UltraRapid Communication

Mechanisms of Atrial Fibrillation Termination by Pure Sodium Channel Blockade in an Ionically-Realistic Mathematical Model

James Kneller*, Jérôme Kalifa*, Renqiang Zou, Alexey V. Zaitsev, Mark Warren, Omer Berenfeld, Edward J. Vigmond, L. Joshua Leon, Stanley Nattel, José Jalife

Abstract—The mechanisms by which Na\(^{+}\)-channel blocking antiarrhythmic drugs terminate atrial fibrillation (AF) remain unclear. Classical “leading-circle” theory suggests that Na\(^{+}\)-channel blockade should, if anything, promote re-entry. We used an ionically-based mathematical model of vagotonic AF to evaluate the effects of applying pure Na\(^{+}\)-current (I\(_{Na}\)) inhibition during sustained arrhythmia. Under control conditions, AF was maintained by 1 or 2 dominant spiral waves, with fibrillatory propagation at critical levels of action potential duration (APD) dispersion. I\(_{Na}\) inhibition terminated AF increasingly with increasing block, terminating all AF at 65% block. During 1:1 conduction, I\(_{Na}\) inhibition reduced APD (by 13% at 4 Hz and 60% block), conduction velocity (by 37%), and re-entry wavelength (by 24%). During AF, I\(_{Na}\) inhibition increased the size of primary rotors and reduced re-entry rate (eg, dominant frequency decreased by 33% at 60% I\(_{Na}\) inhibition) while decreasing generation of secondary wavelets by wavebreak. Three mechanisms contributed to I\(_{Na}\) block–induced AF termination in the model: (1) enlargement of the center of rotation beyond the capacity of the computational substrate; (2) decreased anchoring to functional obstacles, increasing meander and extinction at boundaries; and (3) reduction in the number of secondary wavelets that could provide new primary rotors. Optical mapping in isolated sheep hearts confirmed that tetrodotoxin dose-dependently terminates AF while producing effects qualitatively like those of I\(_{Na}\) inhibition in the mathematical model. We conclude that pure I\(_{Na}\) inhibition terminates AF, producing activation changes consistent with previous clinical and experimental observations. These results provide insights into previously enigmatic mechanisms of class I antiarrhythmic drug-induced AF termination. The full text of this article is available online at http://circres.ahajournals.org (Circ Res. 2005;96:e35-e47.)

Key Words: atrial fibrillation ■ mathematical model ■ class I drugs ■ sodium channels
theoretical exploration of the effects on AF of INa inhibition in the absence of collateral effects on other ion channels. In the present study, we applied this approach to determine whether Na<sup>+</sup>-channel blockade alone is sufficient to terminate AF in the model, and if so, to: (1) evaluate the relationship between the intensity of INa inhibition and the likelihood of AF termination; (2) investigate the mechanism(s) of AF termination by INa inhibition; and (3) relate any effects to associated changes in wavelength and other electrophysiological parameters. To assess the relevance of the model to biological conditions, we performed experiments in isolated perfused sheep atria with the specific Na<sup>+</sup>-channel blocking toxin tetrodotoxin (TTX) to produce pure INa inhibition.

### Materials and Methods

#### Model Description and Implementation

The Ramirez-Nattel-Courtenean (RNC) model of the canine atrial action potential (AP) was implemented.<sup>8</sup> Total ionic current (I<sub>ion</sub>) is given by:

\[
I_{ion} = I_{Na} + I_{K1} + I_{Ca} + I_{Cl} + I_{NaCa} + I_{NaK} + I_{NaK} + I_{NaCa} + I_{Cl} + I_{K1}
\]

An expression for the acetylcholine (ACh)-activated potassium current (I<sub>K(ACh)</sub>) included in the model is to simulate vagal effects. For AF simulation, a 5×10 cm rectangle of atrial tissue was modeled as 25 processors of a 64-processor Enterprise 10000 (Sun). In this way, simulations of 5 s of activity, requiring the solution of 4×14×10<sup>6</sup> coupled equations over 2×10<sup>5</sup> time steps, were accomplished in 12 hours.

#### 2D Simulation Protocols

I<sub>Na</sub> block was modeled by scaling I<sub>Na</sub> conductance (g<sub>Na</sub>). A maximum INa reduction of 65% was used to compare with 60% to 70% sodium channel inhibition (estimated from drug-induced conduction slowing) terminating experimental AF.<sup>4,6,9</sup> Model ERP was measured by simulating experimental protocols.<sup>5,6</sup> All stimuli (180 μA/cm<sup>2</sup>, 1-ms duration) were applied via a 0.2×0.4-cm electrode, such that the stimulus strength was 1.5 to 2×threshold at 65% block. Proximal segments were stimulated from rest at 200 and 400 ms cycle lengths (CLS), and a single premature stimulus (SPS) was delivered after every 15 basic (S<sub>b</sub>) stimuli. ERP was defined as the longest S<sub>b</sub>-S<sub>p</sub> failing to initiate a propagated action potential. CV was calculated during S<sub>b</sub> pulse trains in the longitudinal direction. The wavelength (ERP/CV) was computed as defined previously.<sup>2,3</sup> APDs were recorded from distal segments during S<sub>b</sub> pulse trains to study changes in waveform morphology and AP duration to −60 mV (AP duration [APD]<sub>90</sub>), the approximate voltage at which excitability is normally restored.<sup>10</sup> Maximum phase-0 upstroke velocity (V<sub>max</sub>) was computed at 10-μs resolution with Matlab v5.3.

