Atrial Tachycardia Remodeling of Pulmonary Vein Cardiomyocytes
Comparison With Left Atrium and Potential Relation to Arrhythmogenesis

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Background—The pulmonary veins (PVs) are important in the pathophysiology of atrial fibrillation (AF), as is atrial tachycardia (AT) remodeling. The relative importance of AT remodeling in PVs versus other atrial sites is unknown. The present study assessed AT-induced cellular changes in PVs versus left atrium (LA) and their relationship to arrhythmogenesis.

Methods and Results—We studied ionic currents (single-cell patch clamp) and action potentials (APs; coronary-perfused multicellular preparations) in the PVs and LA free wall of dogs after 7-day AT pacing (400 bpm), as well as in nonpaced control dogs. In controls, rapid (I\textsubscript{Kr}) and slow (I\textsubscript{Ks}) delayed-rectifier currents were larger in PVs; transient-outward (I\textsubscript{o transient}) and inward-rectifier (I\textsubscript{K1}) currents and AP duration were smaller. AT remodeling reduced I\textsubscript{Ca} and I\textsubscript{o transient}, left I\textsubscript{K1} and I\textsubscript{Ks} unchanged, and increased I\textsubscript{K1} in both LA and PV. AT reduced action potential duration in both LA and PV. LA–PV AP differences became smaller in AT than in control dogs. Premature extrasystoles induced atrial tachyarrhythmias at 4.5±2.8% (mean±SEM) sites in 6 control multicellular preparations compared with 64.2±7.3% sites in 9 AT-remodeled preparations (P<0.001). Resection of all PVs failed to alter atrial tachyarrhythmia inducibility in AT-remodeled preparations (67.5±13.1%). PV resection did not significantly change tachyarrhythmia duration (mean 3.9 seconds per heart, range 0.7 to 15.7 seconds before resection; mean 7.0 seconds per heart, range 0.9 to 36.0 seconds after resection) or cycle length (120±6 ms before resection, 115±8 ms after resection).

Conclusions—AT produces qualitatively similar ionic remodeling in LA and PVs but reduces PV–LA AP differences. PVs are not essential for AT-induced atrial tachyarrhythmia promotion in this model, which may relate to the failure of PV isolation to prevent AF in some patient populations. (Circulation. 2005;111:728-735.)

Key Words: ion channels ■ atrium ■ fibrillation

The pulmonary veins (PVs) play an important role in clinical atrial fibrillation (AF). Cheung1 found that PV myocardial sleeves can show slow spontaneous activity, and Chen et al4–7 showed that PV-wall cells generate various forms of rapid abnormal activity. However, Chen et al studied isolated myocytes, which can be substantially affected by the isolation procedure, and other investigators8–10 have been unable to reproduce arrhythmic activity in normal multicellular PV preparations.

Atrial tachycardia (AT) produces atrial arrhythmogenic remodeling, which promotes AF and contributes significantly to its pathogenesis.11,12 Cardiomyocytes in the PV sleeves are remodeled by AT,5 but PV AT remodeling has never been compared directly to AT remodeling in other atrial regions. Furthermore, the importance of PV remodeling for the arrhythmogenic effect of AT is incompletely understood. We undertook the present study to compare directly the changes in ionic currents (by patch clamp of isolated myocytes) and action potentials (APs; with standard microelectrodes in intact multicellular preparations) in PVs with those in a reference atrial region, the left atrial (LA) free wall. Furthermore, we examined the importance of PVs in AT-induced atrial arrhythmia promotion by surgically removing the PVs from isolated, coronary-perfused LA PV preparations.

Methods

The animal model and cell isolation followed previously described procedures.10,13 Animal protocols followed Canadian Council on Animal Care guidelines and were approved by the local Animal Research Ethics Committee. In total, 71 adult dogs (weight 19 to 39 kg) were divided into control (n=37) and AT (n=34) groups, with 7 control and 4 AT dogs used for AP analysis, 6 control and 9 AT dogs for arrhythmia-induction studies, and the rest for ionic-current measurements. AT dogs were anesthetized with diazepam (0.25 mg/kg IV), ketamine (5.0 mg/kg IV), and halothane (1% to 2%) for transvenous insertion of right ventricular (RV) and right atrial (RA)
unipolar pacing leads connected to pacemakers implanted in the neck. Pacing began 24 hours after pacemaker implantation. AV block was created by radiofrequency ablation, and the RV demand pacemaker was programmed to 80 bpm. The RA was then tachypaced (400 bpm) for 1 week.

