Electrical Remodeling in Fibrillating Canine Atrium

Action Potential Alternans During Rapid Atrial Pacing and Late Phase 3 Early Afterdepolarization After Cessation of Rapid Atrial Pacing

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Summary

Sustained atrial fibrillation (AF) was induced by atrial burst pacing, and monophasic action potentials (MAPs) were recorded. MAP alternans was observed at a cycle length (CL) of 167.5 ± 28.2 msec before burst pacing and 201.3 ± 40.2 msec after burst pacing. AF > 5 minutes duration was induced in 1 dog in the control condition but in all 8 dogs after burst pacing. The difference in RA MAPD80 of the first spontaneous beat and steady-state sinus rhythm was significantly larger after atrial burst pacing than before atrial burst pacing (31.5 ± 15.9 msec versus 8.2 ± 9.0 msec). In 4 dogs, late phase 3 early afterdepolarization was observed after rapid atrial pacing. Rapid atrial pacing-induced electrical remodeling includes APD alternans during rapid atrial pacing and also causes an increase in the MAPD of the initial several beats and the development of late phase 3 early afterdepolarizations after a sudden increase in CL. (Int Heart J 2010; 51: 354-358)

Key words: Atrial fibrillation, Action potential duration, Alternans, Early phase 3 afterdepolarization, Electrical remodeling

Both rapid atrial pacing and atrial fibrillation (AF) change the electrophysiologic characteristics of the atria.1,2,3 Electrical remodeling is thought to promote the inducibility and persistence of AF. Thus, AF can become sustained in patients having lone paroxysmal AF.4 In chronically instrumented goats in which AF is artificially maintained, the fibrillation itself markedly shortens the atrial refractory period (atrial electrical remodeling) and increases the inducibility and stability of further AF.5 In addition, rate adaptation of the atrial refractory period is markedly reduced or reversed.6,7 Thus, as noted by Wijffels et al.,8,9,“AF begets AF.” A previous paper demonstrated that rapid atrial pacing induced action potential changes are induced by ionic channel remodeling.10-12 The changes in intracellular Ca2+ handling by rapid atrial pacing might also influence atrial action potentials. Recent in vitro studies have shown that rapid atrial pacing induces early and/ or delayed afterdepolarization and dynamic changes in action potential duration (APD).13-15 However, to the best of our knowledge, there has been no in vivo study regarding atrial action potential dynamics before and after induction of AF by rapid atrial pacing. Therefore, we recorded contact monophasic action potentials (MAPs) to determine the changes in static and dynamic atrial action potential characteristics in dogs in which rapid atrial pacing was imposed for several weeks to induce sustained AF.

Methods

Animal preparation: The care of all animals used in the study conformed to the position of the American Heart Association on the use of research animals in accordance with accepted guidelines for the care and treatment of experimental animals at Nihon University School of Medicine. Eight mongrel dogs weighing 15-22 kg (19.4 ± 3.9 kg) were used. Dogs were immobilized with ketamine (15 mg/kg, i.m.) and anesthetized with pentobarbital sodium (25 mg/kg, i.v.). Anesthesia was maintained with pentobarbital sodium (100 mg) as needed. A surface lead II electrocardiogram was continuously monitored during the experiment. The dogs were then intubated and placed on a volume controlled animal ventilator (Model 613, Harvard Apparatus, South Natick, MA, USA). Intravenous Ringer’s solution was infused as needed to replace lost fluid through a 6F sheath placed in the left femoral vein. Complete atrioventricular block was created by radiofrequency catheter ablation with a 7F, 4-mm-tip catheter electrode (EP Technologies, Inc., San Jose, CA, USA) advanced from the right femoral vein to the atrioventricular junction under fluoroscopic guidance. An RFG-3E RF generator (Radionics, Inc., Wetteren, Belgium) was used for ablation. In a sterile manner, right ventricular pacing was performed by implanting a single chamber pacemaker placed in a left cervical submuscular pocket and a screw-in pacing lead (Medtronic, Inc., Minneapolis, MN, USA) was placed in the right ventricular apex via the left external jugular vein. Ventricular pacing was programmed as follows: rate, 60 beats/minute; pulse amplitude, 3.0-5.0 V;
pulse width, 0.35-0.5 msec; sensitivity, 2.5 V; refractory period, 300 msec. Another screw-in pacing lead (Medtronic, Inc.) was placed in the right atrial appendage via the right external jugular vein and connected to a high frequency pacemaker (Inrepr II, Medtronic) that was placed in a right cervical submuscular pocket. After the incision was closed and the dogs awakened from anesthesia, they were maintained for 2 days in the recovery room before being moved to routine care. The dogs were given cefazoline prophylactically at 25 mg/kg, i.m. once before surgery and for 2 days after surgery. They were allowed to recover for 1 week. After recovery, intermittent right atrial (RA) burst pacing was performed (rate, 50 Hz; amplitude, 6-8 V; pulse width; 0.45 ms with a 1.5 second on-1.0 second off duty cycle). An electrocardiogram was recorded at 4 weeks after the start of atrial burst stimulation and every week thereafter, and sustained AF was defined as AF lasting > 2 hours after the pacemaker was switched off.

