# Chloroquine Terminates Stretch-Induced Atrial Fibrillation More Effectively than Flecainide in the Sheep Heart

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## Abstract

**Background**—Blockade of inward-rectifier $K^+$ channels by chloroquine terminates reentry in cholinergic atrial fibrillation (AF). However, it is unknown whether inward-rectifier $K^+$ channels and reentry are also important in maintaining stretch-induced AF (SAF). We surmised that reentry underlies SAF, and that abolishing reentry with chloroquine terminates SAF more effectively than traditional $Na^+$-channel blockade by flecainide.

**Methods and Results**—Thirty Langendorff-perfused sheep hearts were exposed to acute and continuous atrial stretch, and mapped optically and electrically. AF dynamics were studied under control and during perfusion of either chloroquine (4 μM, N=7) or flecainide (2–4 μM, N=5). Chloroquine increased rotor core size and decreased reentry frequency from 10.6±0.7 Hz in control to 6.3±0.7 Hz (p<0.005) just before restoring sinus rhythm (7/7). Flecainide had lesser effects on core size and reentry frequency than chloroquine and did not restore sinus rhythm (0/5). Specific $I_{Kr}$-blockade by E-4031 (N=7) did not terminate AF when frequency values were >8Hz. During pacing (N=11) flecainide reversibly reduced conduction velocity (~30% at cycle length 300, 250 and 200 ms, p<0.05) to a larger extent than chloroquine (11% to 19%, cycle length 300, 250 and 200 ms, p<0.05). Significant action potential duration prolongation was demonstrable only for chloroquine at CL 300 (12%) and 250 ms (9%) (p<0.05).

**Conclusions**—Chloroquine is more effective than flecainide in terminating SAF in isolated-sheep hearts by significantly increasing core size and decreasing reentry frequency. Chloroquine's effectiveness may be explained by its inward-rectifier $K^+$ channel blockade profile and suggest that reentry is important to maintain acute SAF.

### Keywords

arrhythmia (mechanisms); atrial fibrillation; drug therapy; electrophysiology mapping

## Introduction

Atrial fibrillation (AF) is the most common sustained arrhythmia in clinical practice.[1] Although the mechanisms that sustain AF are incompletely understood, strong evidence in humans[2] and in animal models[3, 4] supports the hypothesis that high-frequency reentrant sources (rotors) are essential to maintain AF. It is possible to demonstrate discrete sites of high frequency periodic activity during AF, along with frequency gradients between left and

right atria.[3] Occasionally, a long-lasting rotor is identified within the area of maximum dominant frequency ($DF_{max}$).[5]

In the human heart, atrial dilatation and stretch predisposes to AF with DF values directly related to left atrial (LA) pressure.[6] Similarly, in a sheep model of stretch-induced AF (SAF), the posterior left atrium (PLA) plays a major role in the maintenance of the arrhythmia.[4]

Less clear is the role of rotors in SAF, where focal discharges (FDs) and reentrant sources interact in such a way that FDs can destabilize and terminate rotors but also can give raise to new wavebreaks and rotor formation.[5] The mechanism underlying FDs is uncertain with either triggered activity or reentry being possible; a more depolarized resting membrane potential (RMP) and the activation of stretch-activated non selective-cationic channels (SACs) enable the generation of after depolarizations[7] that might explain FDs (breakthroughs) in the optically-mapped areas. However, FDs might also be the surface reflection of intramural reentrant sources.[8]

Inward-rectifier $K^+$ currents ($I_{K1}$, $I_{K-Ach}$ and $I_{K-ATP}$) play important roles in controlling rotor dynamics.[2, 9, 10] Recently, Noujaim et al have demonstrated that chloroquine blocks the pore-forming subunits Kir2.1, Kir3.1 and Kir6.2 responsible for the inward-rectifier $K^+$ current ($I_{K1}$), the acetylcholine-sensitive $K^+$ current ($I_{K-Ach}$) and the ATP-sensitive $K^+$ current ($I_{K-ATP}$), respectively.[11] Interestingly, chloroquine depolarizes the RMP and increases automaticity, which can be explained by its blocking effects on $I_{K1}$.[12] The latter might increase FDs underlying a triggered activity mechanism.

We hypothesized that, if reentry plays a critical role sustaining SAF, then chloroquine should effectively restore sinus rhythm (SR) based on its ability to preferentially block inward-rectifier $K^+$ currents. We therefore compared the effects of chloroquine with those of flecainide on SAF and excitation properties using optical mapping in Langendorff-perfused sheep hearts. The rationale for such a comparison was based on the fact that, similar to amiodarone[13] and the most recently introduced drugs dronedarone[14] and vernakalant,[15] flecainide[16] does not block $I_{K1}$ within the therapeutic range of concentrations. Flecainide is more effective than amiodarone in early reversion of recent-onset AF[17] (<48 h) and it is highly recommended in patients with no underlying structural heart disease.[18] However our data demonstrate that chloroquine is more effective than flecainide in restoring SR in this model. Taken together, our results support the hypothesis that rotor activity underlies SAF and that blockade of inward-rectifier $K^+$ currents may be a viable approach for its termination.

## Methods

### Experimental set up

All procedures were approved by the University of Michigan Committee on Use and Care of Animals (UCUCA) and complied with National Institutes of Health guidelines. Thirty 6 month-old sheep (weight: ~35 kg) were included in the study. Anesthesia was induced with 4–6 mg/Kg of propofol and 60–100 mg/Kg of sodium pentobarbital. Hearts were removed via thoracotomy and connected to a Langendorff perfusion system with re-circulating oxygenated (95% $O_2$, 5% $CO_2$) Tyrode's solution at constant flow rate of 240–270 ml/min, pH 7.4 and 35.5–37.5 °C. The Tyrode's composition (in mM) was: NaCl 130, KCl 4.0, MgCl2 1, CaCl2 1.8, NaHCO3 24, NaH2PO41.2, Glucose 5.6, and Albumin 0.04 g/L. Blebbistatin 10 μM was used to reduce the contractile force.

