Reinduction of Atrial Fibrillation Immediately After Termination of the Arrhythmia Is Mediated by Late Phase 3 Early Afterdepolarization–Induced Triggered Activity

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Background—Atrial fibrillation (AF) at times recurs immediately after termination of the arrhythmia. The mechanism(s) responsible for the extrasystole that reinduces AF is largely unknown. We hypothesized that abbreviation of action potential duration (APD) would permit very rapid rates of excitation, known to induce intracellular calcium loading, which in turn could promote delayed and/or early afterdepolarizations (EADs).

Methods and Results—Acetylcholine (ACh, 1 μmol/L) was used to abbreviate atrial APD and permit rapid-pacing induction of AF in isolated coronary-perfused canine right atria. Transmembrane action potentials, pseudo-ECG, and tension development were recorded. AF or rapid pacing was associated with an increase in tonic tension. Termination of AF or rapid pacing (cycle length, 150 to 80 ms) resulted in a dramatic rise of phasic tension, prolongation of repolarization of the initial beats at the regular rate (cycle length, 700 ms), and the development of late phase 3 EADs and extrasystoles. These extrasystoles initiated AF in 15 cases (involving 9 right atria) within the first 11 seconds after termination of AF or rapid pacing. This novel EAD mechanism is observed only in association with marked APD abbreviation. The calcium channel blocker nifedipine reduced, and the sarcoplasmic reticulum calcium release blocker ryanodine eliminated, the post–rapid pacing–induced increase in phasic tension, late phase 3 EADs, and extrasystoles that initiate AF.

Conclusions—These data suggest that calcium overload conditions present after termination of vagally mediated AF contribute to the development of late phase 3 EAD-induced triggered activity and that this mechanism may be responsible for the extrasystolic activity that reinitiates AF. (Circulation. 2003;107:2355-2360.)

Key Words: action potentials ■ fibrillation ■ calcium ■ triggered activity

A number of studies have documented the reinitiation of atrial fibrillation (AF) by an atrial extrasystole arising soon after termination of the arrhythmia.1–4 Although a reentrant mechanism is thought to underlie the maintenance of the arrhythmia, the mechanism(s) responsible for the premature beat(s) that reinduce AF is largely unknown. Immediate recurrence of AF (IRAF) was found to be unexpectedly high in patients with implanted atrial defibrillators, occurring after 27% of successful shocks.5 Patients who experience early AF recurrence after cardioversion have been shown to display briefer mean AF cycle lengths (CLs) than those who do not.6 In addition, acute treatment with the calcium channel blocker verapamil has been shown to reduce IRAF after electrical cardioversion in the clinic.4 These observations highlight the importance of rapid rates and calcium homeostasis in the early reinitiation of AF.

Like electrical remodeling,7 vagal influences are known to cause a marked abbreviation of atrial action potential duration (APD), permitting activation of atrial cells at much higher frequencies than otherwise possible. We hypothesized that immediately after termination of these rapid rates, intracellular calcium loading may promote delayed (DAD) and/or early (EAD) afterdepolarization activity. The present study was designed to test this hypothesis in a cholinergically mediated model of AF.

Methods

Arterially Perfused Whole Canine Right Atrium

Dogs (Martin Creek Kennels, Williford, Ark) weighing 20 to 25 kg were anticoagulated with heparin and anesthetized with pentobarbital (30 to 35 mg/kg IV). The chest was opened via a left thoracotomy, and the heart was excised, placed in a cardioplegic solution consisting of cold (4°C) Tyrode’s solution containing 8.5 mmol/L [K⁺], and transported to a dissection tray. Three-fourths of both ventricles were quickly removed. The ostium of the right coronary artery was cannulated with polyethylene tubing (ID, 1.75 mm; OD, 2.1 mm), and the preparation was perfused with cold Tyrode’s solution (12°C to 15°C) containing 8.5 mmol/L [K⁺]. With continuous coronary perfusion, all ventricular branches of the right coronary artery were immediately clamped with metal clips. The total time from excision of the heart to cannulation and perfusion of the artery was <4 minutes. The entire right atrium was carefully dissected from the remaining tissues, and then the preparation was unfolded. Ventricular right coronary branches and the cut atrial branches were ligated.
with silk thread. The preparation was placed in a temperature-controlled bath (8 × 6 × 3 cm) and perfused at a rate of 8 to 10 mL/min with Tyrode’s solution (36.5 ± 0.5°C). The perfusate was delivered to the artery by a roller pump (Cole Parmer Instrument Co). An air trap was used to avoid bubbles in the perfusion line. The composition of the Tyrode’s solution was (in mmol/L) NaCl 129, KCl 4, NaH2PO 0.9, NaHCO 20, CaCl 1.8, MgSO 0.5, and D-glucose 5.5, buffered with 95% O2 and 5% CO2.