On study days, dogs were anesthetized (pentobarbital 30 mg/kg IV) and artificially ventilated. Hearts and adjacent lung tissue were excised via left thoracotomy and immersed in oxygenated Tyrode solution. For cell isolation, the circumflex coronary artery was cannulated, and distal ends of PV myocardial sleeves were marked with silk thread, before enzyme perfusion with collagenase (100 U/mL, Worthington, type II), to facilitate identification after enzymatic digestion. After isolation, cells were stored at 4°C and studied within 12 hours.

For standard microelectrode experiments and atrial tachyarrhythmia induction, tissue preparations that included the LA and attached PVs were mounted in a Plexiglas chamber and perfused via the circumflex coronary artery with oxygenated Tyrode solution. For cell isolation, the circumflex coronary artery was excised via left thoracotomy and immersed in oxygenated Tyrode solution. For cell isolation, the circumflex coronary artery was excised via left thoracotomy and immersed in oxygenated Tyrode solution. For cell isolation, the circumflex coronary artery was excised via left thoracotomy and immersed in oxygenated Tyrode solution.

Cellular Electrophysiology

Currents were recorded with whole-cell patch clamp at 36±0.5°C. Borosilicate glass electrodes had tip resistances between 1.5 and 3.0 MΩ when filled. Compensated series resistance and capacitive time constants averaged 3.3±0.1 MΩ and 262±5 ms, respectively. Cell capacitance averaged 89±3 pF for LA control, 88±3 pF for PV control, 91±3 pF for LA AT, and 90±3 pF for PV AT cardiomyocytes (n=90/group, P=NS). To control for cell-size variability, currents are expressed as densities (pA/pF). Junction potentials averaged 9.7±0.7 mV and were not corrected.

Solutions

The solution for cell storage contained (in mmol/L) KCl 20, KH2PO4 10, dextrose 10, mannitol 40, l-glutamic acid 70, β-OH-butyric acid 10, taurine 20, EGTA 10, and 0.1% BSA (pH 7.3, KOH). Tyrode (extracellular) solution contained in mmol/L): NaCl 136, KCl 5.4, MgCl2 1, CaCl2 1, NaH2PO4 0.33, HEPES 5, and dextrose 10 (pH 7.35, NaOH). For delayed-rectifier current recording, nifedipine (5 μmol/L), 4-aminopyridine (2 mmol/L), and atropine (200 nmol/L) were added to suppress L-type Ca2+ current (Ica), transient outward current (Ito), and 4-aminopyridine-dependent muscarinic K+ currents, respectively. 10 Dofetilide (1 μmol/L) was added for slow delayed-rectifier current (Ik) recording, and rapid delayed-rectifier current (Ikr) was recorded as chromanol 293B (50 μmol/L)-resistant current. For Ica, inward-rectifier current (Ii) recording, Ic was suppressed with CdCl2 (200 μmol/L). Ik was studied in the presence of 10 mmol/L tetraethylammonium-Cl to suppress delayed-rectifier currents. Ik was recorded as 1 mmol/L Ba2+-sensitive current on stepping from −40 mV to voltages between −120 and −10 mV. Na+ current contamination was avoided by use of a holding potential of −50 mV and/or by substitution of equimolar Tris-HCl for NaCl. The internal solution for K+-current recording contained (in mmol/L) K-aspartate 110, KCl 20, MgCl2 1, Mg ATP 5, Li-GTP 0.1, HEPES 10, Na-phosphocreatine 5, and EGTA 5 (pH 7.3, KOH).