After atrial trans-septal puncture the intra-atrial pressure was increased to 14 cm $H_2O$ to induce continuous atrial stretch. Bipolar electrograms from each of the pulmonary veins (PVs), top and roof of the left atrial appendage (LAA) and right atrial appendage (RAA) were obtained (sampling rate, 1.0 kHz). All vein orifices were sealed except for the inferior vena cava, which was cannulated and used for controlling the level of the intra-atrial pressure.

Optical mapping movies (5 sec) were obtained using a Little Joe CCD camera (80×80 pixels, 500–1000 frames per second). After a bolus injection of 5 to 10 ml Di-4-ANEPPS (10 mg/mL) (Sigma-Aldrich) voltage-sensitive fluorescence was acquired from the RAA and LAA (area: ~14 $cm^2$). In seven experiments epicardial mapping from the LAA was complemented with endocardial mapping of the PLA (area: ~3.7 $cm^2$) using a dual-channel rigid borescope (see supplemental Methods and supplementary Figure 1).

## Experimental Protocols

Baseline action potential duration at 70% repolarization ($APD_{70}$) was optically measured on both atrial appendages (n=5) at progressively shorter pacing cycle lengths (CLs; 300–250–200 ms). The pacing electrode was placed on the top of the LAA. Mean $APD_{70}$ was obtained for RAA and LAA surfaces by averaging $APD_{70}$ from all pixels. AF was then induced via burst pacing (12 Hz) and allowed to continue for 15 min of baseline control. Thereafter, the antifibrillatory effects of either 4 µM chloroquine diphosphate (N=7) or 2 µM flecainide (N=5) (Sigma-Aldrich) dissolved in Tyrode's solution were investigated. The concentrations were based on the highest therapeutic level for flecainide[19] and chloroquine.[20] Drug perfusion was maintained for 20 min, after which the concentration was doubled and maintained for 30 additional min, as long as SR was not already restored. AF was terminated by DC shock in those hearts in which AF persisted for 50 min. Burst pacing was subsequently applied after AF termination to re-induce AF. Finally, AF re-induction was attempted again after a washout period of 30 and 45 min for flecainide and chloroquine, respectively.

To better delineate the specific roles of $I_{K1}$ versus $I_{Kr}$ we conducted experiments (N=7) using the same stretch-induced AF model to study the antifibrillatory effects of specific $I_{Kr}$-blockade. After 15 min of baseline AF, 0.5–1 µM E-4031 was perfused for 20 and 30 min as above.[21]

An additional set of experiments was carried out to investigate the electrophysiological effects of 4 µM chloroquine (N=5) and 4 µM flecainide (N=6). Pacing protocols were completed under basal conditions, after 15 min of either chloroquine or flecainide, and after washout. No AF was induced in these hearts. Activation times were determined at 50% of action potential amplitude and conduction velocity (CV) was calculated.[22] Supplemental Figures 2A and 2B illustrate the time-line of all experimental protocols.

## Frequency analysis

Dominant frequency (DF) maps were obtained for each optical movie in AF after applying a fast Fourier transform (FFT) of the fluorescence signal recorded at each pixel.[23] Bipolar electrograms recorded during AF were high-pass filtered at 3Hz and low-pass filtered at 35 Hz. FFT was also applied to the 5-sec bipolar signals synchronized with the optical movies.

## Atrial fibrillation dynamics

Phase movies were constructed using the Hilbert transformation.[24] In each movie, a rotor was defined as a point of phase-convergence (singularity point; SP) lasting more than one rotation. A breakthrough was defined as a wave appearing inside the field of view and

propagating outward (supplemental Figure 3). During AF, 5-sec movies were acquired and analyzed frame by frame. Rotor analysis was carried out on the PLA and LAA by measuring the total number of rotations in 5 sec, regardless of the life-span of individual rotors, but considering their frequency of rotation. The number of breakthroughs in 5 sec was also quantified on the PLA, since PVs represent the region where FDs are identified in some cases of AF.[25]

Reentry meandering around SPs was analyzed further to quantify the respective effects of chloroquine and flecainide on the unexcited core size. A detailed description of the method is described in Figures 4–6 of the online supplement.

## Statistical Analyses

Results are reported as mean±SEM. DF data were verified (Shapiro-Wilk) and other continuous measurements were assumed to distribute normally. One-way or two-way repeated measures ANOVA or a mixed model ANOVA was used for continuous measurements as appropriate. Fisher's exact test was used to demonstrate significant differences on AF termination and re-inducibility. *Post hoc* comparisons were employed following a Bonferroni correction. $P<0.05$ was considered statistically significant.

# Results

## Stretch-induced AF in the control

Figure 1A (left) shows examples of simultaneously obtained single pixel recordings from the LAA (red) and RAA (black) at 300 ms CL. As summarized graphically on the right, $APD_{70}$ was significantly shorter in LAA than RAA (n=5) at three different CLs (300, 149.3±9.3 vs 175.7±8.2 ms; 250, 138.7±6.0 vs 153.3±4.5 ms; 200, 123.5±5.0 vs 132.8±3.6 ms, p<0.05).