Transmembrane action potential recordings were obtained with standard or floating glass microelectrodes (2.7 mol/L, KCl 10 to 25 MΩ; DC resistance) connected to a high-input impedance amplification system (World Precision Instruments). The signals were displayed on oscilloscopes, amplified, digitized, and analyzed (Cambridge Electronic Design) and stored on computer hard drive or compact disk. Action potentials were recorded throughout the right atrial preparation.

An ECG (pseudo-ECG) was recorded with 2 electrodes consisting of AgCl half-cells attached to Tyrode’s-filled tapered polyethylene electrodes that were placed in the bath solution 1.0 to 1.2 cm from the opposite ends of the atrial preparation.

Isometric contractile force was recorded by attaching the intercalary band to a force-displacement transducer (Grass Instruments Co).

**Drugs**

Acetylcholine (ACh) and ryanodine (both dissolved in distilled water) and nifedipine (dissolved in 100% alcohol) were prepared fresh as a stock of 1 mmol/L before each experiment.

**Experimental Protocols**

Coronary-perfused, spontaneously beating atrial preparations were equilibrated in the tissue bath until electrically stable, usually 30 minutes, and then paced at a basic CL of 700 ms with a pair of thin silver electrodes insulated except at their tips. Stimuli were bipolar rectangular pulses of 2 ms duration and twice threshold intensity. Four sets of experiments were performed; the same preparation was usually used for 2 or 3 of these. In the first set, the fastest possible pacing rate was determined before and after addition of ACh (1 μmol/L). In a second experimental series, AF was induced by a single premature impulse or rapid pacing protocol (CL of 50 to 100 ms for 5 to 20 seconds) in the presence of ACh (1 μmol/L). In a third set, we examined the effects of a 10-second period of rapid pacing (CL, 300 to 80 ms) on electrical and mechanical activity under control conditions and in the presence of ACh (1 μmol/L). In a fourth experimental series, we compared mechanical and electrical activity of the atrial preparation in the presence of ACh (1 μmol/L) alone and 15 minutes after the addition of the L-type calcium channel blocker nifedipine (1 μmol/L) or the sarcoplasmic reticulum (SR) calcium release blocker ryanodine (1 μmol/L). At a minimum, we allowed a 2-minute period of pacing at a normal rate (CL, 700 ms) between periods of rapid pacing.

**Statistics**

Statistical analysis was performed with a Student’s t test for paired or unpaired data and ANOVA followed by Bonferroni’s test, as appropriate. All data are expressed as mean±SD. A value of P < 0.05 was considered significant.

**Results**

ACh dramatically abbreviated atrial repolarization (APD90 from 196±11 to 36±7 ms at a CL of 700 ms, n = 10, P < 0.001), permitting very rapid pacing of the preparation. The shortest possible pacing CL was much briefer after ACh than in control (63±8 versus 152±11 ms, respectively, P < 0.001, n = 10).

AF could be induced in 100% of atria in the presence of ACh (n = 42). AF was persistent in 20 right atria. Seventy-five episodes of self-termination of AF were observed in 22 preparations. The duration of these self-terminated AFs was always <1 minute. An increase of tonic tension was observed during episodes of AF, and termination of AF resulted in a transient dramatic increase in phasic tension during the initial postfibrillatory beats, suggesting a significant increase of intracellular calcium loading during this period (Figure 1A and B).

Spontaneous IRAF was observed in 9 cases involving 5 different preparations (Figure 1B), precipitated by an extrasystole arising within 5.2±3.6 seconds (range, 1 to 11 seconds) after spontaneous termination of the arrhythmia. Extrasystoles that failed to reinitiate AF were recorded in 4 additional preparations. The average duration of the self-terminating AF leading to IRAF was longer than that unaccompanied by IRAF (19±12 versus 9±8 seconds, n = 9 and 66, respectively, P < 0.05).

Because of the intense contractions that accompanied the first few beats after termination of AF, it was very difficult to maintain a microelectrode impalement. However, in 7 cases in which we were able to record transmembrane action potentials at the moment of spontaneous termination of AF, we observed a significant prolongation of the terminal phase of repolarization of the atrial action potential, which in 2 cases was observed to take the form of late phase 3 EADs (Figure 1C).

The unpredictability of the timing of AF termination hampered our ability to evaluate the mechanism(s) involved. Thus, to further assess the cellular mechanism(s) responsible
for the reinduction of AF, we mimicked AF and its termination in 17 preparations by repeatedly pacing the atria for a period of 10 seconds at a CL as short as 80 ms.