Conditions for sustained AF were based on in vivo levels of vagally mediated ERP shortening and spatial heterogeneity<sup>11,12</sup> achieved by varying ACh concentration (0 to 0.015 μmol/L) across the grid according to a sinusoidal distribution. Re-entry was initiated using a cross-shock protocol.<sup>7</sup> The core area of primary-generator spirals was calculated by dividing rotor-tip points into cycles (n≈10) and constructing polygons traced by the tip for each cycle.<sup>7</sup>

### Potential Maps

Propagating wavefronts over the computational substrate were visualized by constructing potential maps.<sup>7</sup> After each millisecond, transmembrane potential at the center of each 6×6 cell-square (the approximate size of a space constant) on the computational grid was subsampled to a 50×100 pixel display grid, providing color maps of transmembrane potential.<sup>11</sup> Corresponding movie supplements are provided in the data supplement, available online at http://www.circresaha.org.

### Signal Analysis

Unipolar electrograms were computed as described previously.<sup>7</sup> Spectral analysis of model electrograms was performed with fast Fourier transformations (FFTs). Spectral signals were normalized to the dominant frequency (DF) to facilitate intersite comparison of activation rates. Activity sampled at 1000 Hz (1 ms) for 5000 frames (<~5 s) provided spectral resolution of 0.2 Hz.

### Experimental Preparations

Animals were handled according to National Institutes of Health guidelines. Ten young sheep (18 to 25 kg) were anesthetized with pentobarbital (35 mg/kg IV). Whole hearts were rapidly excised, placed in cold cardioplegic solution, and connected to a Langendorff apparatus. The coronary arteries were perfused continuously at 200 mL/min via a cannula in the aortic root with warm (36° to 38°C) Tyrode’s solution, pH 7.4, equilibrated with 95% O<sub>2</sub>/5% CO<sub>2</sub>, containing 5 μmol/L ACh. The Tyrode’s solution contained (in mmol/L): 130 NaCl, 24 NaHCO<sub>3</sub>, 12 NaH<sub>2</sub>PO<sub>4</sub>, 1.0 MgCl<sub>2</sub>, 5.6 glucose, 4.0 KCl, and 1.8 CaCl<sub>2</sub>.

### Experimental Protocol

Seven sheep hearts were used to study the effects of increasing doses of TTX on AF. Sustained AF was initiated by burst pacing (15 Hz, 10 s). After initiation, AF was sustained throughout the protocol time until AF termination and was also sustained after reinitiation during the washout period. After a control period, TTX was incrementally perfused at 3, 6, and 20 μmol/L, with at least 10 minutes of perfusion at each concentration, followed by a 15-minute washout period. Three sheep were used to determine the effects of TTX on atrial conduction to relate experimental drug effects to those observed in the model. The left atrium was paced and the progression of activation was determined by optical mapping. Because the primary index evaluated was activation time, which was determined as described previously and does not require contraction arrest for measurement,<sup>14</sup> no electromechanical uncoupling agent was used. The same drug concentrations were used as in the studies of drug effects on AF. Because drug effects were not found to be significantly frequency dependent, we took TTX effect at a basic CL of 300 ms to reflect the action on conduction of the drug.

### Optical Mapping

We used a high-resolution video-imaging system and the potentiometric dye di-4-ANEPPS as detailed previously.<sup>13,14</sup> No chemicals or drugs were needed to suppress contractility during AF, during which no significant movement artifacts were present. Recordings from left atrial free wall (~3×3 cm<sup>2</sup>) yielded 5-s optical movies with 128×128 pixels (16 384 pixels in grid) at 300 frames/s using one camera.

### Electrical Recordings

To monitor the atrial electrical activity throughout experiments, 2 bipolar electrode sets were used to record electrical activity continuously from the left and right atrial free walls.

### Frequency Analysis

As in the model, FFTs of electrograms in the left atrial free wall were obtained using FFT.<sup>14</sup> DFs were organized in well-demarcated domains of contiguous pixels with similar DF value. The maximum DF in the field of view was noted as DF<sub>max</sub>. The level of organization of
the activity in the mapped area was quantified by the number of domains larger than 10 pixels in the field of view.

**Phase Analysis**

Phase movies were obtained as described previously.\textsuperscript{15} Phase values (\(-\pi\) to \(\pi\)) represent the state of the AP (upstroke, plateau, repolarization, resting). Phase singularities (PSs) were detected automatically: all pixels were tested for completeness of the AP phases at a circle with a predefined radius. Those complying with the test with a predefined tolerance were assigned to be PS points as described previously.\textsuperscript{16} PSs <3 pixels apart were considered to be a single PS. Results were subjected to sample manual validation. The number of rotors (defined below) per second of AF was assessed.

**Statistics**

Experimental results were expressed as mean\(\pm\)SEM for experimental data, where \(n\) is the number of hearts per observation. For modeling data, we use SD as the measure of variability because results were not obtained as replicates in different subjects with the same experimental protocol but in simulations with different conditions. One-way ANOVA with Bonferroni post hoc correction was used to compare experimental measurements in each group (Origin-Lab Inc.); \(P<0.05\) was considered to be significant.