Once SAF stabilized, the $DF_{max}$ locations were identified across the mapped regions. Figure 1B shows DF maps, sample local activations and corresponding power spectra from the PLA, LAA and RAA after 14 min of control AF in a representative heart. The $DF_{max}$ was localized at the right PVs with a frequency gradient from PLA (13.2Hz) to LAA (12.0Hz) and RAA (7.1 Hz).

The regional $DF_{max}$ was consistently localized either at one of the PVs or somewhere on the PLA (Figure 1C, top). As expected,[4] a statistically significant frequency gradient was observed from PLA to LAA and RAA (Figure 1C, bottom) (10.8±0.3, 8.5±0.2 and 7.0±0.1 Hz, respectively. N=12, p<0.001).

## Chloroquine terminates SAF

To test the hypothesis that reentry is essential to maintain SAF we determined the effect of 4 μM chloroquine in terminating AF, based on its ability to block the inward-rectifier $K^+$ currents.[11] As shown in Figure 2, 4 μM chloroquine reduced AF frequency and restored SR after 4 to 19 min (N=7). Figure 2A shows representative PLA DF maps in control AF ($DF_{max}$ 9.6 Hz) and just before AF termination ($DF_{max}$ 6 Hz). Single pixel optical activations from the $DF_{max}$ region and electrograms from the left superior PV (LSPV) are shown below the maps. Figure 2B shows the time-course of the $DF_{max}$ location in control AF and after chloroquine. The $DF_{max}$ sharply decreased from 10.6±0.7 to 6.3±0.2 Hz (N=7) in the first few min after chloroquine onset.

Five additional experiments were conducted using 1–2 μM chloroquine to increase the selectivity of $I_{K1}$-blockade.[12] At 1 μM, chloroquine terminated AF in 4 out of 5 AF

episodes after 3.5 to 9.5 min. Increasing the concentration to 2 μM restored SR in the fifth AF episode after 14 min. The $DF_{max}$ decreased from 10.5±0.8 Hz in control to 6.7±0.2 Hz before resumption of SR (supplementary Figure 8).

### Flecainide does not terminate SAF

$Na^+$-channel blockade may affect reentrant activity and rotor dynamics leading to AF termination.[26] As illustrated in Figure 3A, after 15 min of control AF, perfusion with 2 μM flecainide for 20 min and 4 μM for 30 additional min significantly decreased the $DF_{max}$ from 11.1±1.3 to 8.1±1.3 Hz (p=0.0014) but did not restore SR (N=5). As shown in Figure 3B, flecainide was significantly inferior to chloroquine in its ability to restore SR (0/5 vs. 7/7, respectively, p=0.0013). In two hearts the initial $DF_{max}$ was slower than in the other three cases (8.0±0.1 vs. 13.1±1.8 Hz) and AF converted to atrial tachycardia (AT). Figure 3C shows DF maps and bipolar recordings from the PLA during control AF and after 4 μM flecainide. Interestingly, after 40 min flecainide, AF converted to AT, which was sustained by a long meandering rotor located in the roof/PLA-LAA junction (supplemental Movie 1).

### Sustained AF is inducible in the presence of flecainide but not chloroquine

As shown in Figure 4A, only non-sustained AF or AT was induced in the presence of chloroquine (N=7). Conversely, upon cardioversion, AF (N=4) or AT (N=1) was re-induced and sustained for 15 min in the presence of flecainide. After the washout period, AF (N=6, chloroquine. N=5, flecainide) or AT (N=1, chloroquine) was re-induced and sustained in both groups. In Figure 4B, the bipolar electrograms illustrate how initial rapid pacing in the presence of chloroquine was followed by a very brief AF episode, in contrast to sustained AF under flecainide.

### Chloroquine terminates AF where flecainide does not

We determined the effects of chloroquine in the 5 experiments in which flecainide was unable to restore SR. Thus, AF was re-induced after 30 min of flecainide washout. Fifteen min of control AF were registered and optically mapped. Figure 4C shows the evolution of the $DF_{max}$ on the PLA. $DF_{max}$ values were significantly higher than at the beginning of the protocol (16.9±2.0 vs 11.1±1.3 Hz, p=0.018), which might be due to a decrease in optimal conditions of the heart after 3 hours of artificial perfusion. Higher $DF_{max}$ may also imply a role of other inward-rectifiers $K^+$ currents, possibly $I_{K,ATP}$. Nevertheless, 4 μM chloroquine effectively terminated AF (N=5). Time to AF termination was longer (10–29 min) relative to the first application of chloroquine (4–19 min, Figure 2B).

### Chloroquine's effects on reentry and breakthroughs

To determine the basis for chloroquine superiority we studied the effects of each drug on AF patterns of excitation. In Figure 5A, the number of identifiable rotations was quantified on the PLA and LAA every 2 min in control AF and every min after chloroquine. Data were normalized as number of rotations/$cm^2$. In control AF, the PLA (N=2; n=16; where N is the number of animals and n is the number of episodes analyzed) harbored twice as many identifiable rotations/$cm^2$ compared to LAA (N=7; n=56) (3.2±0.3 vs.1.6±0.1). On chloroquine, the last 2 min before termination showed a drastic reduction in the number of rotations in both PLA (3.2±0.3 to 0.7±0.3, p=0.003) and LAA (1.6±0.1 to 0.6±0.2, p=0.0002) (see also supplemental Figure 7A). Supplemental movies 2 and 3 show, respectively, PLA movies of short-lasting rotors (1–2 rotations) in control AF and during a short time before termination. Quantification of the core width using the new method illustrated in supplemental Figures 4–6, showed a significant increase before AF termination under chloroquine (Figure 5B). A larger core due to preferential $I_{K1}$-blockade may reduce the probability of stable rotor initiation as shown in Figure 5C, where a rotor around a SP is

observed after a wavebreak and curling of the wavefront. However the rotating front is annihilated by a new wavefront coming from the PLA.

