Activation of Inward Rectifier Potassium Channels Accelerates Atrial Fibrillation in Humans

Evidence for a Reentrant Mechanism

Felipe Atienza, MD, PhD; Jesús Almendral, MD, PhD; Javier Moreno, MD; Ravi Vaidyanathan, MS; Arkazdi Talkachou, MS; Jérôme Kalifa, MD; Angel Arenal, MD; Julian P. Villacastín, MD, PhD; Esteban G. Torrecilla, MD, PhD; Ana Sánchez, MD; Robert Ploutz-Snyder, PhD; José Jalife, MD; Omer Berenfeld, PhD

Background—It is unclear whether atrial fibrillation (AF) drivers in humans are focal or reentrant. To test the hypothesis that functional reentry is involved in human AF maintenance, we determined the effects of adenosine infusion on local dominant frequency (DF) at different atrial sites. By increasing inward rectifier potassium channel conductance, adenosine would increase DF of reentrant drivers but decrease it in the case of a focal mechanism.

Methods and Results—Thirty-three patients were studied during AF (21 paroxysmal, 12 persistent) using recordings from each pulmonary vein–left atrial junction (PV-LAJ), high right atrium, and coronary sinus. DFs were determined during baseline and peak adenosine effect. In paroxysmal AF, adenosine increased maximal DF at each region compared with baseline (PV-LAJ, 8.03±2.2 versus 5.7±0.8; high right atrium, 7±2.2 versus 5.4±0.7; coronary sinus, 6.6±1.1 versus 5.3±0.7 Hz; $P=0.001$) and increased the left-to-right DF gradient ($P=0.007$). In contrast, in persistent AF, adenosine increased DF only in the high right atrium (8.33±1.1 versus 6.8±1.2 Hz; $P=0.004$). In 4 paroxysmal AF patients, real-time DF mapping of the left atrium identified the highest DF sites near the PV-LAJ, where adenosine induced an increase in DF (6.7±0.29 versus 4.96±0.26 Hz; $P=0.008$). Finally, simulations demonstrate that the frequency of reentrant drivers accelerates proportionally to the adenosine-modulated inward rectifier potassium current.

Conclusions—Adenosine accelerates drivers and increases frequency differently in paroxysmal compared with persistent human AF. The results strongly suggest that AF is maintained by reentrant sources, most likely located at the PV-LAJ in paroxysmal AF, whereas non-PV locations are more likely in persistent AF. **(*Circulation*. 2006;114:2434-2442.)**

Key Words: ablation ■ adenosine ■ atrium ■ fibrillation ■ Fourier analysis ■ reentry

The mechanisms of human atrial fibrillation (AF) are poorly understood.[1,2] Experimental studies have demonstrated that cholinergic AF is maintained by high-frequency reentrant sources (drivers) that result in a consistent left-to-right frequency gradient.[3–6] More recently, clinical studies have confirmed the existence of a hierarchical organization in the rate of activation of different regions in the atria of patients with paroxysmal and chronic AF.[7–9] Although the maximal dominant frequency (DF) sites were found to play a crucial role in the maintenance of AF in some patients,[9] it is unclear whether AF drivers in humans are focal or reentrant and whether changes in the driver activity would alter spatial frequency gradients. To test the hypothesis that localized functional reentry also maintains AF in humans, we determined the effects of adenosine infusion on local DFs at different sites of both atria. By increasing potassium conductance through activation of specific inward rectifier channels, adenosine shortens action potential duration and refractoriness and reduces excitability and automaticity.[10,11] In the present study, we demonstrate that, as expected from a reentrant mechanism of AF maintenance, adenosine infusion increases DFs primarily at sites that activate at the highest rate at baseline. Moreover, as expected from the different distribution of DFs in patients with persistent compared with paroxysmal AF, we demonstrate a different adenosine response in the 2 groups.

Clinical Perspective p 2442

Methods

Patients

Patients admitted for ablation treatment of drug-refractory AF were included in this protocol, as approved by the research and ethics

Received August 31, 2005; de novo received April 17, 2006; revision received September 19, 2006; accepted September 25, 2006.

The online-only Data Supplement is available with this article at http://circ.ahajournals.org/cgi/content/full/CIRCULATIONAHA.106.633735/DC1.

From Hospital General Universitario Gregorio Marañón (F.A., J.A., J.M., A.A., J.P.V., E.T., A.S.) and Hospital Clínico San Carlos (J.M., J.P.V.), Madrid, Spain, and Institute for Cardiovascular Research, SUNY Upstate Medical University, Syracuse, NY (R.V., A.T., J.K., R.P.-S., J.J., O.B.).

Correspondence to José Jalife, MD, Institute for Cardiovascular Research, SUNY Upstate Medical University, 766 Irving Ave, Syracuse, NY 13210. E-mail jalifej@upstate.edu

Circulation **is available at http://www.circulationaha.org** **DOI: 10.1161/CIRCULATIONAHA.106.633735**

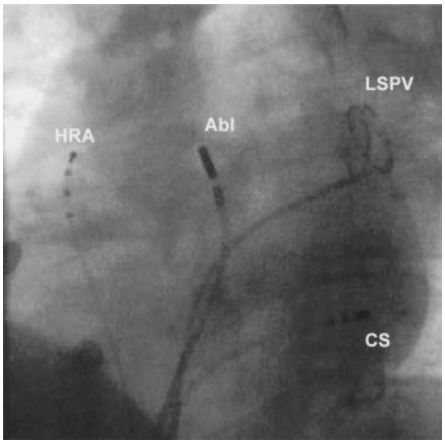

Figure 1. Left anterior oblique fluoroscopic view of heart and electrode positions during adenosine infusions: (1) tetrapolar catheter in HRA near the posterior interatrial septum, (2) deflectable mapping catheter in the distal CS, (3) decapolar circular mapping catheter at the PV-LAJ of the LSPV ostium, and (4) ablation catheter (Abl) at the right superior PV-LAJ.

committees of our institutions. All patients gave informed consent. Exclusion criteria were clinical history of bronchospasm or asthma and prior treatment with dipyridamole or aminophylline. Self-terminating AF lasting <48 hours was defined as paroxysmal; sustained AF lasting >1 month before the procedure or requiring external cardioversion to sinus rhythm was defined as persistent. All antiarrhythmic and β-blocking agents were withheld ≥5-half lives before the study. Amiodarone treatment was terminated at least 2 weeks in advance.

Electrophysiological Study

The electrophysiological study was performed under sedation and additional periodic heparin bolus administrations. Four recording catheters were used (Figure 1) as follows: a high right atrium (HRA) catheter, a mapping catheter in the distal coronary sinus (CS), a decapolar circular mapping lasso catheter at the pulmonary vein–left atrial junction (PV-LAJ) of the left superior pulmonary vein (LSPV) ostium, and an ablation catheter in the left atrium (LA). In a subset of patients (n=6), a single 20-pole catheter was used to record the HRA and CS simultaneously (not shown). The 3-dimensional geometry of the LA chamber was reconstructed using the Navistar catheter and the CARTO navigation system (Biosense Webster, Inc, Diamond Bar, Calif). In patients arriving in sinus rhythm, AF was induced by burst pacing. Only episodes lasting >3 minutes were included in the study.