**Terminology**

AF is defined by rapid and irregular electrograms with varying morphologies. Potential maps were required to demonstrate propagation along lines of block and ongoing wavefront breakup that changed on a cycle-to-cycle basis.\textsuperscript{16}

Wavebreak is a discontinuity permitting contact between a wavefront and its own repolarization tail.\textsuperscript{16}

PS is a point around which all phases of the waveform are encountered, generally where the depolarizing front and repolarizing tail of a wavefront meet.\textsuperscript{16} PSs arise from wavebreaks, are located at the instantaneous center of rotational activity, and were detected by a computer algorithm with manual verification.\textsuperscript{13}

Rotor is a spiral wave of excitation rotating around a PS for one or more cycles.\textsuperscript{16}

Chirality is the sense of rotation, clockwise or counterclockwise, around a PS.\textsuperscript{16}

Core is the area circumscribed by the core-tip trajectory of a PS, consisting of excitable but unexcited tissue, around which a rotor rotates.\textsuperscript{13}

Wavelet is segment of excitation wavefronts flanked by two PSs or a PS and a boundary.\textsuperscript{16}

**Results**

**Results of Model Simulations**

**AF Termination Rates and Intensity of \(I_{Na}\) Inhibition**

We first defined 5 different [ACh] distribution conditions that resulted in sustained AF. Varying degrees of \(I_{Na}\) inhibition were then applied after \(\approx\)500, 750, or 1000 ms of simulated sustained AF for each of the 5 conditions (75 simulations). \(I_{Na}\) inhibition effects were related to intensity of \(Na^+\)-channel blockade. Moderate \(I_{Na}\) inhibition (as little as 30%) could terminate AF, but 100% efficacy required 65% block (Figure 1A).

**Effects of \(I_{Na}\) inhibition on Distributed Properties**

ERP increased slightly but progressively as \(I_{Na}\) inhibition increased. ERP\textsubscript{500} (ERP at 200 ms CL) increased from 146 to 187 ms at 65% inhibition (Figure 2A), with values converging toward ERP\textsubscript{400} at 65% block. \(I_{Na}\) inhibition prolonged the re-entry period (Figure 2B), closely paralleling ERP changes. Means and SDs of re-entry period increased 29% and 190%, respectively, at the strongest \(I_{Na}\) inhibition, in agreement with

29% and 200% increases caused by moricizine in canine atria\textsuperscript{17} and indicating greater increases in re-entry period variability than in period per se. The core area increased from 2.78\(\pm\)0.42 cm\(^2\) to 3.77\(\pm\)1.92 cm\(^2\) (\(P<0.01\)). CV (Figure 2C) progressively decreased with \(I_{Na}\) inhibition, from a control value of 105 cm/s to 66 cm/s at 65% inhibition (37% reduction). In sheep heart experiments, TTX slowed conduction by 33%, 46%, and 82% at 3, 6, and 20 \(\mu\)mol/L, respectively, such that CV slowing at 3 and 6 \(\mu\)mol/L TTX corresponded approximately to the conduction-slowing effects of 50% and 65% \(I_{Na}\) inhibition in the model. These concentrations were therefore appropriate for comparison with AF termination in the mathematical model, encompassing the range of \(I_{Na}\) inhibition for significant (>20%) and substantial (100%) termination (50% and 65% \(I_{Na}\) reduction, respectively). The conduction-slowing effect of 20 \(\mu\)mol/L
TTX was greater than the maximum observed in the mathematical model because I_{Na} inhibition >65% in the model was generally associated with inexcitability. The wavelength (Figure 2D) decreased progressively, from a control value of 17.3 cm to 13.0 cm at 65% inhibition.

Effects of Sodium Channel Block on AP Properties

APD and AP amplitude progressively decreased with I_{Na} inhibition (Figure 3A). APD_{50} decreases became particularly important at >40% inhibition (Figure 3B). APD abbreviation with increased ERP (Figure 2A) reflects postrepolarization refractoriness, typical of class I drugs. As observed experimentally, strong I_{Na} inhibition (>50%) resulted in 2:1 capture in the model at rapid rates (Figure 3B). This observation is consistent with the development of transient lines of block during intracardiac re-entry, which progressed along the refractory lower part of the grid, curving about the single PS (*) point into a re-entering wavefront (165 ms). This PS remained at the core-tip of a generator rotor that maintained activity. Emerging impulses impinged on refractory tissue, fractionating into wavelets with variable chirality, with numerous counter-rotations between adjacent transient circuits. At 2.5 s, the initial primary spiral died out along the bottom right border (Figure 4C). A second rotor of opposite chirality originating from wavebreak at 2.3 s then assumed dominance and sustained fibrillation for the duration of the simulation. Short-lived spirals (one to three rotations) resulting from the 48 of 74 (65%) of attempted counter-rotations that were successfully completed (1 attempted per 68-ms interval) competed with the primary spiral generator. Selected frames show examples of attempted counter-rotations (Y_{success} [475, 525, 2312, 3706 ms]; X_{failure} [3145 ms]).