In the stretched atrium, chloroquine's effects on IK112 might increase automaticity and FDs, which may underlie breakthroughs in the field of view. However, the number of breakthroughs/cm$^2$ decreased ($12.3\pm0.8$ to $6.8\pm0.7$, p=0.03. Figure 5D) suggesting that such breakthroughs represented intramural reentrant activity. AF terminated despite the fact that just before termination the number of breakthroughs/cm$^2$ was higher than the number of identifiable rotations/cm$^2$ in control AF ($6.8\pm0.7$ vs. $3.2\pm0.3$, respectively), which supports the essential role of reentrant activity to sustain SAF.

### Reentry and breakthroughs under flecainide

As shown in Figure 6A, B, the number of identifiable rotations decreased after 4 $\mu$M flecainide (N=4), both in the PLA ($3.7\pm0.2$ to $2.7\pm0.4$, p=NS) and LAA ($2.0\pm0.2$ to $1.1\pm0.1$, p<0.05) (see also supplemental Figure 7B and supplemental Movies 4, 5). However, only chloroquine significantly decreased the number of rotations and breakthroughs at the PLA ($3.2\pm0.3$ to $0.7\pm0.3$ and $12.3\pm0.8$ to $6.8\pm0.7$, respectively, p<0.05. Figures 6A, D). The core size showed no significant increase after 4 $\mu$M flecainide (Figure 6C).

### Electrophysiological effects of chloroquine and Flecainide

As summarized on the left side of Figure 7A, B, 4 $\mu$M chloroquine significantly increased the APD$_{70}$ at 300 and 250 ms CL ($155\pm3.1$ to $176\pm6.0$ and $144\pm3.4$ to $158\pm5.0$ ms, respectively, p<0.05) compared to 4 $\mu$M flecainide, which had no significant effect at any pacing CL (supplemental Figure 9). On the right side of Figure 7A, B, both chloroquine and flecainide decreased the CV at all CLs. However, flecainide's effects were substantially greater than chloroquine; 33% reduction at 250 ms CL compared to 12% reduction, respectively.

### Role of $I_{Kr}$ in acute SAF

Chloroquine, while nominally an $I_{K1}$ blocker, also blocks other currents, including $I_{Kr}$. An additional series of experiments (N=7) using the same AF model showed that $I_{Kr}$-blockade after $0.5$–$1$ $\mu$M E-4031 restored SR in 4 out of 7 animals (Figure 8A). When we classified those experiments based on DF$_{max}$ values at baseline (Figure 8B, left); E-4031 restored SR in those cases in which the AF DF$_{max}$ was <8Hz (4/4), but not in cases with DF$_{max}$>8Hz (0/3) ($7.1\pm0.4$ vs $9.2\pm0.5$ Hz, respectively, p=0.029). Unlike E-4031, chloroquine effectively terminated SAF when DF$_{max}$ was >8 Hz (7/7 vs 0/3, p=0.008. Figure 8B, right).

## Discussion

The present study demonstrates that chloroquine, but not flecainide, effectively converted SAF to SR in a sheep heart model that approximates AF in the human heart with pressure overload and atrial dilatation.[6] SAF termination by chloroquine is preceded by a significant increase in rotor core size, which correlates with a reduction in AF frequency and number of breakthroughs. Altogether these data support the idea that reentry and fibrillatory conduction underlie the overall dynamics of SAF in this model. The Na$^+$-channel blocker, flecainide converted AF to AT but did not restore SR. Preferential blockade of the inward-rectifier K$^+$ currents by chloroquine[11] terminated fast reentrant sources persisting after flecainide washout. In addition, unlike E-4031 chloroquine terminated AF episodes whose DF$_{max}$ was >8Hz. Thus, even though E-4031 terminated some of the episodes, the above results demonstrate the superiority of chloroquine over E-4031 in terminating SAF. Nevertheless, chloroquine being a "dirty drug",[12] its antifibrillatory effect is likely not due solely to $I_{K1}$-

blockade but to some combination of $I_{K1}$ and $I_{Kr}$-blockade, which would vary depending on the dose being used.

Since $I_{K-ATP}$ plays a prominent role in ischemia[27] and $I_{K,ACh}$ underlies vagally-mediated AF,[11] it seems reasonable to consider the role of IK1 in the present SAF model. Rotor acceleration and stabilization of the reentry have been associated with IK1 overexpression.[9] In humans, gain of function of $I_{K1}$ due to mutation in *KCNJ2* is also associated with familial AF[28].

## Why is Chloroquine more effective than Flecainide in SAF?

The greater effectiveness of chloroquine over flecainide in terminating SAF in this model may be related to the fact that atrial dilatation decreases the CV and increases the degree of spatial heterogeneities in conduction.[29] Therefore, under conditions of stretch, anatomical and functional obstacles may become prominent and facilitate initiation and maintenance of reentrant activity. Flecainide further decreases CV, which increases core size and promotes attachment to and rotation around an obstacle. It therefore results in slower rotating activity. This would explain the decrease in DF values and conversion to AT in the presence of flecainide. Under certain critical conditions, unexcitable obstacles may destabilize propagation, causing the formation of self-sustained vortices. Wavefront instabilities have been demonstrated after blockade of $Na^+$-channel conductance leading to detachment of the wavefront from the edge of the functional/anatomical barrier and initiation of reentry.[30]