Adenosine Infusions and Recording Protocol

A central venous bolus infusion of 12-mg adenosine was repeated up to 5 times during AF once all recording catheters were in a stable position. Figure 2 shows the timeline of infusion and recording protocol. Catheters remained at fixed positions in the HRA, CS, and PV-LAJ of the LSPV for the duration of all infusions. For every infusion, the Navistar mapping catheter was positioned randomly at a different PV-LAJ and at a high-frequency site in the posterior LA wall. A 5-second period before infusion and a 5-second period surrounding the longest RR interval at peak adenosine effect were analyzed.

Spectral Analysis

DF was determined as previously described.[7,9] All signals were visually inspected, and spurious DFs were excluded or corrected. See the online-only Data Supplement for further details.

Real-Time Frequency Mapping

In 4 additional patients, DF maps were generated during ongoing AF using a novel CARTO system incorporating online spectral analysis.[9] Primary and secondary sites with highest DF (DF_{max} sites) were identified (ie, the primary DF_{max} site is the site with the highest DF throughout the mapped areas).

Radiofrequency Ablation of AF

After the adenosine infusion protocol, radiofrequency (RF) ablation was applied around the PVs, regardless of the spectral analysis results, aiming at terminating AF. In most patients, the technique used was circumferential LA ablation.[12] In a subset of 6 patients, the ablation technique used was PV electrical isolation.[13]

Statistical Analysis

Continuous variables are reported as mean±SD or median and interquartile range (IQR), depending on whether they were normally or nonnormally distributed. Categorical variables are reported as number and percentage. The effect of adenosine infusions on activation rate and regularity at each atrial region was compared using a 3-factor, mixed-model analysis of variance design that included 2 repeated-measures (within-subject) factors and 1 independent-measures factor. The 2 within-subject factors were time (baseline versus peak) and atrial sites (HRA, CS, PV-LAJ), and the independent-measures factor was AF type (paroxysmal versus persistent AF). The model used a priori pairwise contrasts comparing baseline and peak adenosine infusions within location and AF using Bonferroni correction for multiple comparisons. Statistical significance was established at $P<0.05$.

Computer Simulations

We used a 5×5-cm^2 model with realistic human atrial kinetics with heterogeneous $I_{K,ACh}$ density and increasing the acetylcholine (ACh) concentration from 0.003 to 0.1 μmol/L, simulating the transition from baseline to peak adenosine.[10,11,14] See the online Data Supplement for further details.

The authors had full access to the data and take full responsibility for their integrity. All authors have read and agree to the manuscript as written.

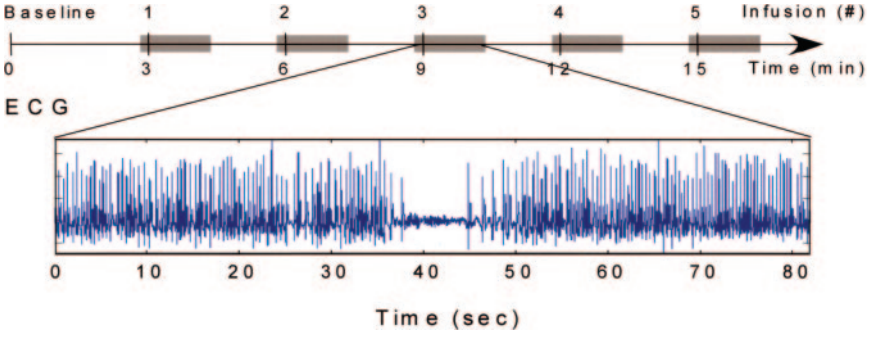

Figure 2. Adenosine infusion protocol. Top, Timeline for each adenosine infusion (infusions 1 through 5) and recording (gray bars). Bottom, Representative surface ECG during the third infusion showing the analyzed time periods: baseline recording (0 to 5 seconds) and peak adenosine effect recording (38 to 43 seconds) as demonstrated by the presence of AV block.

Patient Characteristics

	Paroxysmal AF (n=21)	Persistent AF (n=12)	P
Age, y	51.6±8.7	49.3±9.9	0.52
Male, n (%)	15 (71)	10 (83)	0.66
Body mass index, kg/m^2	27.9±3.5	27.7±3.5	0.84
Structural heart disease, n (%)	3 (14)	1 (8)	0.62
First AF episode, n (%)			
<1 y	5 (24)	...	...
1–3 y	3 (14)	3 (25)	...
>3 y	13 (62)	9 (75)	0.16
Sustained AF duration, mo	NA	17.9±7.9	...
LA size, mm	38.4±6	46.6±4.9	<0.001
LA area, cm^2	18.4±5.8	37.2±13.9	0.03
RA area, cm^2	15±2.8	23.5±12.8	0.01
LV ejection fraction, %	61.5±5.6	60±2.1	0.28
LV mass, g	162±35.7	181±52.5	0.31
Antiarrhythmics used, n	2±0.6	2.2±0.9	0.55
Prior amiodarone treatment, n (%)	10 (48)	5 (42)	0.74

LV indicates left ventricular.

Results

Patients

Thirty-three patients (age, 51±9 years; 76% male) were studied during ongoing AF (21 paroxysmal, 12 persistent) before ablation. There were no clinical differences between paroxysmal and persistent AF patients (Table). However, persistent AF patients had significantly larger LA and RA areas and tended to have a longer history of AF. Twelve patients (6 paroxysmal, 6 persistent AF) presented with mild mitral regurgitation, but only 1 paroxysmal AF patient had moderate mitral regurgitation. No patient had significant left ventricular dysfunction, heart failure, or coronary artery disease.

On arrival at the electrophysiology laboratory, 20 of 21 paroxysmal AF (95%) and 0 of 12 persistent AF (0%) patients were in sinus rhythm. AF was induced during catheter manipulation in 5 of 21 patients (24%); AF was provoked by incremental CS pacing in the remaining 15 patients. Only 1 paroxysmal AF patient required reinduction as a result of spontaneous AF conversion to sinus rhythm during the recording period.

Adenosine Infusion

Overall, a total of 123 adenosine infusions were performed (median, 4 infusions per patient; IQR, 3). There was no significant difference (P=0.21) in the number of infusions between paroxysmal (median, 3; IQR, 3) and persistent (median, 5; IQR, 2) AF patients. The 12-mg adenosine bolus produced significant bradycardia or transient atrioventricular (AV) block in most patients (95%), with a median AV block duration of 4.4 seconds (IQR, 3.6 seconds). There was no significant difference (P=0.18) in the duration of adenosine-induced AV block between paroxysmal (median, 5.2 seconds; IQR, 1.9 seconds) and persistent AF patients (median, 3.3 seconds; IQR, 0.6 seconds). In 1 case, the first 12-mg bolus produced significant AV block (13.5 seconds) without clinical impact, but in subsequent boluses, doses were reduced to 6 mg, producing a mean of 7.7-second AV block episodes. Only 1 patient required an increase in the adenosine bolus dose (18 mg) to obtain the desired effect. One patient developed mild bronchospasm after the first adenosine bolus but recovered spontaneously and completely. The total time required to sequentially perform the proposed adenosine infusions and to record from all attempted PVs (from the baseline of the first infusion to the end of the last recording) was 15 minutes 37 seconds±7 minutes 8 seconds. Protocol duration was similar in paroxysmal (14 minutes 5 seconds±7 minutes 6 seconds) and persistent (17 minutes 47 seconds±8 minutes 5 seconds; P=0.44) AF patients. In a subset of patients (n=6), only mild conscious sedation was used.