Experimental protocols: Electrophysiologic studies were performed before the start of atrial burst pacing (control condition) and 30 minutes after cardioversion of sustained AF under a closed-chest condition. Ventricular pacing was stopped during electrophysiologic study. At the time of study, dogs were anesthetized with pentobarbital sodium (25 mg/kg, i.v.), and atropine (0.04 mg/kg, i.v.) and propranolol (0.2 mg/kg, i.v.) were administered for total autonomic block. Under fluoroscopic guidance, a Franz combination catheter (EP Technologies) was placed in the high lateral right atrium for MAP recording, and a quadripolar catheter electrode was placed adjacent to the Franz catheter for pacing. MAPs were recorded at a filter setting of 0.05-500 Hz, and monophasic action potential duration (MAPD) at 80% repolarization (MAPD<sub>80</sub>) was measured. Right atrial pacing was performed at twice the late diastolic threshold and a pulse width of 2 msec. Atrial pacing was started at a cycle length slightly shorter than the sinus rhythm for 120 beats, and the cycle length was decreased by 20 ms after a 10 second pause until 2:1 atrial capture occurred or AF developed. If AF persisted > 5 minutes, external direct current cardioversion was performed starting from 30 W, and increased by 20 W until sinus rhythm was restored. In 2 dogs, an octapolar electrode catheter was placed in the coronary sinus, and intraatrial conduction time (from the pacing spike to recording of the distal coronary sinus electrogram) was measured before and after atrial burst pacing. MAPD alternans was defined as beat-to-beat changes in MAPD<sub>80</sub> > 20 ms lasting more than 10 beats.

Statistical analysis: Values are expressed as the mean ± SD. Data were analyzed using the Wilcoxon signed-rank test. Fisher’s exact probability test was used for contingency comparisons. A P value of < 0.05 was considered statistically significant. StatView 5.0 software (SAS Institute, Cary, NC, USA) was used for data analysis.

**RESULTS**

Atrial burst pacing was continued for 6.9 ± 1.5 weeks (4-9 weeks) for induction of sustained AF. **Rate-dependent changes in steady-state atrial electrophysiology:** Before burst pacing, the longest cycle length at which 2:1 atrial capture occurred was 132.9 ± 25.0 ms, but after burst pacing, the longest cycle length at which 2:1 atrial capture occurred could not be measured because of the sustained AF. The mean RA MAPD<sub>80</sub> values at pacing cycle lengths of 400 ms and 200 ms before and after atrial burst pacing are shown in Table I. RA MAPD<sub>80</sub> at a pacing cycle length of 400 ms was significantly shorter after (versus before) atrial burst pacing, but RA MAPD<sub>80</sub> at a pacing cycle length of 200 ms did not change significantly.