## Controversies in SAF

The mechanisms sustaining acute SAF are controversial. Stretch induces depolarization of the RMP and generation of after depolarizations.[7] Stretch promotes the occurrence of breakthroughs in the LAA,[5] which might be interpreted as being produced either by local triggered activity or intramural reentry. In the present study, a higher number of breakthroughs compared to reentrant activity were also identified at the endocardial side of the PLA. However after either chloroquine or flecainide the number of breakthroughs decreased concomitantly with reentry, suggesting the possibility that the breakthroughs represent intramural reentry. More interesting is the role of chloroquine, which upon preferential blockade of the inward-rectifier $K^+$ channels strongly affects reentry and terminates AF. Patterns of activation on the PLA before AF termination also show that the number of breakthroughs is higher than the number of rotors in control AF but the arrhythmia terminates. The latter suggests that reentrant activity is essential to sustain SAF and that intramural reentry in the PLA was responsible for the majority of those breakthroughs.

## Clinical Perspective

Atrial dilatation and stretch predispose to AF with DF values directly related to LA pressure.[6] Similar to the human heart,[2] SAF in the sheep heart results in DF gradients, where DF at PLA > LAA > RAA. We demonstrate that a reentry-based strategy of blocking the inward-rectifier $K^+$ channels effectively terminates SAF. Conversely, $Na^+$-channel blockade by flecainide was unable to restore SR during atrial stretch, similarly to the loss of antifibrillatory effect that is observed also in patients with atrial dilatation.[31]

Although attractive, $I_{K1}$-blockade is currently not a clinical strategy to treat AF because of the lack of anti-arrhythmic drugs having this property at therapeutic concentrations, along with the fear that reducing $I_{K1}$ would cause diastolic depolarization and might subsequently increase the propensity for triggered arrhythmias.[32] However, such a fear may be reduced by considering that heterozygous Andersen syndrome patients, who lack one allele of the *KCNJ2* gene with loss-of-function of Kir2.1 channels, have mild QTc prolongation and

seldom undergo sudden cardiac death.[33] In addition, chloroquine does not induce early after depolarizations in single myocytes under current-clamp conditions, even at 10 $\mu$M.[12] Finally, Noujaim et al demonstrated that rather than being pro-arrhythmic, $I_{K1}$-blockade is antiarrhythmic as evidenced by chloroquine's ability to terminate reentry in cholinergic induced-AF.[11] Here we have used a well-established model,[4] in which if at all present, the pro-arrhythmic effect of blocking $I_{K1}$ should be more evident.[5, 7] Clinical AF cases reported in the 50's also demonstrated high rates of AF termination after high doses of chloroquine.[34]

Based on the expression profile of the Kir2.X subfamily, Kir2.3 transcripts are more concentrated in the human atrium than in the ventricles.[35] Protein crystallization, molecular modeling and interactions with modulators (e.g. PIP2)[36] might lead to new pharmacological strategies focusing on selective blockade of Kir2.3 subunits. The latter becomes especially relevant in the setting of congestive heart failure, in which $I_{K1}$ downregulation may be associated with increased susceptibility to ventricular arrhythmias.[37]

## Limitations

Although the chloroquine concentrations we have used preferentially block the inward-rectifier K$^+$ channels, termination of AF might also be mediated in part through its less prominent effects on $I_{Kr}$, $I_{Na}$, and $I_{CaL}$. Additional experiments with 1 $\mu$M chloroquine ($I_{K1}$-blockade, 74%; $I_{Kr}$, 38%; $I_{Na}$, 14%)[12] and E-4031 support the role of preferential $I_{k1}$-blockade to terminate SAF. Further experiments would be required to rule out the possibility that the effectiveness of chloroquine in terminating SAF depends at least in part on a drug action on SACs.[38] Stretch-activated K$^+$ channels (TREK-1) might also be involved in the absence of APD prolongation after flecainide.

The antiarrhythmic effects of chloroquine need to be tested in persistent AF, in which $I_{K1}$ up-regulation might be involved. In addition, the role of the autonomic system is difficult to mimic in isolated hearts. However in previous experiments using the same model and adreno-cholinergic stimulation, reentrant activity played a major role in the sustainability of the arrhythmia after abolishing triggered activity.