Adenosine Accelerates AF Frequency

Of a total of 195 LA sites with simultaneous recordings for HRA and CS, both at baseline and after adenosine infusion, 29 were excluded because of poor signal quality. Twenty-eight and 27 patients had simultaneous recordings in the HRA and CS, respectively, during the first infusion. Twenty-three and 22 patients had recordings from those sites, respectively, during at least 2 infusions (up to 5). In 6 patients, no PV lasso recordings were obtained.

Figure 3 shows representative recordings illustrating the time course of the effect of adenosine on the activity of the LSPV in the same patient as in Figure 2. The data on top demonstrate that DF increases at 2 different locations in the PV-LAJ from ≈5 Hz at baseline to >6 Hz at the peak of the effect, followed by a return to approximate baseline values at recovery. The 3 power spectra of electrode pair 8,9 were extracted at specific time points indicated by the red arrows on the color-coded spectrogram presented at the bottom, which shows the temporal evolution of DF. Clearly, after a stable control period of ≈25 seconds, the DF at the LSPV increased from baseline to a peak value of 6.2 Hz, concomitant with AV block (see Figure 2). This was followed by a return to baseline 15 to 20 seconds after the peak effect. The relatively full return to baseline at both locations indicates DF stability for periods of at least 1 minute. Baseline DFs at the beginning of each of the 5 infusions fluctuated with an average standard deviation of 0.25 and 0.21 Hz in paroxysmal and persistent AF patients, respectively, with no temporal trend during the protocol.[9] When we compared the DFs at baseline and at peak adenosine effect for the first 3 consecutive infusions at all sites and patients, we found no significant differences with time. Thus, the temporal stability of the DFs and reproducibility, at both baseline and peak adenosine effect, was validated in the LSPV, as well as in the HRA and CS.

As illustrated in Figure 4A, in paroxysmal AF patients, DF was somewhat higher in the PV-LAJ (5.7±0.8 Hz) than in the HRA and CS (5.4±0.7 and 5.3±0.7 Hz, respectively; P=NS). Upon adenosine infusion, the local DF increased in all regions compared with baseline (PV-LAJ, 8.03±2.2 versus 5.7±0.8 Hz; HRA, 7±2.2 versus 5.4±0.7 Hz; CS, 6.6±1.1 versus 5.3±0.7 Hz; P=0.001). However, the effect was much larger in the PV-LAJ, which resulted in a signifi-

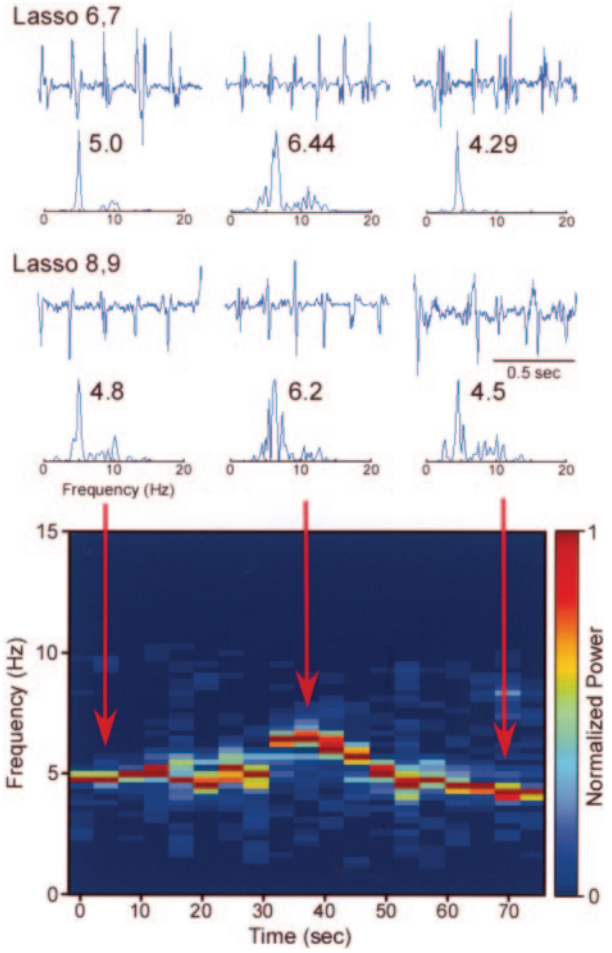

Figure 3. Adenosine infusion effect on DFs at the LSPV in a paroxysmal AF patient. Top, Bipolar signals and corresponding fast Fourier transforms obtained simultaneously from 2 electrode pairs (6,7 and 8,9) of a lasso catheter. Left shows baseline; center, peak adenosine effect; and right, recovery. Numbers on top of fast Fourier transforms indicate DFs (in Hz). Bottom, Spectrogram (frequency vs time) of lasso 8,9 bipole illustrating temporal evolution of DF during adenosine infusion. The entire period is divided into sequential 4096-ms segments; power spectra are calculated for each segment. Colors indicate power: blue shows low power; red, high power (ie, DF). After a stable control of ≈25 seconds, DF increases from baseline to peak value, concomitant with the AV block (see Figure 2), followed by a decrease to baseline value.

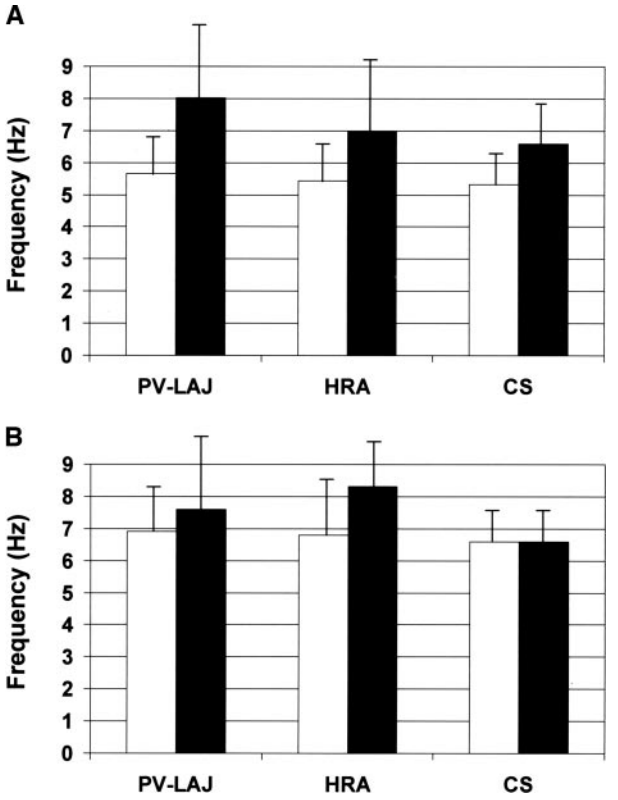

Figure 4. Mean±SD of DF for PV-LAJ, CS, and HRA at baseline (white) and peak adenosine effect (black) in paroxysmal (A) and persistent (B) AF patients. At baseline, paroxysmal AF patients showed no frequency gradient; after adenosine infusion, a significant left-to-right gradient was elicited (P=0.007). In persistent AF patients, there were no baseline differences; after adenosine infusion, DFs significantly increased only in HRA (P=0.004).

