ERP, causing wavefronts to impinge on zones of greater refractoriness in regions with low [ACh]. The spiral wave in Figure 4C rotated in a region of high [ACh] with a DF (15.3 Hz) close to the mean reentry rate for \(0.015\text{ mol/L (≈67 ms; 14.9 Hz)}\) uniform ACh (Figure 3A).

Baseline APD values ranged between 28 and 81 ms in Figures 4A through 4C, with differences being greater at the largest distributions (smallest electrotonic attenuation). Although this analysis provides a useful approximation of the dispersion of repolarization, variations in local activation rate during AF change APD on a cycle-to-cycle basis: this adaptation is important to AF-like dynamics. At rapid rates of AF (DF ≈12.2 Hz), regions of block result in local episodes of 2:1 conduction that increase the range of APD values. In contrast to the static APD values shown in Figure 4B, APD was 65 ± 23.6 ms (range, 15 to 111 ms) in cells with 0 \(\mu\text{mol/L ACh}\) and 34.3 ± 3.3 ms (range, 30.1 to 34.9 ms) in cells with 0.015 \(\mu\text{mol/L}\).

Results in Figure 6 indicate that AF only occurred for certain heterogeneity conditions. Figure 7 shows an analysis of the relationship between the combinations of concentrations and spatial distributions and AF occurrence. Each entry in the graphic represents results from 1 of 67 simulations. The minimum [ACh] was 0 and the maximum for each simulation is shown on a logarithmic scale on the vertical axis, with [ACh] distributions (distances between maximum and minimum [ACh]) given on a logarithmic scale along the horizontal axis. “Sustained AF” was defined as AF >5 seconds. Conditions were classified as “fibrillatory” if AF-like activity was not sustained. Conditions were said to be “nonfibrillatory” when no wavebreak occurred. Fibrillatory distributions ranged from 1.25 to 2.5 cm at low [ACh] to 1.25 to 7.5 cm at high [ACh]. Sustained AF occurred only at high [ACh] when APD gradients were large and distributed between 1.88 and 5.0 cm (mean 3.3 cm). Results in Figure 7 suggest that the...
ACh effect must be sufficiently strong to permit sustained AF, and that as ACh concentration increases, larger spatial distributions are needed for fibrillation. To determine the effect of higher ACh concentrations with smaller differences between peak and trough concentrations, 7 additional simulations were conducted with peak [ACh] of 0.03 μmol/L and trough [ACh] of 0.015 μmol/L (at all distributions); non-fibrillatory activity was observed in all cases (results not shown).

Vagal stimulation reduces mean ERP while increasing ERP heterogeneity in vivo. Figure 8 illustrates the role of concentration gradients in ACh-related AF as cholinergic tone increases. Potential maps show the initiation of reentry, and subsequent activity at the simulation time (ms) indicated in the upper left corner. In Figure 8A, 0.0003 μmol/L mean [ACh] was distributed at 2.5 cm, and concentration oscillations (the difference between maximum and minimum [ACh]) were imposed in increments of ±10% of baseline. When concentration oscillation amplitude was increased from ±40% (left panels) to ±50% (right panels), wavefront thinning (arrows) became clear at 1130 ms, resulting in a transition from flutter-like to AF-like dynamics (1175 ms). FFT alterations reflected the increased disorganization. Figure 8B shows results with a 10-fold increase in mean [ACh] (0.003 μmol/L). This time, a transition from flutter-like to AF-like behavior was observed when concentration oscillation amplitude was increased from ±60% to ±70%. Results at ±50% are also shown, as wavebreak (arrows) was clearly observed at ±60% (497 and 1420 ms), yet without a change in the overall activation pattern. The instabilities at ±60% were reflected in electrogram changes, although AF-like signals were only observed at ±70%. These results demonstrate that as baseline [ACh] increases, larger [ACh] oscillations are needed for fibrillation. DFs were at 8 Hz (Figure 8A) and 11 Hz (Figure 8B). These rates are comparable to the reentry rate at a homogeneous [ACh] (Figure 3A; cycle-lengths 125.4 and 92.5 ms, respectively) equivalent to the mean under each condition, indicating that mean concentrations were the primary determinants of DFs. Otherwise, spectral power was markedly reduced after breakup, suggesting frequency modulation by competing transient circuits. As in Figure 6, breakup occurred because reentrant activity
Mechanisms of AF Maintenance