## Conclusions

Chloroquine effectively terminates SAF in the sheep heart, and is more effective than flecainide in restoring SR, which may be explained by a preferential blockade of the inward-rectifier k$^+$ channels and reentry termination. The results may open novel therapeutic strategies for clinical AF.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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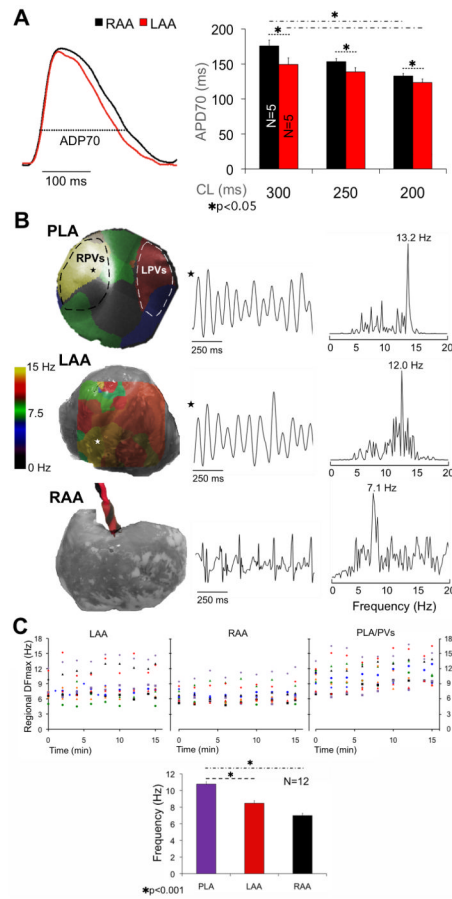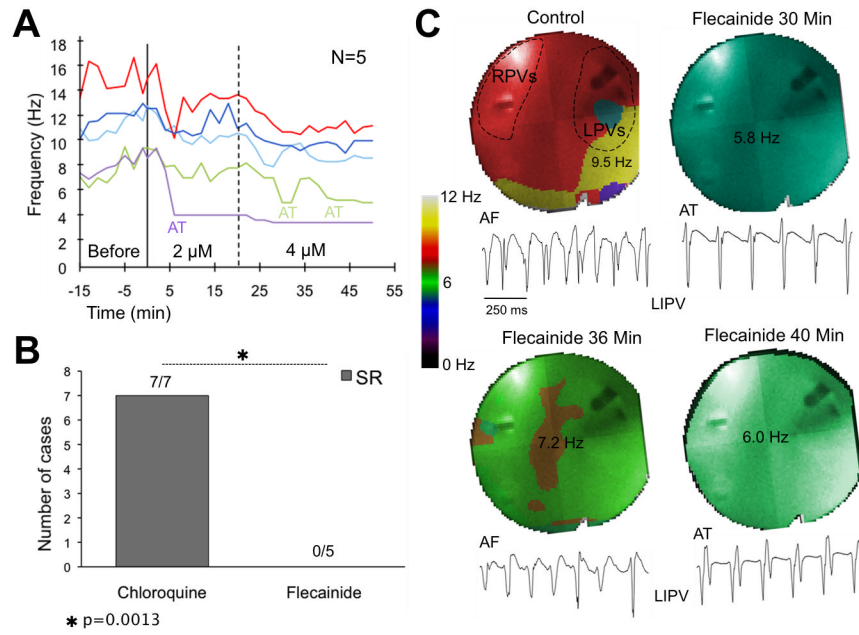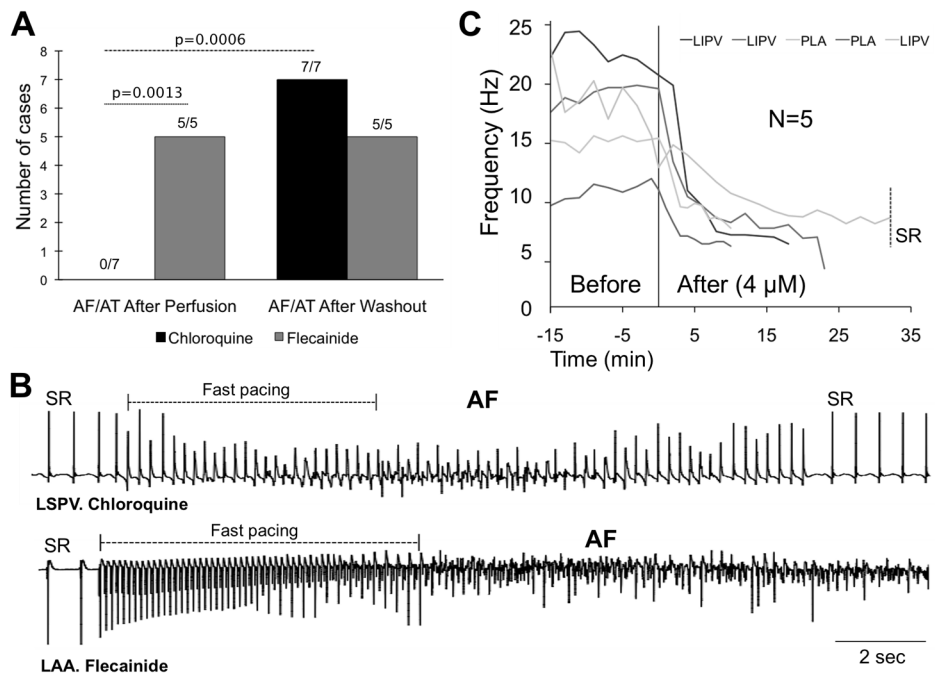
**Figure 1.**
A, left, single pixel AP from the LAA (red) and RAA (black). Right, $APD_{70}$ at varying pacing CLs is shorter in LAA than RAA. $APD_{70}$ significantly shortens in both appendages from 300 to 200 ms CL. B, left column, DF