cant left-to-right DF gradient (P=0.007). In general, patients with persistent AF demonstrated higher maximal baseline DFs (PV-LAJ, 6.9±1.1 Hz; HRA, 6.8±1.2 Hz; CS, 6.6±0.8 Hz) than paroxysmal AF patients (P<0.001). As shown in Figure 4B, adenosine infusion in persistent AF patients increased local DFs only in the HRA (8.33±1.1 versus 6.8±1.2 Hz; P=0.004). The increase in the PV-LAJ was not statistically significant (7.6±1.7 versus 6.9±1.1 Hz; P=0.1), and there was no change in the CS DF (6.6±0.7 versus 6.6±0.8 Hz; P=NS). When considering the ablation outcome in those patients, we observed no differences in DFs either at baseline or upon adenosine infusion between paroxysmal AF patients with versus without sinus rhythm conversion during RF ablation (Persistent AF patients were not analyzed because of the small sample size; see the online Data Supplement).

In 4 additional paroxysmal AF patients, the adenosine effect was measured at the primary and secondary DF_{max} sites in the LA (see Methods) that play an important role in AF maintenance.[9] Maps of the LA were obtained using real-time DF analysis. We acquired 81±17 points per patient, 74% of which were valid. Figure 5 shows a representative example in which the AF frequency was relatively slow (<5 Hz) and 3 DF_{max} sites were identified with a primary DF_{max} site near the RIPV (red arrow). Figure 5B and 5C show that, although the adenosine infusion practically abolished the ventricular activity as detected by V_5, the DF at the primary DF_{max} site accelerated from 4.64 to 6.35 Hz. An additional adenosine infusion performed while measuring activity at the secondary DF_{max} sites also showed an increase in DF, but to a lesser extent. In this patient, the arrhythmia terminated during postmapping ablation at the primary DF_{max} site, demonstrating again its critical role in AF driver location.[9] Compared with baseline, adenosine significantly accelerated the primary and secondary DF_{max} sites in these 4 patients from 4.96±0.26 to 6.7±0.29 Hz (P=0.008), demonstrating that the sites involved in the maintenance of AF are clearly affected by adenosine.

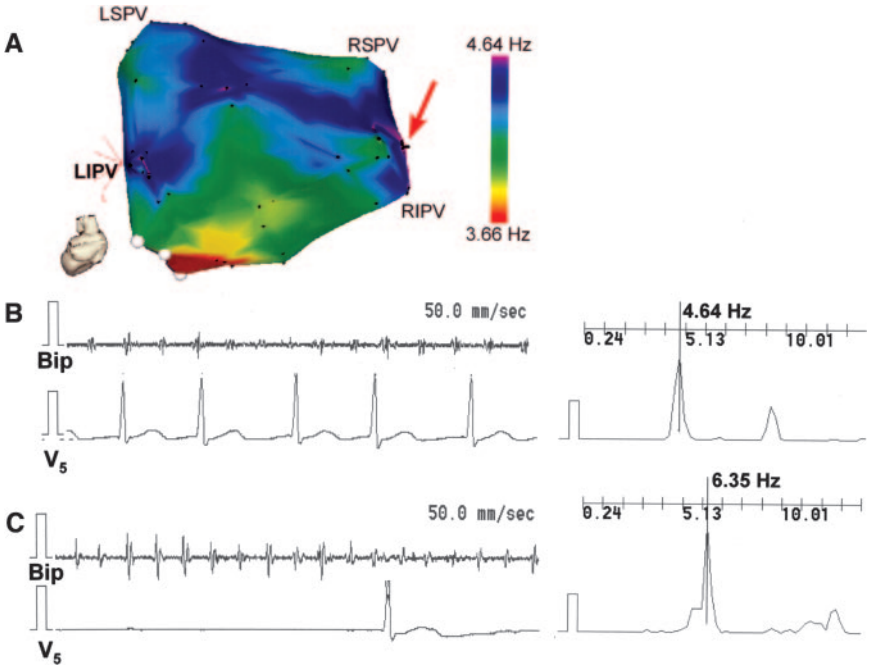

Figure 5. A, LA posterior view DF map from a paroxysmal AF patient. The DF map was produced by the real-time frequency-mapping CARTO system before infusion of adenosine. Red arrow indicates primary DF_{max} site near the RIPV. B, Baseline recording at the primary DF_{max} site with its power spectrum and simultaneous V_5 reference. C, Recording at the primary DF_{max} site with power spectrum and simultaneous V_5 reference during peak adenosine effect showing an increase in the DF. LIPV indicates left inferior pulmonary vein; RSPV, right superior pulmonary veins; and Bip, bipolar catheter.

Computer Simulations

A simplified in silico 2-dimensional model of the atria was used to provide mechanistic insight into the effects of adenosine on DF_{max} and the LA-to-RA DF gradient. The adenosine effect was simulated by activation of $I_{K,ACh}$. Figure 6 shows consecutive snapshots during a simulation of paroxysmal AF at baseline (Figure 6A) and peak adenosine effect (Figure 6B). In Figure 6A, stable reentry in the left half of the sheet acted as the high-frequency source (a; DF=9 Hz) of waves that propagated toward the right (b; DF=4.4 Hz). In Figure 6B, $I_{K,ACh}$ activation increased the rotation frequency and consequently the DFs in the LA (15.9 Hz) and RA (9 Hz). When conditions of persistent AF were established and a reentrant source was simulated in the LA (see movie clips in the online Data Supplement), $I_{K,ACh}$ activation increased the DF in the LA (11 to 14.6 Hz) and unified the DFs in the RA from a range of 8.8 to 11 Hz during baseline to 9.8 Hz during $I_{K,ACh}$ activation. Overall, although the AF frequencies are somewhat higher in the simulations than in the patients, qualitatively, the numerical results demonstrate that $I_{K,ACh}$ activation by adenosine increased the source region DF and consequently the left-to-right DF gradient in both paroxysmal (4.6 to 6.9 Hz) and persistent (maximum, 2.2 to 4.8 Hz) AF. In general, the degree of DF increase was significantly higher in paroxysmal (LA, 77%; RA, 100%) compared with persistent (LA, 33% LA; RA, 0%) AF.