An average of 2.7±1.4 wavelets were present, with up to 7 at times of peak disorganization (Figure 4D). Electrograms recorded near (Figure 4B, +) and distal to (Figure 4C, ○) the primary sources are clearly fibrillatory, with DFs of 11.3 and 10.7 Hz respectively.

Figure 5A shows termination of the AF shown in Figure 4 by I_{Na} inhibition (animation, online-only Movie 2).
tions identical to those in Figure 4 were used, but 65% \( I_{Na} \) inhibition was applied after \( \approx 900 \) ms. A single wavelet persisted after block (at 920 ms). Wavefront curvature was greatly expanded and the wavelet corresponding to the uppermost PS (smaller asterisk) promptly extinguished along the top boundary (1010 ms). The PS at the core-tip of the original primary rotor (larger asterisk) followed a more linear trajectory than before \( I_{Na} \) inhibition, rotating around a markedly enlarged core. Re-entry failed along the top border (1336 ms) after nearly one complete cycle, terminating AF. In this case, \( I_{Na} \) inhibition enlarged the primary rotor to the extent that it could no longer be accommodated by the tissue substrate, leading to rapid termination. The spatial APD gradient was reduced to 50 ms (range 20 to 70 ms), representing a 15-ms reduction in the maximum value relative to control (Figure 4), and an 8-ms decrease overall. No further wavebreak occurred as the enlarged primary rotor conducted slowly in the presence of decreased APD heterogeneity.

Figure 5B shows termination under the same AF conditions by a lesser degree (30%) of \( I_{Na} \) inhibition (animation, online-only Movie 3). Circuits were enlarged and wavelets tended to coalesce, such that the number of successfully completed counter-rotations was reduced to 12 of 35 (34% versus 65% in control) before termination (\( \approx 1 \) attempted per 103-ms interval) because fewer PSs were separated by the critical inter-PS distance needed for ongoing re-entry. Enlarged daughter wavelets interfered with the primary generator rotor and the dominant PS died out along the bottom boundary at \( \approx 3.22 \) s. Other PSs present at this time blocked (3255 ms). Subsequent wavebreak produced a final rotor, which failed to establish sustained generator function, completing one rotation before annihilation along the left bound-

**Figure 4.** AF in the absence of \( I_{Na} \) inhibition. A, Left top, [ACh] distribution; below, APD distribution; other panels, selected potential maps show activity during sustained AF at times indicated (ms). Asterisks indicate spiral tip, Y successful re-entry of counter-rotating spirals. Electrograms were recorded near the source (B) and distally (C). Corresponding FFTs are at the right. D, Number of wavelets present at each time.
ary (3415 ms), terminating AF (3510 ms). The DF during AF decreased from 11.3 Hz (Figure 4B) to 10.7 Hz (Figure 5C) during 30% $I_{Na}$ inhibition. In this example, the spatial APD gradient (57 ms; range 27 to 84 ms) was negligibly changed relative to control (Figure 4A); thus, wavelet alterations resulting in AF termination did not require reductions in APD heterogeneity.

Figure 6A shows an example of AF maintained by a single spiral wave in the absence of $I_{Na}$ inhibition (animation, online-only Movie 4). A rotor with clockwise chirality located near $\frac{\pi}{4}$ acted as the primary source of activity. The trajectory of the dominant PS is plotted in black on the APD distribution map. A region of increased APD in a low-[ACh] zone served as a functional anchor for the generator spiral wave tip. Wavelets tended to multiply from the primary rotor and would at times coalesce to form a large unstable circuit, reducing the wavelet count to one. Several PSSs with opposite chirality completed multiple re-entry cycles near the right border. On average, 3.6 ± 1.2 wavelets were present, with 7 at peak disorganization (Figure 6B). Electrogram activity was fibrillatory, but the underlying order imposed by the primary spiral wave generator was evidenced by a discrete, narrowly peaked FFT (Figure 6B).

Figure 6C shows termination of this AF by 60% $I_{Na}$ inhibition applied after $\approx 750$ ms of activity (animation, online-only Movie 5). $I_{Na}$ inhibition caused rapid termination of the generator rotor. A secondary wavelet generated counter-rotating spiral waves (760, 1195, 1224 ms) that evolved through 4 cycles before one spiral (PS indicated by smaller asterisk) terminated against the lower boundary. The core-tip (PS indicated by larger asterisk) of the remaining rotor then sustained generator function until termination. The trajectory of the initial spiral wave generator is plotted on the APD distribution map (black, before $I_{Na}$ inhibition; black and white-after block). The tip trajectory of the secondary generator after block is shown in yellow. $I_{Na}$ inhibition greatly slowed re-entry (Figure 6D, DF decreased from 11.2 to 7.1 Hz) and reduced the spatial APD gradient (Figure 6C, from...
40 to 33 ms in this example). These reduced the anchoring effect of low-[ACh] zones and wavebreak against refractory tissue. Reduced anchoring, along with regions of transient conduction block, caused hypermeandering of the primary-generator tip PS, continuing until the primary PS reached the lower boundary and extinguished after 20 cycles. Because of reduced wavebreak, other PSs were unavailable to assume generator function. Despite the increased meaner of the generator rotor, the electrogram was more organized after INa inhibition (Figure 6D), indicating that wavebreak contributed significantly to the disorganized electrograms in control (Figure 6B). The area of core-tip meander, 2.0 cm² under control conditions, increased to 7.7 cm² with 60% INa inhibition.