maps of the PLA and LAA, and electrode location on the RAA. Center column; single pixel activations (PLA, LAA) and bipolar electrograms (RAA). Right Column, power spectra showing $DF_{max}$ at each location. C, top, LAA, RAA and PLA/PVs $DF_{max}$ during 15 min of control AF. Bottom, bar graph of mean ±SEM $DF_{max}$ during control AF shows significant DF gradient between PLA and LAA/ RAA.
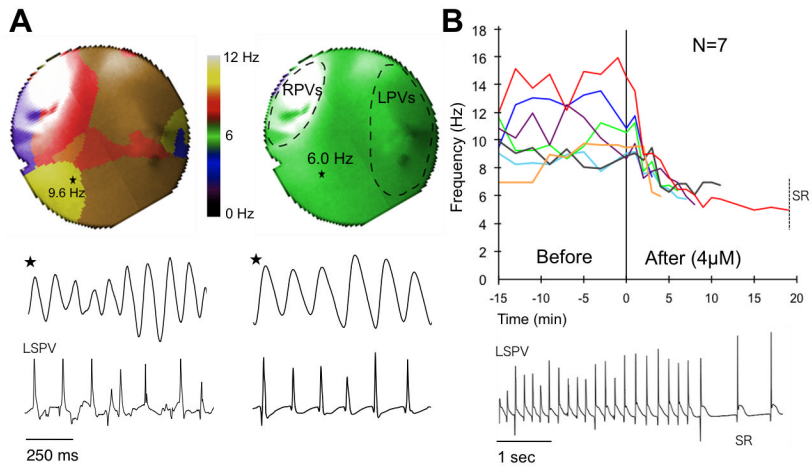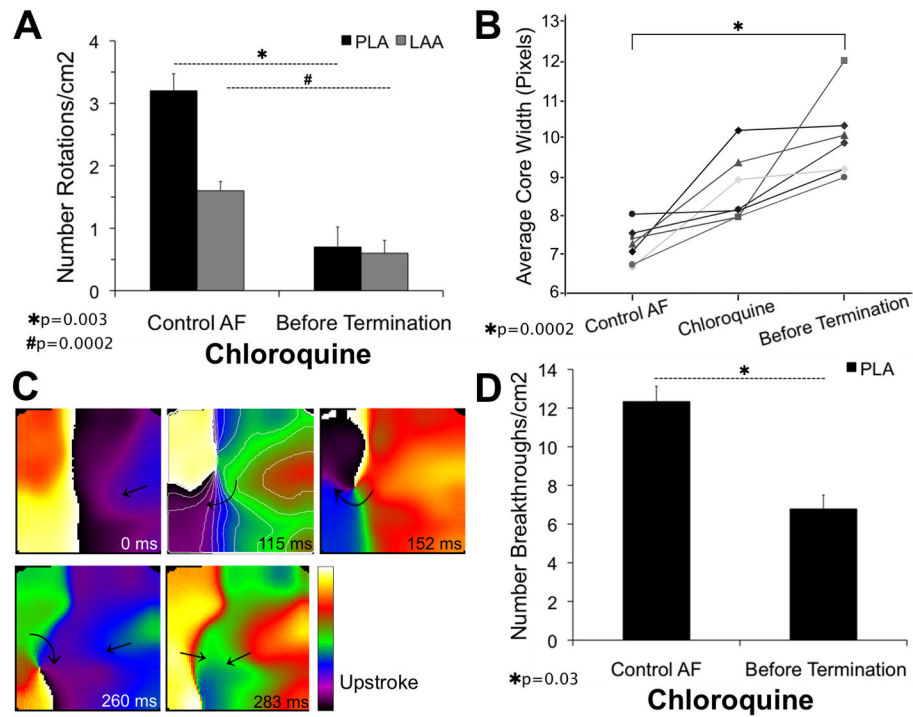
**Figure 2.**
Chloroquine terminates AF. A, top: DF maps of PLA during control AF and before termination; middle and bottom: single pixel recordings and electrograms from LSPV. B, top: time course of $DF_{max}$, before and during chloroquine; bottom: electrograms from LSPV at the moment of termination.