Dissociated PV Potentials

The response of dissociated PV potentials (DPVPs) to adenosine infusion (12-mg bolus) was assessed after PV isolation. DPVPs were observed in 4 patients (14%). We observed slow (cycle length >1200 ms) DPVPs at baseline in 2 paroxysmal and 1 persistent AF patients; rapid (cycle length <400 ms) DPVPs were observed in 1 paroxysmal patient. Figure 7 shows an example of a rapid DPVP (mean cycle length, 185 ms) after PV isolation and conversion to sinus rhythm. During peak adenosine effect, there was a marked acceleration (mean cycle length, 120 ms) and regularization of the dissociated PV local activity, with a significant increase in DF from 6.68 Hz at baseline to 9.3 Hz at peak adenosine effect. In contrast, adenosine infusion transiently suppressed slow DPVPs in all patients, as illustrated by the example in Figure 8.

Discussion

The major finding of the present study is that, in humans, activation of $I_{K,ACh}$ channels by adenosine accelerates AF drivers and increases the excitation frequency differently in paroxysmal compared with persistent AF. In paroxysmal AF patients, adenosine infusion increases local DFs, particularly at the PV-LAJ region, amplifying a left-to-right frequency gradient. In persistent AF patients, DF is significantly higher than in paroxysmal AF patients in all atrial regions surveyed, with the highest adenosine increase in frequencies outside the PV region. As confirmed by computer simulations, adenosine-induced driver acceleration is strongly suggestive of a reentrant mechanism in both groups of AF patients. These results are a natural extension of our previous experimental and clinical studies[2–6,9,15] and support the idea that AF in humans is maintained by high-frequency reentrant sources that can be accelerated by intravenous administration of adenosine. Final proof of this idea, however, requires a more direct demonstration of both high-frequency rotors in the human atria and their acceleration by adenosine.

AF Mechanisms

The mechanisms underlying AF maintenance in humans remain poorly understood. The multiple wavelet hypothesis led to the consensus that AF is the result of the random propagation of multiple wavelets across the atria.[16] However, experimental studies suggest that at least some cases of AF may be maintained by high-frequency reentrant sources

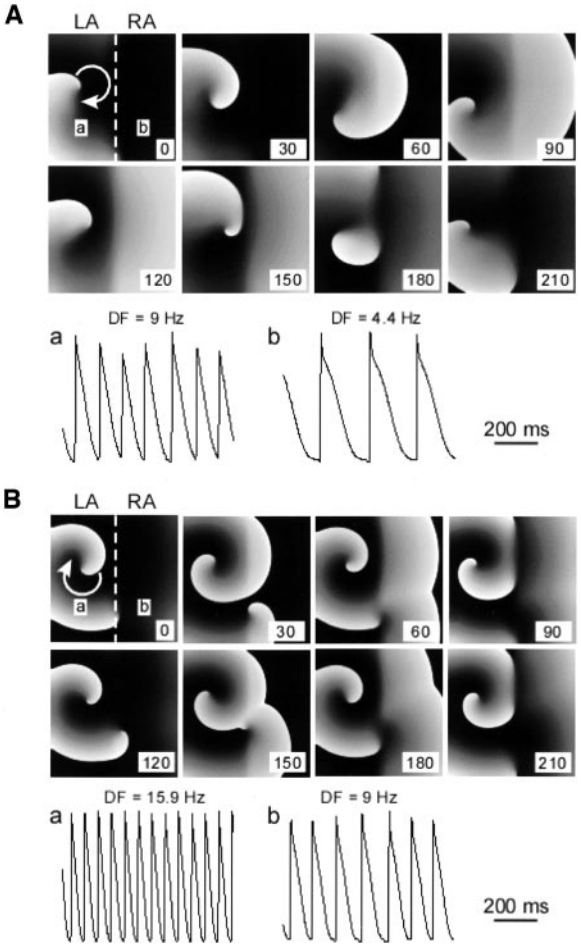

Figure 6. Computer simulations in a 2-dimensional sheet of paroxysmal AF at baseline (A) and peak $I_{K,ACh}$ effect (B). For each snapshot, stable reentry in the left half of the sheet acted as the high-frequency source of fibrillatory waves in the LA (a) that propagated toward the RA (b) in the right part of the sheet. At baseline (A), LA DF (a; 9 Hz) is higher than RA DF (b; 4.4 Hz); left-to right gradient is 4.6 Hz. At peak adenosine effect (B), DFs in the LA (a) is 15.9 Hz and in the RA (b) is 9 Hz; the left-to-right gradient is 6.9 Hz. Numbers in frames indicate relative time in milliseconds.

(drivers), usually located in the posterior LA, that result in spatially distributed frequency gradients.[2–6] Recently, clinical studies have confirmed the existence of a hierarchical organization in the rate of activation of different regions in the atria of patients with paroxysmal and persistent AF.[7–9] Moreover, ablation of high-frequency sites results in AF termination in a significant proportion of patients with paroxysmal AF, confirming their role in the maintenance of AF in humans.[9] To date, however, whether such sites are automatic, triggered, or reentrant remains unresolved. The present combined electrophysiological/pharmacological study was designed to address that issue specifically.

Adenosine Provides Mechanistic Insight in AF

Adenosine and ACh are known to activate the same Kir3.x subfamily of inward rectifier potassium channels through different signaling pathways.[17] The current that arises from such activation is the same and called either $I_{K,ACh}$ or $I_{K,Ado}$, depending on whether ACh or adenosine is the agonist.[10,11,17] By increasing K^+ conductance in the atrium, both ACh and adenosine hyperpolarize the cell membrane, abbreviate the action potential duration and refractory period, and inhibit spontaneous pacemaker discharge, as well as early and delayed depolarizations.[10,11] Experimentally, ACh increases atrial activation frequency in a dose-dependent manner, with the effect being larger in LA than RA, leading to an increase in LA-to-RA frequency gradient.[15] As with the simulations shown here (Figure 6), changes in rotor frequency were responsible for the changes in DF_{max}.[15] Thus, the adenosine-induced acceleration of DF_{max} points toward reentry as the mechanism of AF maintenance in the patients surveyed here and all but rules out an automatic or triggered mechanism.