Organization of AF by INa Inhibition
Figures 5 and 6C demonstrate how INa inhibition may increase primary-circuit size, promote primary-circuit meander, and reduce wavefront density, thereby slowing and organizing AF before conversion. These changes occurred in all cases of AF termination and agree with experimental observations of class I drugs.4,24 Figure 7 illustrates the progression of changes caused by INa inhibition in another case. Activity is shown under control conditions (Figure 7A), with 30% (Figure 7B) and 60% (Figure 7C) INa inhibition applied at the times indicated.

Under control conditions, electrograms were fibrillatory with a DF of 10.3 Hz, and 3.5±1.5 (range 1 to 7) wavelets. The trajectory of the dominant PS is shown in black. With
30% \(I_{\text{Na}}\) inhibition, the fibrillation rate slowed to 9.4 Hz. The trajectories of the dominant spiral tip before (black) and after (yellow) blockade are shown, with the introduction of \(I_{\text{Na}}\) block marked by an orange dot. With 60% \(I_{\text{Na}}\) block, activity became flutter-like, with discrete spectral peaks including higher harmonics (arrows) of the DF. \(I_{\text{Na}}\) block caused the generator to meander along an erratic course before stabilizing in a region of low APD. The trajectories of the dominant spiral tip (large asterisk on potential map) before (black) and after (yellow) \(I_{\text{Na}}\) block are shown. In this example, two counter-rotating PSs (small asterisks on potential map) present at the time of block persisted for the duration of the simulation (green trajectories), stabilizing activity into a system of large, highly uniform counter-rotating circuits. The electrogram frequency was 6.4 Hz and the wavelet count was reduced to 2.3 ± 1.4 (range 1 to 5).

**Figure 7.** Progression of changes caused by \(I_{\text{Na}}\) inhibition in another case of AF. Activity under control conditions (A), and with 30% (B) and 60% (C) \(I_{\text{Na}}\) inhibition (block) applied at the times indicated. A representative potential map during AF, APD distribution map with trajectory of the dominant PSs, electrogram recordings with corresponding FFTs, and wavelet count are shown for each condition. CTL indicates control. For discussion, see text.

**PS Lifespan Analyses**

To obtain a quantitative index of the effect of \(I_{\text{Na}}\) inhibition on activity during AF, we analyzed the lifespan of all PSs with the use of an automated algorithm. Results are shown in Figure 8. Simulations (5 s) were performed for each of 5 [ACh] distributions associated with sustained AF and then repeated with 30% and 60% \(I_{\text{Na}}\) block after 500 ms of activity. PS lifespans were measured between 500 and 4500 ms, and PS lifespan histograms were constructed for all simulations under control (A), 30% \(I_{\text{Na}}\) inhibition (B), and 60% \(I_{\text{Na}}\) inhibition (C) conditions. Consistent with experiments, the mean lifespan of PSs lasting <1 s under control conditions was ≈ 30 ms, with 90% lasting <50 ms. PSs lasting >1 s belonged to the core tips of primary-generator spirals. After 30% \(I_{\text{Na}}\) inhibition, the number of PSs was reduced by 37%. The number of short-lasting PSs was reduced (for example, 310 lasted <10 ms compared with 420 under control conditions); however, PSs lasting <50 ms remained >90% of the total. After 60% \(I_{\text{Na}}\) inhibition, the total number of PSs was drastically reduced to 3.6% of control. The mean lifespan was increased to 70 ms, with only 70% lasting <50 ms.
Experimental Observations

Effects of $I_{Na}$ Inhibition with TTX on Maintenance of Experimental AF and Activation Frequency Domains

The infusion of TTX terminated experimental AF in a concentration-dependent way. As in the mathematical model, 100% AF termination was achieved with maximal $I_{Na}$ inhibition. Figure 1B shows the effectiveness of termination of experimental AF as function of TTX concentration ($n=7$ animals). Because the type of data from experiments is different in voltage and spatial resolution from mathematical modeling data, we could not analyze the data from experiments in an identical fashion. Therefore, we selected analytical tools for the experimental data that would reflect key findings in the mathematical model. We first addressed the rate and organization of activity during arrhythmia as reflected by the local frequency of activation in DF domains. Figure 9A shows a representative example of changes in $DF_{max}$ in control (top left panel) after perfusion with 3 (top right panel) and 6 (bottom left panel) μmol/L TTX before AF termination and after subsequent washout (bottom right panel). The number of frequency domains was progressively decreased by TTX, indicating greater organization of AF dynamics. Figure 9B shows quantification of this effect, indicating that the number of different frequency domains substantially decreased just before termination (from $80.2 \pm 3.6$ to $28.2 \pm 8.5$; $P<0.01$).