AF Maintained by a Single Spiral Wave

Figure 10 shows an example of AF maintained by a single spiral wave. The distribution of [ACh] (1.67 cm), an APD distribution map, electrogram recording sites, and a representative potential map during AF are shown (Figure 10A). Animation of activity in Figure 10A is also provided as an online-only movie (see online Movie 3). A functionally determined rotor with clockwise chirality was located near (+) and acted as a primary source of activity, generating impulses that fractionated into multiple independent wavelets along the heterogeneous substrate. Most died out without incidence. Several PSs with opposite chirality attached to functional anchors near the right border and persisted for 1 to 3 rotations. An average of 3.5±1.2 wavelets were simultaneously present, with 6 to 7 at times of peak disorganization (Figure 10D). Wavelets tended to multiply from the primary spiral and would at times coalesce to form a large unstable circuit, reducing the wavelet count to 1. These episodes of global organization were short-lived, however, as activity cycled through varying degrees of complexity. Sequences of spatiotemporal periodicity were evident throughout.

Representative examples of electrograms recorded near (+, Figure 10B) and distal to (o, Figure 10C) the primary circuit are shown. The irregular propagation patterns were associated with decreased electrogram amplitude and increased morphological variability at the distal site. Although the power of the major spectral peak was also reduced, DFs at both locations were the same (11.2 Hz) and correlated with the mean cycle length of the dominant circuit (89.3 ms), as expected for AF arising from a relatively stable periodic source. Frequency content was narrow-banded with discrete spectral peaks between 8.7 and 19.7 Hz. The DF was slower than the reentry rate with uniform ACh (Figure 3A; 13.1 Hz) at the same mean concentration (0.0075 μmol/L), indicating that heterogeneity slowed reentry. The reduced rate is due to anchoring and slowing of the dominant spiral about a functional obstacle and the effects of electrotonic modulation, through which influences from regions of low [ACh] increase APD in regions of high [ACh].

AF Maintained by Multiple Spiral Waves

AF sustained by multiple sources has also been observed, which may appear sporadically or be spawned several centimeters away from the point of initiation. Figure 11 shows an example of AF maintained by up to 2 spiral waves. The distribution of [ACh] (3.33 cm), an APD distribution map, electrogram recording sites, and a representative potential map of activity are shown (Figure 11A). Animation of activity in Figure 11A is also provided as an online-only movie (see online Movie 4). AF initially arose from a primary rotor below (+). This spiral failed to anchor, increasing the variability of the PS trajectory. Emanating impulses impinged on refractory tissue, fractionating into wavelets with variable chirality. This time, many more PSs completed multiple reentry cycles, competing with the primary spiral. At ≈2.5 seconds the initial primary spiral died out along the bottom right border, at the time indicated by the black vertical line in Figure 11C. A second generator of opposite chirality origi-
nated from wavebreak at ~2.3 seconds, then assumed dominance and sustained fibrillation for the duration of the simulation (indicated as “generator 2” in Figure 11C). No appreciable difference in the reentry periods of the generator spirals was found. Figure 11A of the figure shows activity when both generator spirals coexisted. An average of 3.0±1.4 wavelets were simultaneously present, with as many as 7 at times of peak disorganization (Figure 11D). Sequences of

Figure 10. AF maintained by a single spiral wave. A, Top left, [ACh] over the grid; Bottom left, APD distribution; Right, a representative transmembrane potential map at one time point during AF. Asterisk indicates tip of the spiral; solid arrows, direction of wavefront propagation; and dashed arrow, chirality of the spiral. The larger symbols correspond to the primary spiral; smaller symbols to transient wavelets generated by the primary spiral. Electrograms were recorded near the source (B) and distally (C). Corresponding FFTs are at the right. D, Number of wavelets present at each time.

Figure 11. AF maintained by multiple spiral waves. A, Top left, [ACh] over the grid; Bottom left, APD distribution; Right, a representative transmembrane potential map at one time point during AF. Asterisk indicates tip of the spiral; solid arrows, direction of wavefront propagation; and dashed arrow, chirality of the spiral. In this example, one primary spiral maintained AF until 2.5 seconds of the simulation. This was then replaced by a second spiral originating from wavebreak at 2.3 seconds, which sustained AF for the rest of the simulation. Map in A was obtained at an instant when both the initial generator (1) and the second spiral (2) coexisted. Electrograms were recorded near the source (B) and distally (C). Corresponding FFTs are at the right. D, Number of wavelets present at each time.
spatiotemporally periodic propagation patterns arising from both generators were evident. Heterogeneity was such that the minimum path length for reentry of transient circuits was clearly not determined by the wavelength for all cycles, as calculated in Figure 3D and Wang et al.\textsuperscript{21} and Rensma et al.\textsuperscript{22}

Electrograms recorded at all sites were characteristic of AF. Representative examples of electrograms recorded near (+, Figure 11B) and distal to (o, Figure 11C) the primary sources are shown. Matching DFs (9.7 Hz) in Figures 11B and 11C indicated that activity at both sites arose from the same generator-type spiral. The rate was slower than in Figure 10 because refractory regions were broader, increasing the cycle length around functional obstacles. The amplitude of signals near the primary sources was reduced by the proximity of the cores, whereas the spectral profile at the distal site was much less organized due to the competing influence of other transient spirals.