Adenosine Amplifies DF Gradient in Paroxysmal AF

Because ablation at the highest-DF sites is associated with AF termination in 87% of paroxysmal AF,[9] adenosine infusion during ongoing AF could be used as a diagnostic tool (see the online Data Supplement). Indeed, the present study shows that a baseline dominance of the LA is further enhanced by adenosine infusion and can potentially point to the location of the source. According to Sanders et al,[9] the highest-DF sites were found in the PV-LAJ region at baseline in 60% of the cases, whereas upon adenosine infusion, the highest-DF sites were localized in the PV-LAJ region in the 76% of patients. Our preliminary results using high-density real-time DF mapping confirm the ability of adenosine to highlight sites driving paroxysmal AF that could be targeted for ablation (Figure 5). The left-to-right gradient in DFs is in agreement with prior reports.[7,9] Lazar et al[7] demonstrated the presence of a similar gradient in paroxysmal human AF, confirming experimental results.[2–6] Regional disparities of spatial DF distribution between both studies exist, however. Although we found a regional maximal frequency gradient from the LA to RA with lowest DFs in the CS, Lazar et al[7] found that DFs were lowest in the posterior RA and intermediate in the CS. This may be attributed to a different methodology. We obtained a single recording from the HRA, close to the interatrial septum, whereas Lazar et al considered an average value taken from 5 sites in the posterior RA. More extensive mapping studies may account for the differences found in the 2 studies. This is suggested by the agreement of our results with those of Sanders et al,[9] who conducted high-density (126 ± 13 points per patient) frequency mapping of both atria and the CS during AF.[9] Animal studies also support such an idea,[18] which may explain why the highest DFs located in the right PVs can be followed by faster DFs in the RA than in the CS via rapid conduction through the Bachmann bundle. Similarly, Jaïs et al[19] demonstrated slower and more organized activity in the distal CS that does not necessarily reflect the adjacent endocardial LA activity.

Adenosine Effect in Persistent AF

Patients with persistent AF demonstrated higher baseline DFs at each region compared with paroxysmal AF, but we found no spatiotemporal differences between mapped atrial sites. In this group, adenosine infusion increased local DFs only in the

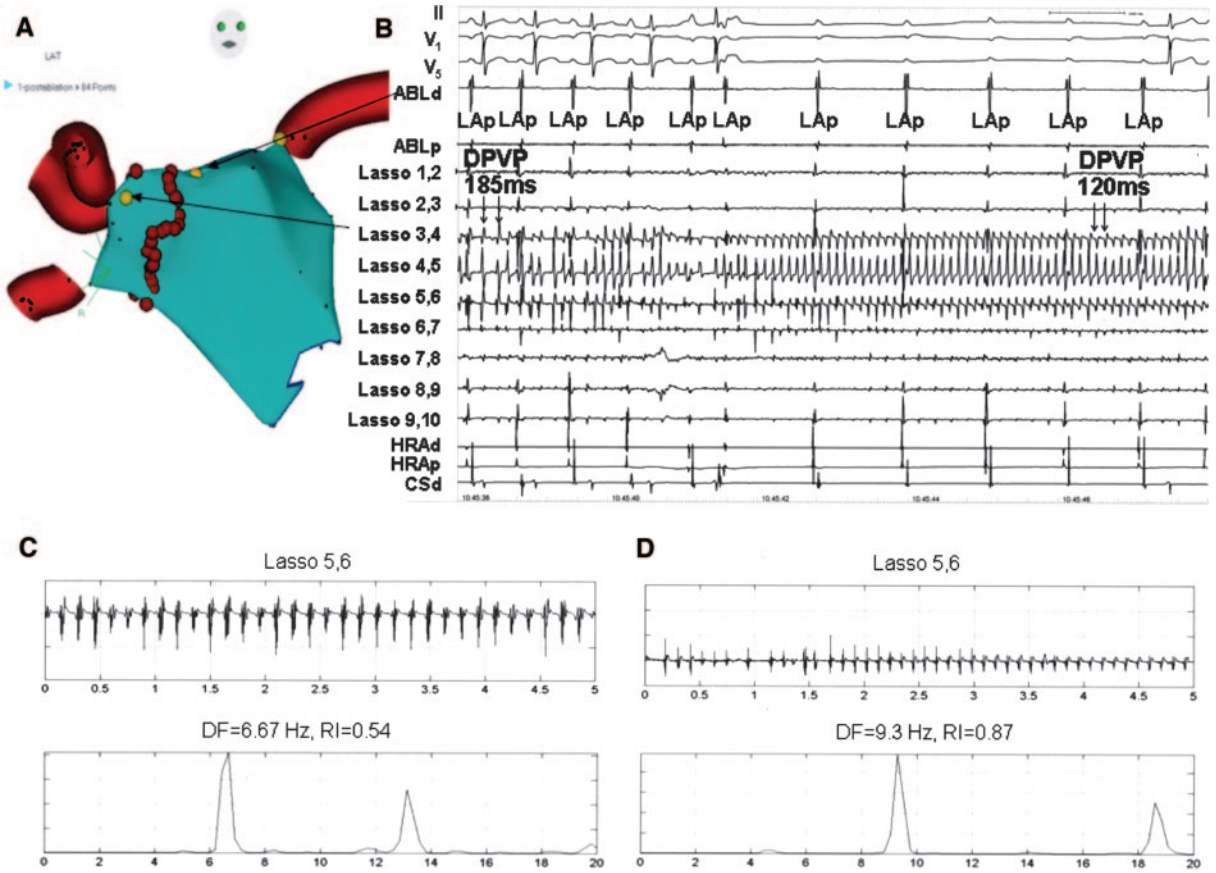

Figure 7. Rapid DPVPs after right superior pulmonary vein (RSPV) isolation and conversion to sinus rhythm. A, Right anterior oblique electroanatomic map projection. Red dots show ablation line; orange dot, ablation (Abl) catheter outside the encircled area; and yellow dot, 5,6 bipole position of a lasso catheter positioned inside the encircled area. B, Tracings during peak adenosine effect are ECG leads II, V_1, and V_5 and intracardiac electrograms recorded from lasso catheter within RSPV, ablation catheter outside the encircled area, CS catheter, and HRA catheter after isolation of right-sided PVs. Note that catheters recording outside the encircled area show stable sinus rhythm (LA potential; LAp); that DPVP recordings from a lasso catheter within the RSPV show AF with a mean cycle length of 185 ms; and that, during peak adenosine effect (complete AV block), there is a marked acceleration (mean cycle length, 120 ms) and regularization of RSPV local activity. Bottom, Effect of adenosine infusion on DPVP DF. C, Baseline lasso 5,6 bipole recordings and corresponding DF. D, DF increases during peak adenosine effect.

HRA, without significant changes at other atrial sites. The acceleration in the HRA suggests that persistent AF also is maintained by high-frequency reentrant sources; however, such sources could be located at non-PV sites. Our data are consistent with those of other investigators who have reported a change in spatiotemporal organization in persistent AF.[9,20,21] In contrast, Lazar et al[7] did not find that persistent AF rates were any faster than paroxysmal AF rates, but they also reported a loss in spatial gradient due predominantly to an increase in the DF of the RA. When high-density mapping techniques were used, however, a left-to-right gradient also was observed in persistent AF patients. Sahadevan et al[8] found the highest-frequency activity in the posterior LA in most patients with chronic AF undergoing intraoperative epicardial mapping, suggesting that the posterior LA may be the driving source maintaining chronic AF. Sanders et al,[9] however, found that patients with permanent AF were more likely to have high-DF sites localized to other non-PV regions of the atria, including the RA.