Effects of $I_{Na}$ Inhibition on AF Dynamics

As shown in Figure 9A, TTX led to a progressive decrease in activation frequency as reflected by $DF_{max}$ values. Figure 10A illustrates activity during AF in the left atrium of one isolated sheep heart, in the form of a phase map (Figure 10D, scale to left). Green corresponds to depolarization, yellow and red to the plateau and repolarization, and blue to the resting membrane potential. Regions of inexcitability were observed at larger TTX concentrations, resulting in a reduced field of activity on optical mapping (eg, right panel). Activity was much less complex after perfusion of TTX, as can be appreciated from Figure 10A, and was much slower, most readily appreciated from online-only Movie 6. Consistent with this slowing, $DF_{max}$ was significantly reduced by TTX in a concentration-dependent way, with a return toward pre-TTX values on washout (Figure 10B). We then examined the number of identifiable PSs in the optical maps. As shown in Figure 10C, TTX decreased PS numbers from $499.9 \pm 68.5$ PSs during control to $304.6 \pm 29.5$ (26±0.1% reduction; $P<0.01$) at 3 μmol/L TTX and to $243.8 \pm 55$ (51±0.1% reduction; $P<0.01$) at 6 μmol/L TTX, with a return toward control values on washout (468.7±51.8). This decrease in PS count was qualitatively similar but quantitatively smaller than...
blockers to terminate clinical AF. Mechanisms contributing to termination included: (1) enlargement of the core size of primary rotors so that they could no longer be accommodated by the substrate; (2) decreased effectiveness of anchoring in zones of greater refractoriness, increasing meander and causing extinction at boundaries; and (3) reduction in the number of daughter waves (resulting from wavebreak) that could provide new primary rotors to maintain AF should the initial generator rotor be extinguished. Experiments with the Na⁺-channel blocker TTX showed concentration-dependent AF termination accompanied by conduction slowing, decreased dominant frequencies during AF, and a decreased number of rotors and PSs, electrophysiological actions in qualitative agreement with model simulations.

Comparison With Previous Studies of AF Termination by Class I Antiarrhythmic Drugs
The major effects of I_{Na} inhibition in the mathematical model were a reduction in the number of wavelets because of decreased wavebreak, increased size and hypermeander of the primary generator rotor, and slowing of re-entry and dose-related AF termination (despite decreased wavelength), with 100% efficacy at 65% Na⁺-channel blockade. Wijffels et al showed that the class I drugs cibenzoline and flecainide terminate sustained AF in an AF-remodeled substrate, while prolonging the AF CL (AFCL) with a concomitant decrease in the wavelength.⁶,²⁵ AFCL was consistently prolonged relative to the ERP during AF (R_{P-AF}), and the only change correlating consistently with AF termination was an increased temporal excitable gap (AFCL-R_{P-AF}).⁶ They suggested that the increased excitable gap might reduce the number of daughter wavelets because of decreased wavebreak, a phenomenon that was quite apparent in our model. The variability of AFCL values was increased by ≈2-fold (cibenzoline)⁶ and 4-fold (flecainide),²⁵ indicating a large drug-induced increase in re-entry rate variability before cardioversion. The mathematical model suggests that these large increases in variability may be attributable to hypermeander of the spiral wave generator rotor that leads to termination by extinction at boundaries. The maximum conduction slowing caused by these drugs was 42% to 44%, corresponding with inhibition of 64% and 69% of Na⁺-channels,²⁵ in the same range as I_{Na} inhibition associated with complete efficacy in the present study. Sinus rhythm was restored in 80% of goats treated with cibenzoline but only 40% of goats receiving flecainide, suggesting differences possibly related to the AF substrate (atrial remodeling in the Wijffels study; ACh in the present study). Nonlinear analysis showed slowing and increased organization of AF by cibenzoline before termination,²⁷ similar to our results. In the present study, TTX concentrations (3 and 6 μmol/L) producing conduction slowing effects equivalent to 50% and 65% I_{Na} inhibition terminated AF in 20% and 60% of hearts studied, in overall qualitative agreement with the 22%, 33%, and 100% termination rates seen with 50%, 60%, and 65% I_{Na} inhibition, respectively, in the mathematical model.

Several studies have examined the effects of class I antiarrhythmic agents in canine vagotonic AF, an experimental paradigm directly analogous to our model. Lidocaine terminates AF

Discussion
We applied a mathematical model of AF to evaluate the effects of pure I_{Na} inhibition. We found that I_{Na} inhibition terminated AF in a fashion consistent with the ability of I_{Na}
maintained by bilateral vagal nerve stimulation, making atrial electrogams broader, slower, and more regular, as in our model (Figure 7). FFTs under control conditions showed broad-banded peaks in the range of 13 and 17 Hz, changing to a narrow-band peak at ~7 Hz after lidocaine. Flecainide and propafenone are also highly effective in canine vagotonic AF, organizing activity and increasing the size of re-entrant circuits before termination. AF termination was attributed to increased wavelength in the latter studies, but estimated $I_{Na}$ inhibition was 55% by flecainide and 68% by propafenone, within the range of $I_{Na}$ inhibition that effectively terminated AF in the present model. Experimental observations are thus consistent with the results of our mathematical model in showing that $Na^+$-channel–blocking drugs terminate AF with high efficacy at concentrations producing substantial $Na^+$-channel blockade, with organization and slowing of atrial activity before termination. The rotors observed in the experimental AF were short-lived and probably secondary rotors rather than primary generators. The lack of observable generator rotors in our experimental studies, as well as the lack of observable termination events, was likely attributable to the limited field of observation during optical mapping. Nevertheless, experimentally observed rotor frequency and wavebreak changes are comparable to those expected based on the behavior of rotors of comparable duration (100 to 300 ms; Figure 8) in the computational data.