**Figure 3.**

A, time-course of $DF_{max}$ before and during flecainide. B, flecainide is significantly inferior to chloroquine in resuming SR. C, DF maps of the PLA and bipolar electrograms from the left inferior PV (LIPV) show control AF and conversion to AT during flecainide.
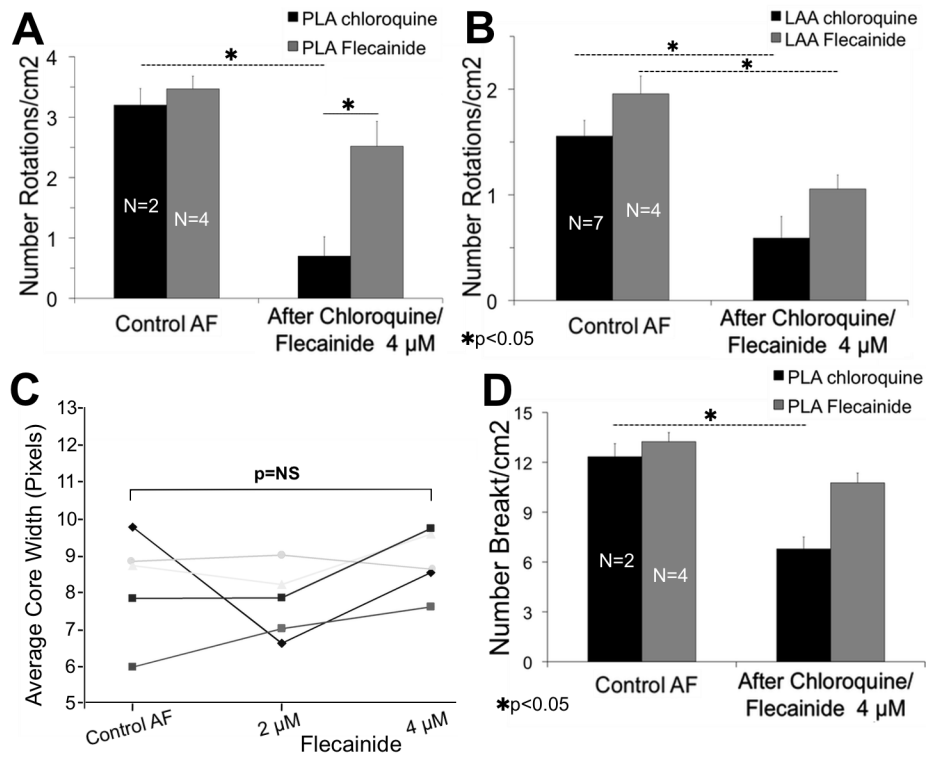
**Figure 4.**
A, left, sustained AF/AT is re-inducible during flecainide but not during chloroquine perfusion. Right, after washout sustained AF/AT is re-inducible in both groups. B, representative traces; rapid pacing at 12 Hz did not capture or re-induce non-sustained AF during chloroquine (top); at the same pacing frequency, sustained AF was readily induced during flecainide (bottom). C, time-course of $DF_{max}$, SR is restored when chloroquine was perfused after flecainide washout.
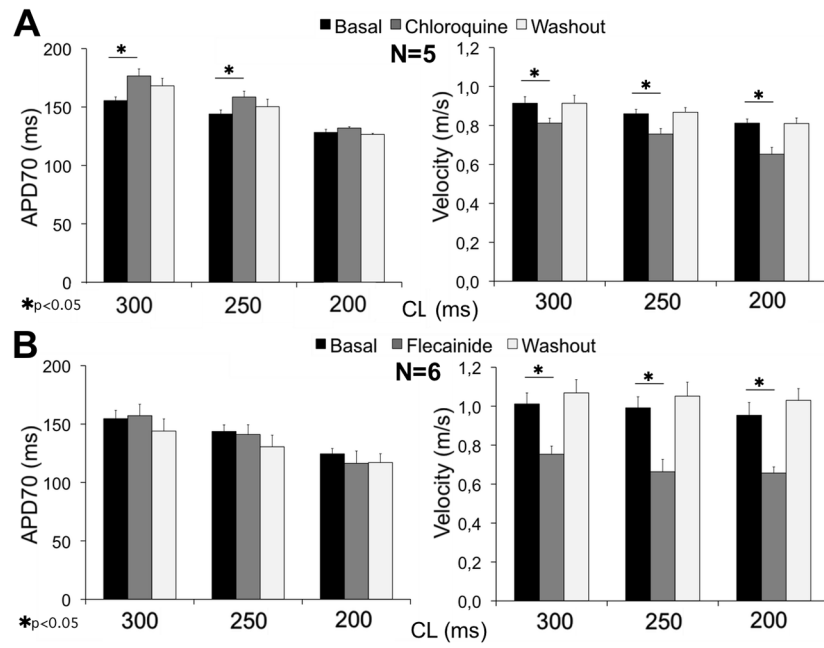
**Figure 5.**
Chloroquine effects on reentry and breakthroughs (5-sec movies). A, number of rotations/cm$^2$ in the PLA/LAA in control and just before chloroquine-induced AF termination. B, average core width significantly increases in size during chloroquine. C, phase maps show a wavebreak and the initiation of a rotor that cannot complete an entire rotation during chloroquine. D, number of breakthroughs/cm$^2$ in control and during chloroquine.
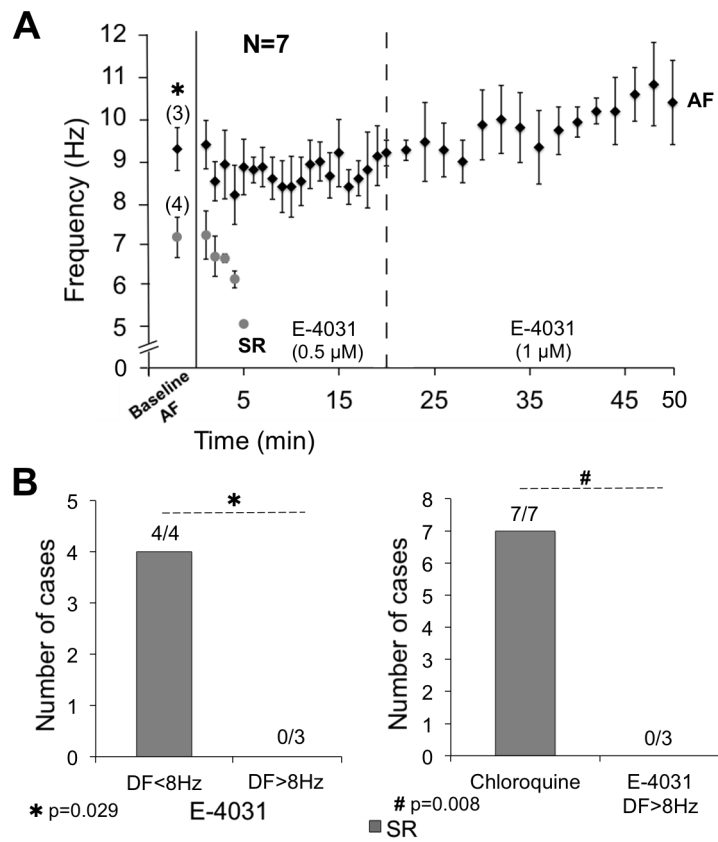
**Figure 6.**
Comparing chloroquine to flecainide. A and B, although flecainide reduces the number of rotations/cm$^2$ at the PLA and LAA (5-sec movies), only chloroquine significantly decrease the number of rotations/cm$^2$ at the PLA. C, the core size does not significantly increase during flecainide. D, Similarly, only chloroquine significantly decreases the number breakthroughs/cm$^2$ at the PLA.

**Figure 7.**
A, left, mean $APD_{70}$ for control, chloroquine and washout at 300, 250, and 250 ms CL; right, mean CV for control, chloroquine and washout; 300, 250, and 250 ms CL. B, left: flecainide does not significantly affect the $APD_{70}$ at any CL; right, flecainide strongly reduces velocity in a reversible manner.

**Figure 8.**
A, time-course of DF$_{max}$ after E-4031 0.5–1 µM. B, E-4031 restored SR in those cases with DF$_{max}$<8 Hz. C, chloroquine was more effective than E-4031 to terminate SAF with DF$_{max}$>8 Hz.