In controls, phasic tension increased as CL was abbreviated to intervals of 200 to 300 ms and was particularly intensified after the development of mechanical alternans (CL, 200 ms; Figure 2B). At more rapid rates (CL, 150 ms), developed tension was reduced (Figure 2A). In the presence of ACh, phasic tension was practically eliminated. A rise in tension was observed at rapid rates (Figure 2A). To further characterize intracellular calcium loading, we measured post–rapid pacing tension development (Figure 2C). Termination of rapid pacing produced a dramatic transient increase in tension development in first few post–rapid pacing beats, both in controls and in the presence of ACh. Note, however, that in the presence of ACh, post–rapid pacing tension increased dramatically only at very rapid rates, not achievable in controls. The amplitude of the post–rapid pacing contraction was clearly rate dependent, with higher contractility manifest at faster rates (Figure 2C).

The data thus far presented clearly indicate significant cellular calcium loading immediately after termination of rapid pacing, which may also have electrical correlates, as previously demonstrated after termination of AF. Repolarization was prolonged significantly in the first few beats after termination of rapid atrial pacing both in the absence and in the presence of ACh (Figure 3). Transient post–rapid pacing APD prolongation was observed in all regions of the right atrial preparation. Under control conditions, both APD50 and APD95 were prolonged, whereas in the presence of ACh, only APD95 was prolonged (Figures 4 and 6); APD50 was abbreviated in the post–rapid pacing beats, resulting in the development of an EAD-like deflection. APD prolongation of the postpacing responses increased as a function of the pacing rate (Figure 3).

Late phase 3 EAD-induced upstrokes (triggered responses) were observed in 9 cases (6 different atrial preparations) immediately after termination of pacing in the presence of ACh but never in control. EAD-induced triggered activity displayed takeoff potentials ranging between −60 and −75 mV. Figure 4 illustrates an example of an EAD (A) and an EAD-induced triggered beat initiating a short run of AF (B).

Such activity was observed only with pacing CLs ≤ 150 ms. Postpacing extrasystoles were recorded with intracellular microelectrodes and/or ECGs in 6 of 17 atrial preparations exposed to ACh but never in controls. Extrasystolic activity arising under these conditions induced AF in 6 cases (4 different atrial preparations) within 2.2 ± 1.6 seconds (range, 1 to 5 seconds) after termination of very rapid pacing (CL, 100 or 80 ms). Postpause tension amplitude tended to be larger in preparations that developed AF after termination of rapid pacing (n = 4) compared with those that did not (n = 13) (23 ± 7 versus 17 ± 6 g; P = NS, for the first postpause regular beat after a pacing CL of 100 ms).

The duration of the QRS complex of the basic beats (CL, 700 ms) before and immediately after rapid pacing was similar (52 ± 10 versus 54 ± 9 ms, respectively; P = NS; n = 6; rapid pacing CL, 100 ms; ACh, 1 μmol/L).

In the continued presence of ACh (1 μmol/L), the addition of nifedipine (1 μmol/L) reduced (Figure 5, A and B) and ryanodine (1 μmol/L) practically eliminated (Figure 5, C and D) post–rapid pacing augmentation of tension development and prolongation of APD. Distinct EADs, triggered activity, and AF were not observed in the presence of nifedipine (in 5 right atria) or ryanodine (in 5 right atria).

![Figure 2](image-url). Developed tension during and after rapid pacing under control conditions in presence of ACh (1 μmol/L). A, Developed tension recordings and corresponding pacing protocols. B, Summary data for phasic tension recorded during rapid pacing protocol in presence (open circles) and absence (closed circles) of ACh. C, Summary data for phasic tension associated with first beat after termination of rapid pacing rate. Statistical analysis was not performed for control data in B because of development of mechanical alternans. n = 10 for each point. *P < 0.05 vs steady-state value at a CL of 700 ms.

![Figure 3](image-url). Rapid pacing produces a transient prolongation of APD in postpacing period. Steady-state CL, 700 ms. APD50 and APD90 of first post–rapid pacing beat are plotted as a function of preceding pacing CL in presence (open circles) and absence (closed circles) of ACh. *P < 0.05 vs steady-state value at CL of 700 ms. n = 6 to 10.
Discussion
IRAF after electrical cardioversion has been reported in a sizable fraction of patients (13% to 27%). The IRAF is caused by an atrial extrasystole, the mechanism of development of which is largely unknown. In the present study, we have developed an experimental model that recapitulates these clinical phenomena, permitting evaluation of the cellular mechanisms involved. The model consists of a coronary-perfused canine right atrial preparation in which cholinergically mediated AF can be readily induced by rapid pacing and in which transmembrane, ECG, and mechanical data can be correlated.