Clinical observations with class I antiarrhythmic agents are also consistent with our model simulations. Flecainide and propafenone increase AFCL before conversion. Cibenzoline transforms highly disorganized atrial activity into much more organized activity before termination, in conjunction with a substantial increase in AFCL.

**Theoretical Aspects**

Gray et al were the first to show experimentally substantial spatial and temporal organization during cardiac fibrillation. They suggested that ventricular fibrillation (VF) is maintained by a limited number of organized rotors that terminate on collision with rotors of opposite chirality or by encountering a boundary condition. Chen et al provided experimental evidence for the relevance of these considerations to mechanisms of AF. We previously showed that such theoretical considerations apply to AF in a computer model of vagotonic AF in a 2D cell grid closely approximating canine atrial properties. In the present study, we found that the termination of AF by $I_{Na}$ inhibition in this computational model follows the principles of spiral wave re-entry and impulse propagation in excitable media.

Mandapati et al showed that decreased excitability resulting from $I_{Na}$ blockade or ischemia expands the core area and reduces the wavefront density while slowing VF. During conduction in excitable media, the safety factor, or source-to-sink ratio, determines propagation velocity. The excitation front curves around functional or anatomical obstacles when the safety factor for conduction is sufficiently large that activated cells in the wavefront are able to excite >1 cell at rest. Because $I_{Na}$ blockade decreases source current, the number of sink cells that can be activated decreases and wavefront curvature flattens. This effect is particularly significantly close to spiral core-tips, where wavefront curvatures become increasingly pronounced. A relatively large source enables the spiral to rotate rapidly around a small core. When the source is reduced by $I_{Na}$ block, the critical curvature becomes large, and the path followed by the spiral wave tip increases, increasing core size and slowing re-entry, $I_{Na}$ blockade stabilizes spiral waves without terminating re-entrant activity in a mathematical model of ventricular tissue, opposing breakup and destabilizing the spiral wave core, thereby promoting meander and increasing the size of re-entry pathways.

**Novel Insights Into Mechanisms of AF Termination by Na\textsuperscript{+}-Channel Blockers**

Experimental studies have pointed to spiral wave re-entry in atrial tissue. They have shown that a variety of antiarrhythmic drugs with $Na^+$-channel blocking activity can terminate AF. Initial studies suggested that AF termination was attributable to increased wavelength. However, more recent work has shown that class I drugs can terminate AF while decreasing wavelength. The experimental evidence is difficult to interpret because of accessory drug actions, including important $K^+$-channel blockade. In a computational model that mimics many properties of experimental vagotonic AF, we found that pure $I_{Na}$ inhibition terminates arrhythmic activity, with the likelihood of termination increasing with increasing $Na^+$-channel block. Effective AF termination in the model is seen at levels of $Na^+$-channel block occurring with doses of class I antiarrhythmic agents that effectively terminate experimental AF. Changes in atrial activation in the model (including slowing, organization, and regularization of atrial activity) are similar to those observed experimentally and clinically. Although wavelength increases could contribute to the AF-terminating ability of some class I agents, the present study indicates that pure $I_{Na}$ inhibition can terminate AF without prolonging, and in fact, despite decreasing, the wavelength.

Therefore, our simulations demonstrate for the first time (to our knowledge) that pure $Na^+$-channel inhibition can terminate AF and that $Na^+$-channel inhibition can account for many of the effects of class I drugs on AF. Our studies also provide potential insights into the mechanisms by which $Na^+$-channel blockers may terminate AF. One mechanism is an increase in the critical angle of curvature of the rotating wavefront, which may enlarge the core diameter of primary rotors so that they exceed the available substrate. This mechanism parallels suggestions based on observations of the effects of flecainide on activation in a rabbit model of 2D ventricular re-entry. The second potential contributor, decreased formation of multiple daughter wavelets, has been postulated based on increases in the temporal excitable gap during experimental AF. The final mechanism for AF termination, increased core meander, causing termination by movement to a boundary condition has not, to our knowledge, been suggested previously. Boundary conditions exist at multiple atrial locations, including the atrioventricular valves, venae cavae, pulmonary veins, and interatrial septum, which contains significant conduction discontinuities. The primary spiral wave generator to a boundary, resulting in extinction as described by Gray et al, could explain AF termination by class I antiarrhythmics at levels of $I_{Na}$ inhibition below those needed to enlarge the spiral beyond the capacity of the re-entry substrate.
To our knowledge, our study provides the first demonstration of AF termination by the pure Na\textsuperscript{+}-channel blocking toxin TTX. This finding is significant because it demonstrates unequivocally the ability of pure $I_{\text{Na}}$ inhibition to terminate AF.

The observations of the present study may help to understand why class I antiarrhythmics terminate experimental and clinical AF but not VF. The effects of $I_{\text{Na}}$ inhibition on activation during VF are qualitatively similar to those on AF, including increased organization with slowing and enlargement of re-entrant rotors.\textsuperscript{31,43} However, the ventricular mass is much greater than that of the atrium, potentially making it much more difficult to enlarge rotors beyond the spatial capacity of ventricular tissue. In addition, boundary conditions are much more plentiful in the atria than the ventricles, making spiral core drift to a boundary much more likely at the atrial level. Thus, Na\textsuperscript{+}-channel blockers slow, regularize, and stabilize VF, while tending to terminate AF.