The external solution for Ikr recording contained (in mmol/L): TEA-Cl 136, CsCl 5.4, CaCl2 2, MgCl2 0.8, HEPES 10, and dextrose 10 (pH 7.4, CsOH). Niflumic acid (50 μmol/L) was added to inhibit Ca2+-dependent Cl− current. The internal solution for Ic recording
was (in mmol/L) CsCl 120, TEA-Cl 20, MgCl₂ 1, Mg ATP 5, Li-GTP 0.1, EGTA 10, and HEPES 10 (pH 7.3, CsOH). Krebs solution (standard microelectrode experiments) contained (in mmol/L) NaCl 120, KCl 4, KH₂PO₄ 1.2, MgSO₄ 1.2, NaHCO₃ 25, CaCl₂ 1.25, and dextrose 5 (95% O₂-5% CO₂, pH 7.4).

Statistical Analysis
Nonlinear curve fitting was performed with Clampfit in pCLAMP6. Group data are presented as mean±SEM. Statistical analysis was performed with the MIXED procedure of the Statistical Analysis Systems (SAS) software package (SAS Institute Inc). Three-way repeated-measures ANOVA was performed using mixed model methodology with group, region, and voltage as main effects, with the dog considered as the unit of analysis. If appropriate, heterogeneous variance-covariance matrices were used for groups. The repeated-measures ANOVA was applied to evaluate interactions between the 3 main effects. In case of a significant interaction, contrasts were used to compare groups for each region at each voltage. However, for display purposes, we avoided cluttering the figures with large numbers of asterisks and simply indicate the statistical significance of main effects and interactions in the text. A 2-tailed P<0.05 was taken to indicate statistical significance. Whenever possible (>90% of cases), we studied ionic currents or AP properties from similar numbers of PV and LA cells from each preparation. Analyses controlled for dog of origin as a variable.

Results
Ionic Remodeling
Iᵥ
Typical Iᵥ was recorded from both LA and PV cells and was reduced by AT in both. Iᵥ recordings had morphologies similar to those we have reported previously,¹⁰ and because of space limitations, we do not show original recordings in this report. Mean Iᵥ density decreased significantly (P<0.001) with AT compared with control for both regions (Figure 2A).

There was no significant region-by-AT interaction to suggest a quantitatively different response to AT in PV versus LA.

Activation voltage dependence of Iᵥ was assessed with the protocol shown in Figure 2, based on the relationship $I(V) = I_{max}(V-V_r)(G/G_{max})$, where $I(V)$=current at voltage $V$; $G$=conductance at voltage $V$; $I_{max}$=maximum current; $G_{max}$=maximum conductance; and $V_r$=reversal potential (Figure 2B). $V_r$, as evaluated by tail currents after 2.2-ms depolarizations to 50 mV, averaged −71.5±1.7, −70.9±0.9, −69.5±1.2, and −70.0±1.4 mV in LA control, PV-control, LA AT, and PV AT, respectively (5 cells/group, P=NS). Activation $V_{t/2}$ based on Boltzmann fits to data in each experiment averaged 10.3±2.5, 10.1±1.6, 10.2±1.0, and 10.0±0.7 mV in LA and PV control and LA and PV AT, respectively (10 cells/group, P=NS). Corresponding slope factors averaged 11.5±0.8, 12.1±0.6, 12.3±0.5, and 12.9±0.8 mV (P=NS). Inactivation voltage dependence was studied with 1000-ms prepulses from −70 mV followed by 200-ms test pulses to 50 mV. Boltzmann fitting provided inactivation $V_{t/2}$ that averaged −30.4±1.4, −30.5±1.0, 29.3±0.4, and −30.5±0.5 mV in LA control, PV control, LA AT, and PV AT, respectively (P=NS). Corresponding slope factors were −6.3±0.5, −7.5±1.1, −6.4±0.4, and −6.6±0.4 mV (n=8 cells/group, P=NS). Iᵥ decay time constants were similar in PV and LA and unchanged by AT (Figure 2C). Time to peak current, an index of activation speed, was not changed by AT. A paired-pulse protocol with identical 150-ms depolarizations (P₁, P₂) from −70 to 50 mV and varying P₁–P₂ intervals was used to analyze recovery kinetics. Current during P₂ normalized to current during P₁ was a monoexponential function of P₁–P₂ interval (Figure 2D). Recovery time constants averaged 30.9±2.6 ms in LA control, 30.2±1.9 ms in PV control, 29.7±1.2 ms in LA AT, and 32.2±2.4 ms in PV AT (n=7 cells/group, P=NS).
As previously reported, $I_{Ks}$ was smaller in LA (Figure 3A) than PV cells (Figure 3B) under control conditions, and the same relationship was observed after AT (Figures 3C and 3D). PV $I_{Ks}$ step current was significantly different from LA ($P<0.001$), and there was no interaction with AT or any significant AT effect (Figure 3E). Voltage dependence of $I_{Ks}$ activation (tail-current analysis) was not altered ($V_{1/2}$ 22.2±1.6, 21.6±1.4, 22.4±1.0, and 21.5±1.4 mV, respectively, in LA control, PV control, LA AT, and PV AT; n=12 cells/group). $I_{Ks}$ activation kinetics at 40 mV were biexponential, with slow-phase time constants averaging 2224±340, 2490±424, 1979±332, and 2435±813 ms in LA control, PV control, LA AT and PV AT, respectively ($P=NS$, n=10 cells/group). Fast-phase time constants were similarly unchanged, averaging 248±25, 256±46, 221±20, and 236±24 ms in LA control, PV control, LA AT, and PV AT, respectively ($P=NS$, n=10 cells/group). Fast-phase time constants were similarly unchanged, averaging 248±25, 256±46, 221±20, and 236±24 ms in LA control, PV control, LA AT, and PV AT, respectively ($P=NS$, n=10 cells/group). Tail-current deactivation time constants after a step to 40 mV averaged 114±17, 118±32, 116±10, and 91±13 ms, respectively ($P=NS$).