The different spatial response to adenosine in paroxysmal versus persistent AF patients could be due to substrate remodeling in persistent AF. Atrial myocytes adapt to a chronic high rate by downregulating $I_{K,ACh}$ to counteract the shortening of the atrial effective refractory period resulting from electrical remodeling.[22] $I_{K,ACh}$ downregulation is counteracted by an increase in I_{K1}, which plays an important role in stabilization and acceleration of functional reentry.[23] Upregulation of I_{K1} in patients with persistent AF results in higher AF frequencies at baseline compared with paroxysmal AF patients. Therefore, as demonstrated by our computer simulations, persistent AF–induced downregulation of $I_{K,ACh}$ channels could result in a significantly blunted response to adenosine. Whether $I_{K,ACh}$ channel downregulation is chamber specific and accounts for the observed differences between paroxysmal and persistent AF warrants further investigation.

Adenosine Effect on DPVPs

We documented a differential response to adenosine infusion of DPVPs that depended on their rate. As noted by Marrouche et al,[24] we observed that slow DPVPs were transiently abolished by adenosine, with complete recovery when the adenosine effect faded out, suggesting automaticity or trig-

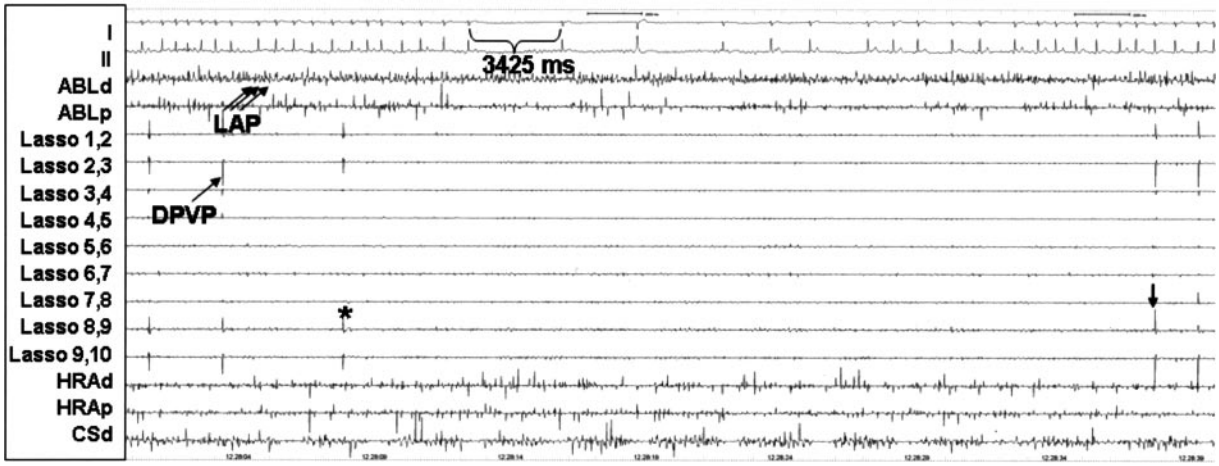

Figure 8. Effect of adenosine infusion on slow DPVPs after right superior pulmonary vein (RSPV) isolation. Tracings show from top to bottom ECG leads I and II and intracardiac electrograms recorded from ablation (Abl) catheter outside the isolated RSPV, lasso catheter within the RSPV, HRA catheter, and CS catheter. Catheters recording outside the isolated RSPV show AF (LAp); lasso catheter shows slow DPVP within the RSPV before adenosine infusion. During peak adenosine effect with marked RR interval, prolongation (3425 ms) causes DPVP transient suppression (*last DPVP before AV block) with subsequent resumption of DPVP activity (arrow). Slow DPVP activity reappears after adenosine effect disappearance. LAP indicates LA potential.

gered activity as the responsible mechanism. In contrast, rapid dissociated activity within the circumferential area isolating the PV from the atrial body responded to adenosine infusion with cycle length shortening and an increase in local frequency and regularity (Figure 7). It is therefore reasonable to surmise that, in contrast to the slow DPVPs, these rapid sources represent rotors confined to the isolated portion of the PV-LAJ.

Study Limitations

The present study has several limitations. First, we cannot rule out the existence of higher-frequency areas outside the mapped regions, especially in persistent AF patients. Second, the effect of sedation on autonomic tone could influence the results. No differences were observed, however, between patients under deep sedation state and those under mild conscious sedation (data not shown). Third, a significant proportion of the paroxysmal AF episodes were induced, as in other studies on mechanisms or ablation of AF.[7,13] Finally, the responses to adenosine infusion could vary, depending on specific location of the AF driver(s) and the sensitivity of the tissue harboring such drivers. The distribution of adenosine specific channels responsible for $I_{K,ACh}$ ($I_{K,Ado}$) is unknown. It is probably heterogeneous throughout the atria and PV region and could be affected by chronic AF remodeling. Furthermore, we must be cautious in interpreting the increase in frequency in certain areas because a shift in the location of the AF driver may occur upon adenosine administration as a result of the transition from automatic to reentrant mechanisms. Nevertheless, the fact that the most notable accelerations occurred in areas that were the fastest at baseline[7–9] and involved in AF termination suggests that the sources maintaining AF did not change. Moreover, it is likely that, in the case of functional reentry, a new AF driver will have a higher frequency than at baseline, whereas the opposite would be expected for the case of automaticity or triggered activity.

Sources of Funding

This work was supported in part by grants from the Spanish Society of Cardiology, Biosense Webster, and Guidant, Inc (F.A.); NIH grants PO1 HL039707, RO1 HL070074, and RO1 HL060843 (J.J.); and American Heart Association Scientist Development Grant 0230311N (O.B.).

Disclosures

O.B., J.J., and R.V. have licensing agreements with Biosense Webster.

References

1. Nattel S. New ideas about atrial fibrillation 50 years on. *Nature*. 2002; 415:219–226.
2. Jalife J. Rotors and spiral waves in atrial fibrillation. *J Cardiovasc Electrophysiol*. 2003;14:776–780.
3. Skanes AC, Mandapati R, Berenfeld O, Davidenko JM, Jalife J. Spatiotemporal periodicity during atrial fibrillation in the isolated sheep heart. *Circulation*. 1998;98:1236–1248.
4. Mandapati R, Skanes AC, Chen J, Berenfeld O, Jalife J. Stable microreentrant sources as a mechanism of atrial fibrillation in the isolated sheep heart. *Circulation*. 2000;101:194–199.
5. Berenfeld O, Mandapati R, Dixit S, Skanes AC, Chen J, Mansour M, Jalife J. Spatially distributed dominant excitation frequencies reveal hidden organization in atrial fibrillation in the Langendorff-perfused sheep heart. *J Cardiovasc Electrophysiol*. 2000;11:869–879.
6. Mansour M, Mandapati R, Berenfeld O, Chen J, Samie FH, Jalife J. Left-to-right gradient of atrial frequencies during acute atrial fibrillation in the isolated sheep heart. *Circulation*. 2001;103:2631–2636.
7. Lazar S, Dixit S, Marchlinski FE, Callans DJ, Gerstenfeld EP. Presence of left-to-right atrial frequency gradient in paroxysmal but not persistent atrial fibrillation in humans. *Circulation*. 2004;110:3181–3186.
8. Sahadevan J, Ryu K, Peltz L, Khrestian CM, Stewart RW, Markowitz AH, Waldo AL. Epicardial mapping of chronic atrial fibrillation in patients. *Circulation*. 2004;110:3293–3299.
9. Sanders P, Berenfeld O, Hocini M, Jaïs P, Vaidyanathan R, Hsu LF, Garrigue S, Takahashi Y, Rotter M, Sacher F, Scavée C, Ploutz-Snyder R, Jalife J, Haïssaguerre M. Spectral analysis identifies sites of high frequency activity maintaining atrial fibrillation in humans. *Circulation*. 2005;112:789–797.
10. Kabell G, Buchanan LV, Gibson JK, Belardinelli L. Effects of adenosine on atrial refractoriness and arrhythmias. *Cardiovasc Res*. 1994;28: 1385–1389.