**Apparent Multiple-Circuit AF**

Figure 12 shows an example of AF maintained by a single spiral wave (≈2.8 cm dispersion), which gives the appearance of multiple-circuit AF. Figure 12A shows a representative potential map during AF and animation of activity is provided as an online-only movie (see online Movie 5). A single spiral-wave generator was located below (+) and persisted for the duration of the simulation, although numerous PSs completed multiple reentry cycles. The mechanism of breakup was the same as in other simulations of AF (Figures 6, 8, 10, and 11). On average, 3.1±1.2 wavelets were present. Electrograms recorded at all sites were characteristic of AF. Electrogram frequency content near the primary source (+, Figure 12B) was narrow-banded, with a dominant discrete peak (12.0 Hz) that corresponded to the mean period of the source (≈80 ms). DFs were greatly reduced in Figure 12C and lower frequencies were more prominent, reflecting a process of spectral transformation\textsuperscript{43} between the generator region and the distal site. It might have been expected that the DF would fall between those in Figures 10 and 11, because the spatial dispersion of [ACh] in Figure 12 is intermediate between those in Figures 10 and 11. The DF was faster, however, likely because of the net effect of less electrotonically-mediated ERP increase in regions of high [ACh] than in Figure 10 (favoring an increased rate of reentry), smaller functional obstacles than in Figure 11, and less interference by competing transient circuits than when AF was maintained by multiple spirals (Figure 11).

**Discussion**

We have developed a mathematical model of canine atrial electrophysiology and cholinergic actions that reproduced experimentally observed AF dynamics. AF in the model is maintained by primary reentry sources, which are often single dominant spiral waves, but may also involve more than one spiral wave in succession with periods of overlap.

**Model Studies of AF**

Since the early 1960s, Moe’s multiple-wavelet hypothesis\textsuperscript{1} has shaped clinical and experimental conceptions of AF. To test his hypothesis, Moe et al.\textsuperscript{1} developed a simple cellular automaton computer model of canine atrial tissue and cholinergic AF containing 992 4-mm diameter tissue-units in a 12.4×12.8 cm atrial sheet. Under control conditions, CV was...
≈80 cm/s and conduction time ≈160 ms (about twice typical experimental values). Units were programmed with rate dependence of the absolute refractory period. CV dropped to 20 cm/s during the relative refractory period (30 ms fixed duration), slowing propagation to extremely low levels during repeated stimulation. ERP heterogeneity was randomly distributed, permitting maximal ERP gradients between adjacent units, as electrotonic modulation was not incorporated.

Moe acknowledged that this substrate bore only a limited resemblance to atrial tissue electrophysiology and recognized severe limitations to the computer representation and capacity. The strength of the model was that it displayed sustained turbulent reentrant activity resembling fibrillation. Limitations included the nonphysiological determinants of the cellular-automaton substrate, totally random ERP distribution (impossible with physiological electrotonic interactions), and CV heterogeneity, on which fibrillation depended. The model suggested that AF is a totally random process maintained by the ongoing multiplication of wavelets, with 23 to 40 needed at any time.

Our model included a complete ionic representation of the canine atrial AP, ensuring that rate dependence and ACh-related changes in rate-dependent properties were realistic (Figure 2). Coupling properties were selected to ensure that important 2-dimensional properties including ERP, CV, CV anisotropy, and wavelength were physiological at all [ACh] used (Figure 3). These features enabled the model to reproduce experimental observations of AF, permitting meaningful analyses of potential underlying mechanisms.

Under control conditions, significant rate adaptation caused unstable reentry and meander that was sufficient to manifest as AF (Figures 4C and 4D), although spontaneous activity was generally self-terminating. Uniform ACh abolished rate adaptation (Figure 2D) and markedly reduced the wavelength (Figure 3D), thereby organizing reentry into a highly stable stationary circuit and flutter-like rhythm (Figure 5D). These results are consistent with the findings of Nygren et al., who recently explored the role of rate adaptation in spiral-wave stability using models of the human atrial AP by Nygren et al. and Courtemanche et al. The Courtemanche AP displayed substantial rate adaptation although the Nygren AP did not; consequently, the Nygren model supported very stable and periodic reentrant activity, whereas the Courtemanche model displayed less stable, aperiodic behavior with stable and periodic reentrant activity, whereas the Courtemanche model displayed less stable, aperiodic behavior with stable and periodic reentrant activity, whereas the Courtemanche model displayed less stable, aperiodic behavior with stable and periodic reentrant activity, whereas the Courtemanche model displayed less stable, aperiodic behavior.