<table>
<thead>
<tr>
<th></th>
<th>CL 400 msec</th>
<th>CL 200 msec</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>149.4 ± 11.2 (n = 7)</td>
<td>128.4 ± 10.6 (n = 7)</td>
<td>0.043</td>
</tr>
<tr>
<td>After atrial burst pacing</td>
<td>133.2 ± 14.7 (n = 8)</td>
<td>128.4 ± 14.7 (n = 8)</td>
<td>0.345</td>
</tr>
<tr>
<td>P</td>
<td>0.043</td>
<td>0.500</td>
<td></td>
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RA MAPD indicates right atrial monophasic action potential duration; CL, cycle length; Control, control condition before atrial burst pacing; and AF, atrial fibrillation.

**Figure 1.** Rate-dependent changes in RA MAPD after 50 days of atrial burst pacing to induce atrial fibrillation. RA MAPD indicates right atrial monophasic action potential duration. Note that macroscopic RA MAPD alternans appeared at a pacing cycle length of 200 msec.

**Figure 2.** Induction of atrial fibrillation by rapid atrial pacing (atrial burst pacing for 29 days) at a pacing cycle length of 200 msec. RA MAPD indicates right atrial monophasic action potential. Note that atrial fibrillation was preceded by RA MAPD alternans.
MAP indicates monophasic action potential; Control, control condition before atrial burst pacing; and AF, atrial fibrillation.

Table II. Pacing Cycle Length Associated With Atrial MAP Alternans

<table>
<thead>
<tr>
<th>Dog</th>
<th>Control</th>
<th>AF</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>200, 180, 160, 140 → AF</td>
<td>220, 200 → AF</td>
</tr>
<tr>
<td>2</td>
<td>160, 140, 120, 100 → AF</td>
<td>160, 140 → AF</td>
</tr>
<tr>
<td>3</td>
<td>140, 140, 120, 100 → AF</td>
<td>140, 140, 120, 100 → AF</td>
</tr>
<tr>
<td>4</td>
<td>200, 180, 160, 140, 120 → AF</td>
<td>200, 180, 160, 200 → AF</td>
</tr>
<tr>
<td>5</td>
<td>140, 130 → AF</td>
<td>140, 130 → AF</td>
</tr>
<tr>
<td>6</td>
<td>150 → AF</td>
<td>140, 120 → AF</td>
</tr>
<tr>
<td>7</td>
<td>140, 120 → AF</td>
<td>140, 120 → AF</td>
</tr>
<tr>
<td>8</td>
<td>140, 120 → AF</td>
<td>140, 120 → AF</td>
</tr>
</tbody>
</table>

Note that RA MAPD of the initial sinus beat was 25 msec longer than that of the 4th beat in the control condition, but that RA MAPD of the initial sinus beat was 6 msec longer than that of the 4th beat after atrial burst pacing.

Figure 3. Dynamic changes in RA MAPD after cessation of rapid atrial pacing before and after atrial burst pacing. RA MAPD indicates right atrial monophasic action potential duration and CL, cycle length. Left panel: Note that RA MAPD during sinus rhythm was shorter after the longer atrial cycle. Right panel: Note that RA MAPD of the initial sinus beat was 6 msec longer than that of the 4th beat in the control condition, but that RA MAPD of the initial sinus beat was 25 msec longer than that of the 4th beat after atrial burst pacing.

MAPD at a pacing cycle length of 200 msec was similar before and after rapid burst pacing. RA MAPD values at pacing cycle lengths of 400 ms and 200 ms after atrial burst pacing did not differ significantly. For conditions before and after atrial burst pacing, RA MAPD, at a pacing cycle length of 400 msec was subtracted from MAPD at a pacing cycle length of 200 msec. The differences were 21.0 ± 8.1 msec and 4.8 ± 10.0 msec, respectively (P = 0.003). These results demonstrate a decrease in rate adaptation of the atrial action potential is mainly due to decreased atrial action potential duration at longer atrial cycle length. Cycle length-dependent changes in intraatrial conduction time did not differ between the control condition and the experimental condition (ie, after atrial burst pacing).