Our study provides evidence in support of the hypothesis that very rapid rates of excitation, as occur during AF, lead to an elevation of intracellular calcium, which in turn can contribute to the development of EAD-induced extrasystoles capable of reinitiating AF soon after termination of the arrhythmia.

The mechanism responsible for the transient increase in contractility immediately after the transition from rapid to normal rates is fairly well established. Rapid activation rates result in an increase of intracellular Na\(^+\), leading to cellular calcium loading mediated by Na/Ca exchange (generating an outward current). A transient period of hypercontractility occurs immediately after return to normal rates because of augmented SR calcium loading and release. The strong SR calcium release at the slow rates then stimulates extrusion of the calcium through Na/Ca exchanger, explaining the transient nature of hypercontractility. The electrogenic inward current generated by the exchanger is most likely responsible for the transient APD prolongation and EAD development.

Support for this hypothesis derives from the following observations: (1) increased tonic tension during AF, indicating an elevation of cytosolic calcium (phasic tension remains low during AF because of the lack of sufficient time for the SR to load); (2) transient appearance of strong phasic contractions immediately after slowing of rate; (3) concomitant appearance of EADs or APD prolongation; and (4) disappearance of electrical and mechanical sequelae in the presence of ryanodine. The relatively small effect of nifedipine to reduce the electrical and mechanical changes is also consistent with the notion that calcium loading is primarily through Na/Ca exchange, with L-type calcium channels playing a lesser role. The absence of extrasystoles in the presence of nifedipine may also be a result of a direct effect of the drug to suppress the upstroke of the premature beat. This finding is consistent with the observation that acute treatment with the calcium channel blocker verapamil reduces IRAF after electrical cardioversion of persistent AF in patients.
Dramatic APD abbreviation, very rapid rates of excitation, and apparently strong SR calcium release were needed to elicit EADs in the initial period after termination of AF or rapid pacing. Moreover, EADs developed as a result of the interruption of the final phase of repolarization of the action potential (late phase 3). These characteristics of the EAD are distinctly different from those generally encountered and represent a hybrid between the EAD and DAD activity normally encountered in ventricular myocardium. To distinguish these EADs from those commonly encountered in the presence of agents with class III antiarrhythmic action, we call these “late phase 3 EADs.”

Although strong post–rapid pacing contractions are recorded both in controls and in the presence of ACh, post–rapid pacing EAD is observed only after ACh. A possible explanation for how and why ACh-induced abbreviation of APD contributes to the development of late phase 3 EADs and triggered activity is summarized in Figure 6. As previously discussed, transient APD prolongation, EADs, and extrasystoles occurred immediately after termination of AF or rapid pacing seem to be secondary to accentuated SR calcium release. Based on the time course of contraction, levels of intracellular calcium (Ca) would be expected to peak during the plateau of the action potential (membrane potential of approximately −5 mV) under control conditions but during the late phase of repolarization (membrane potential of approximately −70 mV) in the presence of ACh. As a consequence, the 2 principal calcium-mediated currents, $I_{\text{Na-Ca}}$ and $I_{\text{Cl(Ca)}}$, would be expected to be weakly inward or even outward ($I_{\text{Cl(Ca)}}$ when APD is normal (control) but strongly inward when APD is very short (ACh). Thus, abbreviation of the atrial APD allows for a much stronger recruitment of both $I_{\text{Na-Ca}}$ and $I_{\text{Cl(Ca)}}$ in the generation of late phase 3 EADs. It is noteworthy that the proposed mechanism is similar to that thought to underlie the development of DADs, the principal difference being that in the case of DADs, $I_{\text{Na-Ca}}$ and $I_{\text{Cl(Ca)}}$ are recruited secondary to a spontaneous release of calcium from the SR, whereas in the case of late phase 3 EADs, these currents are accentuated as a consequence of the normal SR release mechanisms.

Other currents may contribute to the generation of the late phase 3 EAD. Prominent among them are the stretch-activated currents. Strong phasic contraction after the first few post-AF beats is likely to cause a stretch, known to induce premature beats and promote AF. Stretch has been shown to prolong APD and induce triggered beats during late phase 3 or phase 4 but not during phase 2 of the action potential. This property of stretch may also explain the presence of EAD at very short (ACh) but not normal (control) duration of APD (Figure 6).

Although cellular calcium overload plays a determining role, it is clearly not the only factor responsible for IRAF. Indeed, IRAF could be observed up to 11 seconds after the termination of AF, even though the largest tension development occurred after the first post-AF regular beat.

Our findings demonstrate a novel mechanism in which the development of EADs is strictly dependent on the presence of a marked abbreviation of APD. APD abbreviation is traditionally considered to lead to the development of an arrhyth-
References

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