Spectral analysis quantitatively reflected this organization, demonstrating that source frequencies may also be dominant at distal sites (Figures 10 and 11) or altered by spectral transformation along the heterogeneous myocardium (Figure 12). Both results are consistent with experimental studies of the spatial distributions of DFs during AF, demonstrating how activity at various sites may be related, despite seemingly random activity throughout. Berenfeld et al. demonstrated that AF arising from these mechanisms may be characterized by multiple domains with distinct DFs on the atrial epicardium, thereby revealing a hidden organization, independent of the activation sequences or nature of the electrograms. Some studies emphasize the spatial variability of spectral properties, whereas others demonstrate that DFs may form large uniform domains or be spectrally transformed, depending on conditions between sites of comparison. As in these experimental studies, we noted a prominent underlying organization in AF, with the ability of vagal tone to promote sustained AF related both to stabilization of a primary spiral wave (which causes arrhythmia maintenance) and disorganization of propagation by repolarization gradients (which causes fibrillatory dynamics).

Schuessler et al. first demonstrated that ACh exerts an organizing influence on fibrillatory dynamics, and that AF maintenance depends on the strength of this effect. In an in vitro study of cholinergic AF, the number of circuits and wavelets increased with increasing [ACh], but activity was only sustained at high concentrations when a single, relatively stable circuit emerged and dominated activity. The wavelet count was greatest after stabilization. Skanes et al. further established organization during cholinergic AF, demonstrating that complex AF electrograms arose from a small number of dominant frequencies. Both Skanes et al. and Mandapati et al. demonstrated that AF sources produce a left-to-right atrial frequency gradient, as wavefronts sometimes encounter local activity and not all conduc-
tation pathways (Bachmann’s bundle, inferoposterior pathway) are able to capture in a 1:1 manner.

Indications from our model that multiple wavelets are a consequence of AF-generating mechanisms, rather than a cause of AF, agree with the wavelet analysis of Chen et al during cholinergic AF (Figure 9A). In the latter study, the mean PS (n=554) lifespan was 19.5±18.3 ms with a range that varied from 8.33 (1 frame) to 200 ms. The number of waves that entered the mapping field (15.7±1.6) significantly exceeded the number that exited (9.7±1.5). If wavebreak and the resulting wavelets were maintaining AF, the number of wavelets exiting the field of view would have been expected to exceed the number entering. The distribution of surviving PSs was found to be nonrandom, as would occur for activity originating from primary sources.

In the 7 simulations of sustained AF, ongoing breakup of primary circuits formed wavelets that would collectively form unstable circuits (as in Figures 10 through 12), with an average ≈3 wavelets at any time. In canine cholinergic AF, Allessie et al found an average of 3 wavelets (range from 0 to 5) present per atrium. Schuessler et al observed 0 to 15, with 5.1±1.8 present during sustained AF. Liu et al reported 3.3±0.3 wavelets at a time. Similarly, Kumagai et al found an interdependence between unstable and short-lived reentrant circuits and resulting multiple daughter wavelets during experimental AF. Breakup of unstable circuits formed wavelets that were continuously re-forming unstable circuits. In keeping with the present work, the mechanism of fibrillation in these studies may be a ceaselessly correcting balance between organization and disorganization of complex dynamics. Although a perfect balance is never obtained, neither does a flutter-like rhythm or totally random activity ever gain lasting control. Organizing influences exerted by basal levels of ACh appear to be in constant competition with the disorganizing pressure of ACh-related heterogeneity, such that AF represents an ongoing process of breakup and self-organization. It may be the tendency of wavelets to coalesce, rather than to multiply, that is necessary for AF maintenance.

Our results support the notion that single reentrant sources (analogous to Lewis’ “mother wave” concept11) may maintain AF, but also demonstrate that under certain conditions, AF may arise from more than one coexisting spiral wave, analogous to multiple-circuit reentry.53,54 Moe’s classic computer model of AF and multiple-wavelet hypothesis were contributions of fundamental importance, but were limited by the analytical and experimental methodologies available. Modern computing techniques and a wealth of experimental data allowed us to develop a more realistic computer model, which indicates that (1) AF may be maintained by primary sources that in many cases are single rotors, (2) global dynamics during AF are nonrandom, and (3) most wavelets are a consequence of AF-generating mechanisms, not a cause of AF.