RA MAPD alternans: The longest pacing cycle length at which MAPD alternans began was 167.5 ± 28.2 msec in the control condition and 201.3 ± 40.2 msec after atrial burst pacing (P = 0.0173) (Figure 1). MAPD alternans was observed mainly at the phase 3 of action potentials as shown in Figures 1 and 2 and action potential amplitude alternans was not obvious. AF lasting > 5 minutes was induced in 1 dog (12%) in the control condition at a pacing cycle length of 140 msec and in all 8 dogs after atrial burst pacing (100%) at a mean pacing cycle length of 148.8 ± 27.5 msec (P = 0.0007) (Table II). Initiation of AF by rapid atrial pacing was always preceded by RA MAPD alternans (Figure 2). The extent of alternans differed depending on the pacing cycle length, and alternans of the APD before degenerating into AF showed not only a 1:1 pattern but also a 2:1 pattern. We did not conduct quantitative analysis of the extent of alternans. Thus, the development of alternans of the APD at longer cycle length may play an important role in the development and maintenance of AF.

Figure 4. Dynamic changes in right atrial monophasic action potential (RA MAP) after cessation of rapid atrial pacing (after atrial burst pacing for 120 beats). RA MAPD indicates RA MAP duration; CL, cycle length. Left panel: RA MAP during sinus rhythm. Right panel: Late phase 3 afterdepolarization (hump morphology) (arrows) appeared during the initial several spontaneous beats after cessation of rapid atrial pacing.

The shortest pacing cycle length showing 1:1 atrial capture without development of AF was 153.3 ± 29.4 ms in the control condition and 180.0 ± 33.5 ms after atrial burst pacing (P = 0.154). The right atrium was paced for 120 beats at the shortest pacing cycle length showing 1:1 atrial conduction without inducing AF (Table II). The AA interval from the last paced beat of the shortest pacing cycle length to the initial spontaneous beat was 476.0 ± 101.4 ms in the control condition (145.7 ± 14.6 ms, P = 0.0585) even though the AA interval was significantly longer after atrial burst pacing compared to the control condition (516.8 ± 71.7 ms versus 422.2 ± 27.4 ms, P = 0.035).

The shortest pacing cycle length showing 1:1 atrial capture after degenerating into AF was 133.5 ± 20.4 msec which was marginally shorter than RA MAPD after sinus rhythm in the control condition (145.7 ± 14.6 ms, P = 0.0585) even though the AA interval was significantly larger after atrial burst pacing compared to the control condition (516.8 ± 71.7 ms versus 422.2 ± 27.4 ms, P = 0.035).

Late phase 3 early afterdepolarization: There was no early or
late afterdepolarization in the RA MAP after rapid atrial pacing in the control condition, while in 4 of the 8 dogs, late phase 3 early afterdepolarization (hump morphology) was noted within several beats after cessation of rapid atrial pacing (Figures 4 and 5). Thus, atrial burst pacing-induced AF not only affected steady-state atrial APD, but also dynamic changes in atrial APD after rapid atrial pacing.

**DISCUSSION**

**Major findings:** We found that after cardioversion of sustained AF, rate adaptation of atrial APD was reduced. The change in atrial APD after abrupt termination of pacing was altered after cardioversion of sustained AF, ie., when the cardiac cycle was prolonged, the APD was also prolonged at the first beat, and then shortened during several subsequent beats.

**Steady-state changes in APD after cardioversion of sustained AF:** Like others using atrial pacing-induced animal models, we found that rate adaptation of steady-state atrial APDs is abnormal after cardioversion of sustained AF. Wijffels, et al. found that whereas normal goats in sinus rhythm showed a clear shortening of the atrial refractory period at a relatively short cycle length, goats in which AF had been artificially maintained lost this physiological adaptation and showed either a constant refractory period despite the change in cycle length or an inverse adaptation. However, we previously reported that APD alternans occurs during rapid atrial pacing, ie., after 60 minutes of atrial burst pacing; thus, the atrial refractory period changes according to the number of basic atrial stimuli, so APD alternans might be a cause of inverse adaptation of refractory periods after long-term atrial burst pacing.

The longest cycle at which APD alternans appeared was significantly longer after atrial burst pacing than before burst pacing, and induction of sustained AF was always preceded by APD alternans. Previous experimental studies have shown that AF initiation is associated with action potential alternans in pacing-induced AF and myocardial infarction models. Previous clinical studies also uncovered alternans of atrial action potentials as an imminent precursor of AF.