11. Belardinelli L, Shryock JC, Song Y, Wang D, Srinivas M. Ionic basis of the electrophysiological actions of adenosine on cardiomyocytes. *FASEB J*. 1995;9:359–365.
12. Pappone C, Oreto G, Rosanio S, Vicedomini G, Tocchi M, Gugliotta F, Salvati A, Dicandia C, Calabro MP, Mazzone P, Ficarra E, Di Gioia C, Gulletta S, Nardi S, Santinelli V, Benussi S, Alfieri O. Atrial electroanatomic remodeling after circumferential radiofrequency pulmonary vein ablation: efficacy of an anatomic approach in a large cohort of patients with atrial fibrillation. *Circulation*. 2001;104:2539–2544.
13. Haissaguerre M, Sanders P, Hocini M, Hsu LF, Shah DC, Scavée C, Takahashi Y, Rotter M, Pasquié JL, Garrigue S, Clémenty J, Jaïs J. Changes in atrial fibrillation cycle length and inducibility during catheter ablation and their relation to outcome. *Circulation*. 2004;109:3007–3013.
14. Courtemanche M, Ramirez RJ, Nattel S. Ionic mechanisms underlying human atrial action potential properties: insights from a mathematical model. *Am J Physiol*. 1998;275(pt 2):H301–H321.
15. Sarmast F, Kolli A, Zaitsev A, Parisian K, Dhamoon AS, Guha PK, Warren M, Anumonwo JMB, Taffet SM, Berenfeld O, Jalife J. Cholinergic atrial fibrillation: I(k,Ach) gradients determine unequal left/right atrial frequencies and rotor dynamics. *Cardiovasc Res*. 2003;59:863–873.
16. Moe GK, Abildskov JA. Atrial fibrillation as a self-sustaining arrhythmia independent of focal discharges. *Am Heart J*. 1959;58:59–70.
17. Khositseth A, Clapham DE, Ackerman MJ. Intracellular signaling and regulation of cardiac ion channels. In: Zipes DP, Jalife J, eds. *Cardiac Electrophysiology: From Cell to Bedside*. 4th ed. Philadelphia, Pa: WB Saunders Co; 2004.
18. Berenfeld O, Zaitsev AV, Mironov SF, Pertsov AM, Jalife J. Frequency-dependent breakdown of wave propagation into fibrillatory conduction across the pectinate muscle network in the isolated sheep right atrium. *Circ Res*. 2002;90:1173–1180.
19. Jaïs P, Haissaguerre M, Shah DC, Chouairi S, Clémenty J. Regional disparities of endocardial atrial activation in paroxysmal atrial fibrillation. *Pacing Clin Electrophysiol*. 1996;19(pt II):1998–2003.
20. Akar JG, Everett TH, Kok LC, Moorman JR, Haines DE. Effect of electrical and structural remodeling on spatiotemporal organization in acute and persistent atrial fibrillation. *J Cardiovasc Electrophysiol*. 2002;13:1027–1034.
21. Sih HJ, Zipes DP, Berbari EJ, Adams DE, Olgin JE. Differences in organization between acute and chronic atrial fibrillation in dogs. *J Am Coll Cardiol*. 2000;36:924–931.
22. Dobrev D, Graf E, Wettwer E, Himmel HM, Hala O, Doerfel C, Christ T, Schuler S, Ravens U. Molecular basis of downregulation of G-protein-coupled inward rectifying K(+) current I(K,ACh) in chronic human atrial fibrillation: decrease in GIRK4 mRNA correlates with reduced I(K,ACh) and muscarinic receptor-mediated shortening of action potentials. *Circulation*. 2001;104:2551–2557.
23. Samie FH, Berenfeld O, Anumonwo J, Mironov SF, Udassi S, Beaumont J, Taffet S, Jalife J. Rectification of the background potassium current: a determinant of rotor dynamics in ventricular fibrillation. *Circ Res*. 2001;89:1216–1223.
24. Marrouche N, Wazni OM, Martin DO, Rossillo A, Saliba W, Erciyes D, Schweikert R, Khaykin Y, Burkhardt D, Bhargava M, Verma A, Abdul-Karim A, Natale A. Response to pharmacological challenge of dissociated pulmonary vein rhythm. *J Cardiovasc Electrophysiol*. 2005;16:122–126.

CLINICAL PERSPECTIVE

Radiofrequency ablation of high-frequency sites (drivers) results in atrial fibrillation (AF) termination in a significant proportion of patients, consistent with the role of such drivers in the maintenance of AF. However, it is unclear whether AF drivers in humans are focal (automatic or triggered) or reentrant. The answer to this question may lead to novel AF treatment strategies (either pharmacological or invasive). In the present study, we have determined the effects of adenosine infusion on local activation frequency at different sites of the fibrillating atria of patients and used computer simulations to help explain the results. Adenosine shortens refractoriness in the atrium, which accelerates some forms of reentry but suppresses automatic or triggered activity. As expected from a reentrant mechanism of AF drivers, adenosine infusion increased frequency primarily at sites that activated at the highest rate at baseline. In paroxysmal AF patients, adenosine increased activation frequency in the pulmonary vein–left atrial junction. In persistent AF patients, the highest-frequency sources accelerated by adenosine were located in either atria but not at pulmonary vein sites. Ablation of these high-frequency drivers terminated AF in a significant proportion of paroxysmal AF patients. Thus, the response to adenosine is consistent with reentrant drivers maintaining AF that have different locations in paroxysmal compared with persistent AF. Adenosine infusion may be useful to help identify reentrant drivers to guide AF ablation.

Activation of Inward Rectifier Potassium Channels Accelerates Atrial Fibrillation in Humans: Evidence for a Reentrant Mechanism

Felipe Atienza, Jesús Almendral, Javier Moreno, Ravi Vaidyanathan, Arkazdi Talkachou, Jérôme Kalifa, Angel Arenal, Julian P. Villacastín, Esteban G. Torrecilla, Ana Sánchez, Robert Ploutz-Snyder, José Jalife and Omer Berenfeld

Circulation. 2006;114:2434-2442; originally published online November 13, 2006;
doi: 10.1161/CIRCULATIONAHA.106.633735
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7322. Online ISSN: 1524-4539