As shown previously, $I_{Ks}$ was larger than in LA ($P<0.001$). $I_{Ks}$ was not changed by AT (Figure 4A). Voltage dependence of $I_{Ks}$ activation was evaluated from the normalized tail-current/test-potential relation (Figure 4B). Half-activation voltage (tail-current analysis) was not altered: $V_{1/2}$ averaged −11.3±0.6, −11.1±0.5, −13.3±1.5, and −12.6±1.2 mV in LA control, PV control, LA AT, and PV AT, respectively ($P=NS$, n=8 cells/group).

Representative 1-mmol/L Ba$^{2+}$-sensitive $I_{Kr}$ recordings are illustrated in Figures 5A through 5D. $I_{Kr}$ density was larger in LA than PV ($P<0.001$). AT significantly increased $I_{Kr}$ density ($P<0.001$; Figures 5E and 5F), but there was no significant interaction between region and AT effects. $I_{Kr}$ density was smaller in PV and was decreased significantly by AT ($P<0.001$ for each; Figure 6A), but there was no significant region-by-AT interaction. The voltage dependencies of $I_{Kr}$ activation and inactivation were not changed by AT (Figure 6B). $V_r$ determined by linear extrapolation of the ascending limb of the I-V curve to the voltage axis was not different among groups (62.1±1.0, 63.4±0.8, 61.3±0.7, and 64.0±3.0 mV in LA control, PV control, LA AT, and PV AT, respectively; n=10 cells/group). Inactivation $V_{1/2}$ averaged −37.9±1.3, −36.4±1.5, −38.3±1.2, and
−38.1±1.4 mV in LA control, PV control, LA AT, and PV AT, respectively (n=8 cells/group, P=NS), with corresponding slope factors of −6.0±0.7, −6.2±0.5, −5.9±0.5, and −5.8±0.4 mV (P=NS). Activation V_{1/2} averaged −4.8±0.5, −5.2±1.0, −5.1±0.6, and −4.5±1.4 mV in LA control, PV control, LA AT, and PV AT, respectively (n=10 cells/group, P=NS), and slope factors were 5.0±0.2, 4.9±0.2, 4.6±0.1, and 4.8±0.4 mV (P=NS; Figure 6B). I_{Ca} recovery time constants were not different among groups: 32.0±3.9, 31.1±4.2, 31.6±1.0, and 32.5±2.6 ms (n=6 cells/group, P=NS; Figure 6C). I_{Ca} inactivation time constants were also comparable among groups (Figure 6D).