**Nonsteady-state changes in APD in canine models of sustained AF:** Previous *in vitro* studies showed right atrial nonsteady-state action potential potential changes after an abrupt decrease in pacing cycle length. We described the time course of change in fibrillating atria after an abrupt change in cycle length, ie, when the cardiac cycle was prolonged, the APD was also prolonged at the first beat and then shortened during several subsequent beats. Burashnikov, et al. reported that termination of AF induced by rapid pacing resulted in a dramatic rise in phasic tension, prolonged repolarization of the initial beats at regular rate, and the development of late phase 3 early afterdepolarizations and extrasystoles initiating AF in acetycholine-perfused canine atria. In the present study, the mechanisms underlying the initial prolongation of APD late phase 3 early afterdepolarizations were not elucidated, but previous *in vitro* studies showed that these changes were reduced or abolished by the calcium channel blocker nifedipine and sarcoplasmic reticulum calcium release blocker ryanodine.

Our *in vivo* findings are consistent with the previously reported *in vitro* studies, but AF was not initiated during late phase 3 early afterdepolarization activity. Previous studies in acetylcholine + norepinephrine-treated atrial cells and atrial cardiomyocytes subjected to long-term rapid atrial pacing revealed that the pulmonary vein (PV) sleeve showed an increased prevalence of both early and delayed afterdepolarizations and triggered firing. However, Stambler, et al. reported that atrial cells from dogs with rapid ventricular pacing-induced heart failure showed only prolonged APDs and delayed afterdepolarizations. We recorded atrial MAPs only from the right atrium; therefore, the action potential recording site and method used to induce AF might influence the results. Late phase 3 early afterdepolarizations observed after rapid atrial pacing did not result in early recurrence of AF in this experiments. Therefore, the pathogenic role of the late phase 3 early afterdepolarizations still remains to be elucidated in *in-situ* experiments using different type animal models for AF. On the other hand, Dytschaever, et al. reported that the atrial refractory period after 5 minutes of paroxysm of AF resulted in an ultrashort value of the atrial refractory period after 48 hours of electrically maintained AF compared to the control condition. Thus, the dispersion of atrial refractoriness due to shortening of the APD, heterogeneity of its distribution, and development of late phase 3 early afterdepolarizations might contribute to the pathogenesis of immediate recurrence of AF after termination of AF.

**Study limitations:** The present study has the following limitations. The right ventricle was paced at 60 beats/minute for 1 week in the control condition and for an additional 4-9 weeks during atrial burst pacing. We did not compare RA electrophysiologic changes after 4-9 week right ventricular pacing without atrial burst pacing. Thus, the effects of long-term right ventricular pacing might affect the electrophysiologic changes in RA after atrial burst pacing because we did not measure RA
pressure during sinus rhythm and during right ventricular pacing at different pacing rates. However, Nattel and coworkers used a right ventricular pacing rate of 80/min in their series of similar experiments.22 Our results may not show large differences from previous reports. We conducted experiments after induction of sustained AF by atrial burst pacing of 4-9 weeks. Thus, the extent of atrial electrical remodeling might differ between 4 weeks and 9 weeks. The sites of MAP recording in the control condition and after atrial burst pacing were not exactly the same because the recording sites were determined by fluoroscopic guidance in closed-chest dogs. A previous study revealed that atrial tachycardia causes nonuniform remodeling of atrial refractoriness, which plays an important role in increasing atrial vulnerability to AF induction and the duration of induced AF.23 MAPs were obtained from only one site (the RA) and not from the pulmonary vein area in this study. As revealed in experiments22,23,11 and clinical22 studies, the PV sleeve is the most common site for origination of atrial premature beats triggering AF. Recording MAPs from the PV sleeve might be necessary.

Conclusion: Rapid atrial pacing-induced electrical remodeling includes not only shortening of the APD and reduced rate adaptation of APD, but also APD alternans during rapid atrial pacing and an increase in the APD of initial beats and the development of late phase 3 early afterdepolarizations after a sudden increase in cycle length.

References