Clinical AF is most commonly observed in the contexts of chronic AF and pathologies like congestive heart failure (CHF). Like cholinergic AF, tachycardia-induced remodeling in chronic AF decreases ERP and increases ERP heterogeneity.55 Although the ionic mechanisms of action potential changes in tachycardia-remodeled atria are different from those in cholinergic AF,66 comparable changes in action potential properties (decreased APD and APD rate adaptation, increased APD heterogeneity) suggest that basic mechanisms of AF maintenance may be similar. In contrast, CHF primarily alters the atrial substrate by promoting interstitial fibrosis; associated ionic remodeling, although substantial, minimally affects atrial ERP.20,57 CHF-related AF appears to arise, at least in some instances, from a single macroreentrant source.59 Further work is needed to evaluate quantitatively the potential mechanisms of AF based on substrates other than increased cholinergic tone.

Novel Findings and Potential Significance

The present model of AF in a 2-dimensional system with realistic atrial ionic and propagation properties agrees well with experimental observations and has important potential implications regarding AF mechanisms. To our knowledge, it is the first such model described. The model presents a tool that may be useful in the quantitative analysis of hypotheses regarding mechanisms of AF in defined pathological substrates. Weaker left-ventricular inward-rectifier ($I_{K_{1}}$) rectification appears to stabilize single left-ventricular rotors underlying VF, whereas strong $I_{K_{1}}$ rectification in the right ventricle leads to rotor instability, termination, and wavebreaks.59 Our model may similarly be useful in the evaluation of the role of specific ionic currents, providing insights into potential antiarrhythmic drug mechanisms and helping in the development of new antiarrhythmic agents. Preliminary data suggest that the model provides novel and potentially important insights into the mechanisms of Na+ channel–blocking drug-induced AF termination.60

Potential Limitations

Our model representation of canine atrial tissue contains numerous simplifications. No area in the atria comprises a 2-dimensional 5×10 cm² sheet. Rather, the normal atria have substantial areas of conduction discontinuity, in particular in the septum61 and the venae cavae. Although many experimental studies of AF have been performed in isolated, unfolded atrial preparations,32,62 and are therefore more comparable with the present model, even these possess atrial microstructure that may contribute importantly to the stability of AF, as reentry may occur within or be anchored by pectinate muscle bundles projecting from the plane of the tissue.63 In addition, the no-flux model boundary conditions in the model may protect vortices from terminating, thereby influencing the balance between source-type spirals and resulting wavelets.59 Finally, it is possible that the mechanisms of AF may be different in tissues of larger dimension, which might more readily permit the coexistence of more than one primary spiral-wave generator. We have begun to examine this issue and have found that even in a 4-fold larger 2-dimensional sheet (10×20 cm), AF can be maintained by a single primary spiral wave, but that under different conditions activity resembles multiple-wavelet reentry with no long-lived spiral-wave sources. We selected the dimensions of the computational substrate for the present study to reproduce overall conduction times and tissue dimensions in normal dog.
A detailed evaluation of AF mechanisms in a much larger atrial substrate, as might for example occur with severe CHF, is potentially very interesting but goes beyond the scope of the present study.

Acknowledgments

The authors thank the Canadian Institutes of Health Research (CIHR), the Natural Sciences and Engineering Research Council (NSERC), and the Mathematics of Information Technology and Complex Systems (MITACS) Network for research funding. Our work was made possible through access to the infrastructure and resources generously funded by MACI, Multimedia Advanced Computational Infrastructure for Alberta. The authors also thank Dr Yves Léger and the “Réseau Québécois de Calcul de Haute Performance (RQCHP)” for providing essential computing resources. Sincere appreciation is extended to Annie Laprade for secretarial help with the manuscript. James Kneller was supported by a CIHR MD, PhD Studentship and a Merck Pharmacology Fellowship.

References

Cholinergic Atrial Fibrillation in a Computer Model of a Two-Dimensional Sheet of Canine Atrial Cells With Realistic Ionic Properties
James Kneller, Renqiang Zou, Edward J. Vigmond, Zhiguo Wang, L. Joshua Leon and Stanley Nattel

Circ Res. 2002;90:e73-e87; originally published online April 25, 2002;
doi: 10.1161/01.RES.000019783.88094.BA
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/90/9/e73

Data Supplement (unedited) at:
http://circres.ahajournals.org/content/suppl/2002/05/10/90.9.e73.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/