AP Recordings
Examples of APs recorded with standard fine-tipped microelectrodes from multicellular LA PV preparations are shown in Figure 7A. Mean resting potential (RP) was more negative in LA than PV under control conditions (Figure 7B). With AT, RP differences between PV and LA decreased (Figure 7B). Under control conditions, APD was significantly shorter in PV (Figure 7C). AT remodeling reduced APD, with greater changes in LA than PV, which reduced PV–LA differences. No early or delayed afterdepolarizations were seen.

Figure 6. A, Mean±SEM, I_{Ca} density at 0.1 Hz in 13, 15, 14, and 16 cells in LA control, PV control, LA AT, and PV AT, respectively. B, Voltage dependence of I_{Ca} inactivation and activation. Curves are Boltzmann fits to mean data (n=8 cells/group for activation, n=8 cells/group for inactivation). C, I_{Ca} recovery kinetics (n=6 cells/group). D, I_{Ca} inactivation time constants (n=8 cells/group). TP indicates test potential; CTL, control.

Figure 7. A, Superimposed AP recordings at 2 Hz from an LA and a PV cell under control (CTL) conditions (top) and from an LA and a PV cell from AT-remodeled preparation (bottom). Mean±SEM resting membrane potential (B) and APD to 90% repolarization (APD90; C) in LA and PV cells under CTL and AT conditions (n=39, 89, 24, and 34 cells in LA control, PV control, LA AT, and PV AT, respectively).
Role of PVs in Atrial Tachyarrhythmia Promotion by AT Remodeling

To evaluate the role of PVs in atrial tachyarrhythmia promotion by AT remodeling, we studied arrhythmia inducibility in isolated, coronary-perfused LA PV preparations from 6 control dogs and 9 dogs with 1-week AT remodeling. Atrial tachyarrhythmia (>3 beats) induction was attempted with single extrastimuli at S1-S2s just beyond the effective refractory period at CLs of 200, 250, 333, and 500 ms at 5 or more sites per preparation. In AT preparations, tachyarrhythmias were induced at 64.2±7.3% sites per preparation (Figure 8A) compared with 4.5±2.8% sites in control preparations (P<0.001; Figure 8B). PV resection did not alter tachyarrhythmia inducibility, which remained 67.5±13.1% after resection of all PVs. Figures 8C and 8D show typical tachyarrhythmias induced in one preparation before and after PV removal. Induced tachyarrhythmia duration averaged 3.9±1.7 seconds per preparation before and 7.0±4.5 seconds per preparation after PV resection (P=NS). There was wide variation in mean tachyarrhythmia duration per preparation, from 0.7 to 15.7 seconds before PV resection and from 0.9 to 36.0 seconds after PV resection. Tachyarrhythmia CL was unchanged by PV resection, averaging 120±6 ms before resection and 115±8 ms after resection.

Discussion

In this report, we compared AT remodeling effects on ionic currents in PV versus LA cardiomyocytes. AT had qualitatively similar effects in both tissues. After AT remodeling, PV–LA APD differences were attenuated. AT promoted atrial tachyarrhythmia induction in isolated LA PV preparations, an effect that was unaltered by PV resection.

Comparison With Previous Studies of PVs in AT Remodeling

Chen et al studied changes in PV cardiomyocyte electrical activity in dogs subjected to 6 to 8 weeks of AT. They noted downregulation of \( I_{\text{to}} \) and \( I_{\text{Ca}} \), as well as a high prevalence of spontaneous early and delayed afterdepolarizations in isolated PV cells. The main differences between the studies is that we compared LA and PV responses to AT and used specific conditions to isolate individual currents, which Chen et al did, and that we did not observe PV afterdepolarizations. The difference in afterdepolarization behavior may have been due to the fact that we studied more physiologically intact, coronary-perfused multicellular preparations, in which spontaneous arrhythmic PV behavior is absent, possibly related to the effect of cell isolation on PV APs. Cell isolation is known to have potentially significant effects on ionic current properties, which is why we were careful to study APs in coronary-perfused multicellular preparations and why we were careful to measure the same ionic currents in both regions (LA and PV) from each set of isolated cells.