**Potential Limitations**

Our computational substrate is a 2D sheet of cells and does not reproduce the complexities of 3D atrial geometry. Although the model reproduces well the features of vagal AF\textsuperscript{7} and accounted for many experimentally observed effects of $I_{\text{Na}}$ inhibition in the present work, it would be interesting to analyze atrial arrhythmia mechanisms and mechanisms of drug action in more complex models incorporating realistic atrial geometric features.\textsuperscript{44} An advantage of the 2D system is its ability to resolve events and their mechanisms. A recent study in the realistic 3D system\textsuperscript{45} has supported many of the mechanisms underlying cholinergic AF that were suggested previously by our 2D model,\textsuperscript{7} but the complexity of observed behaviors made them considerably more difficult to quantify and analyze. Disease states and atrial remodeling may importantly change the atrial re-entrant substrate.\textsuperscript{46,47} Consequently, they may affect the response to antiarrhythmic agents, including Na\textsuperscript{+}-channel blockers, altering mechanisms of drug action. Further theoretical and experimental work to address this issue would be of considerable potential interest and importance.

The experimental model was performed in sheep hearts rather than dog for technical reasons related to optical mapping requirements in our system. Although it would have been preferable to obtain optical mapping in the same species as used for the modeling studies, the qualitative similarity in the results of experimental and modeling work lend support to the applicability of the modeling results. A much higher ACh concentration was used in the in vivo experiments than incorporated in the modeling work. This is likely because of the very active breakdown of ACh by acetylcholinesterases in the intact cardiac preparation, which greatly reduces the effective ACh concentrations.

We used a simple pore-block model (fixed percentage decrease in maximum Na\textsuperscript{+} conductance) to simulate Na\textsuperscript{+}-channel blockade. This is a reasonable approach for TTX block that produced results consistent with our experimental findings. In addition, our modeling results are compatible with previous clinical and experimental reports of the effects of such Na\textsuperscript{+}-channel blockers as cibenzoline, flecainide, and lidocaine, as discussed above. A more detailed exploration of the impact of state-dependent $I_{\text{Na}}$ block as occurs for all clinically used class I drugs, with the application of a mathematical formulation of such action, would be of great interest but is beyond the scope of the present study. However, the work in this study does demonstrate for the first time that pure reduction of Na\textsuperscript{+} current terminates AF in mathematical simulation and experimental conditions and provides insight into underlying mechanisms.

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**References**


Mechanisms of Atrial Fibrillation Termination by Pure Sodium Channel Blockade in an Ionically-Realistic Mathematical Model
James Kneller*, Jérôme Kalifa*, Renqiang Zou, Alexey V. Zaitsev, Mark Warren, Omer Berenfeld, Edward J. Vigmond, L. Joshua Leon, Stanley Nattel and José Jalife

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Legends for On-line Movie Supplements

**Movie 1.** AF maintained by multiple spiral waves, as shown in Figure 4. AF arose from two primary sources. The first source died out along the bottom right border and was replaced by a second source which had originated from earlier wavebreak. Colors represent transmembrane potentials as indicated by the color grid in Figure 4. Animations are shown at ~1/30 real time to facilitate visualization of events.

“movie_file_1.avi” is for online movie supplement for Figure 4A.

**Movie 2.** Termination of the AF shown in Figure 4 by 65% INa-inhibition, applied after ~900 ms. Colors represent transmembrane potentials as indicated by the color grid in Figure 5B. Animations are shown at ~1/30 real time to facilitate visualization of events.

“movie_file_2.avi” is for online movie supplement for Figure 5A.

**Movie 3.** Termination of the AF shown in Figure 4 by 30% INa-inhibition, applied after ~1000 ms. Colors represent transmembrane potentials as indicated by the color grid in Figure 5B. Animations are shown at ~1/30 real time to facilitate visualization of events.

“movie_file_3.avi” is for online movie supplement for Figure 5B.

**Movie 4.** AF maintained by a single spiral wave, as shown in Figure 6A. A functionally determined rotor with clockwise chirality acted as a primary source of activity, generating impulses that fractionated into multiple independent wavelets along the heterogeneous substrate. Colors represent transmembrane potentials as indicated by the color grid in Figure 6A. Animations are shown at ~1/30 real time to facilitate visualization of events.

“movie_file_4.avi” is for online movie supplement for Figure 6A.

**Movie 5.** Termination of the AF shown in Figure 6A by 60% INa-inhibition, applied after ~750 ms. Colors represent transmembrane potentials as indicated by the color grid in Figure 6A. Animations are shown at ~1/30 real time to facilitate visualization of events.

“movie_file_5.avi” is for online movie supplement for Figure 6C.

**Movie 6.** Phase movies showing one second of atrial fibrillation in control condition, after 3 µmol/L and 6µmol/L TTX (left, center and right movies respectively). The 3 movies were recorded at 300 frames/sec and are presented at the same frame rate of 20 frames/sec. Depolarization is depicted in green, the plateau in yellow and red and the resting membrane potential in blue (see methods and inset Figure 10C). The AF activity becomes slower and wave break formation is decreased after perfusion of TTX.

“movie_file_6.avi” is for online movie supplement for Figure 10E.