Wu et al studied dogs with >48 hours of sustained AF induced by an average of 139 days of AT pacing. They noted a dominant frequency gradient during AF, with LA activity being faster than RA and the most rapid activation occurring in the PV and ligament of Marshall regions. Park et al recently showed that radiofrequency ablation encircling the PVs, ligament of Marshall, and superior vena cava terminates sustained AF (mean duration 17 days) induced by an average of 30.6 days of AT pacing and prevents sustained AF induction. These findings contrast with our observation that PV removal did not affect atrial tachyarrhythmia promotion by 1 week of AT. The discrepancy may be due to the more extensive LA and RA damage caused by the multiple encircling radiofrequency lesions applied to the LA and RA in the study by Park et al, as well as the much longer period of AT (47 days in the study by Park et al versus 7 days in the present study).

Novel Findings and Potential Significance

The present study is the first of which we are aware to compare directly AT-induced cellular electrophysiological remodeling in PV versus LA cardiomyocytes. AT significantly decreased \( I_{\text{Ca}} \) and \( I_{\text{to}} \) and increased \( I_{\text{k1}} \) in both LA...
and PV cardiomyocytes, while leaving \(I_{Ks}\) and \(I_d\) unchanged. PV APD, which was considerably smaller than LA at baseline, became more similar to LA with AT. In addition, with the AT-induced increase in \(I_{Ks}\), RPs in the PV became more negative, attenuating the RP differences present under baseline conditions. There is evidence that PVs may be a privileged source for atrial reentry in the normal dog, at least in part because of shorter APD and less negative RPs that result in smaller phase-0 upstroke velocity in PVs. The loss of PV–LA differences in APD and RP with AT remodeling, as well as the substantial reduction in LA APD, may explain why the PVs are not essential for AT-induced arrhythmia promotion in this model. The present results provide potentially important new information about the topography of AT-induced ionic current and AP remodeling changes in the PV and LA.

The PVs are clearly important in clinical AF, and the elimination of PV input to the LA by PV isolation is effective in suppressing AF. PV isolation is less effective in treating persistent AF than the paroxysmal form, however, and an ablation strategy involving the destruction of LA tissues surrounding the PVs is more effective than PV isolation in AF prevention. It has been suggested that the elimination of LA rotors may be needed to optimally suppress AF and that the elimination of PV sources may at times be insufficient. The present results are consistent with these clinical experiences in indicating that electrical remodeling can produce a substrate in the LA that is capable of sustaining atrial tachyarrhythmias in the absence of PVs. Our results do not dispute in any way the great importance of PV activity in AF, nor do they prove that the PVs cannot play a significant role in AF-related remodeling (eg, by providing triggers). They do, however, show that a substrate for atrial tachyarrhythmias is present in the AT-remodeled atrium that does not necessarily require a contribution from the PVs.

**Limitations of the Study**

We evaluated AT remodeling after 7 days of AT pacing. Different results might have been obtained with longer-duration AT or AF. Nevertheless, we were able to compare quantitatively ionic current and AP remodeling between LA and PV at a time when AT remodeling produces substantial changes. The isolated LA PV preparation is devoid of neural input and is clearly not identical to an intact heart; however, such preparations have been used previously to study potential mechanisms of PV cardiomyocyte involvement in atrial arrhythmia, of AT-related arrhythmias, and of reentrant atrial tachyarrhythmia in the presence of cholinergic stimulation. Atrial tachyarrhythmias in the present model were self-limited and generally much briefer than AF that would require ablation in humans. Great caution is required in attempting to extrapolate our results directly to the clinical setting.

**Conclusions**

AT-induced ionic remodeling is qualitatively similar in LA and PVs, but AT decreases the AP differences between LA and PV cardiomyocytes. AT-remodeled LA preparations have easily inducible atrial tachyarrhythmias in vitro, which do not require the presence of PVs. Thus, PVs are not essential for AT promotion of arrhythmogenesis in this preparation, which may serve as a paradigm for clinical contexts in which the LA can maintain AF in the absence of PV input.

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**References**


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