Intracellular Calcium Dynamics and Autonomic Stimulation in Atrial Fibrillation: Mechanisms and Implications

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While atrial fibrillation is characterized by the co-existence of multiple activation waves within the atria, rapid activations in the pulmonary veins play an important role for the initiation and maintenance of atrial fibrillation. In addition to reentry, non-reentrant mechanisms resulting from abnormal intracellular calcium handling and intracellular calcium overload can also be responsible for these rapid activations in the pulmonary veins. Meanwhile, alterations of autonomic tone, involving both the sympathetic and parasympathetic nervous system, have been implicated in initiating paroxysmal atrial fibrillation. But the effectiveness of autonomic modulation as an adjunctive therapeutic strategy to catheter ablation of atrial fibrillation has been inconsistent. The interactions between the autonomic nervous system and atrial fibrillation are more complex than currently understood and further mechanistic and clinical studies are warranted.

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Pulmonary veins (PVs) play important roles in the genesis of atrial fibrillation (AF). Studies in the past decade have shown that rapid electrical activation in the PVs is the mechanism responsible for triggering of AF,1) and that paroxysmal AF can be cured by ablation of the focal triggers in the PVs.2) Rapid electrical activations in the PVs also play an important role for maintenance of AF in animal models of sustained AF.3) Although reentry could be responsible for the rapid electrical activations in PVs,4) non-reentrant mechanisms5) such as triggered activity or automaticity may also underlie these focal discharges in the thoracic veins. Brunton and Fayer6) were the first to demonstrate independent PV contractions in rabbit hearts in 1872. They observed contractions of the PVs asynchronous to the atria when artificial respiration was discontinued in these anesthetized rabbits. They also noted that, while both atria subsequently ceased to beat, the PVs from both lungs continued to pulse. These seminal observations imply that PVs have contractile myocardial sleeves and are capable of generating electrical activities independent of the atria. Early works by Zipes and Knope7) showed that not only did atrial

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musculature extend for some distance into PVs but also that these muscle sleeves received vagal innervation. Masani et al.\(^8\) showed that node-like cells were present in the myocardial layer of the PVs with juxtaposition of nerve fibers containing small and large vesicles with and without dense cores in rats. The close interaction between the nerve structures and the specialized muscle cells might play a role in the generation of ectopic activities in the PVs. Takahashi et al.\(^9\) reported that vagal excitation is associated with shortening of the fibrillatory cycle length which occurred earlier in the PVs, suggesting that vagal excitation enhances a driving role of the PVs. Clinical observations have noted that denervation of the PVs could improve the success rate of AF ablation in humans.\(^{10}\) Radiofrequency catheter ablation of selected atrial sites (mostly adjacent to the PVs) in which high-frequency stimulation induced vagal reflexes may prevent AF recurrences in selected patients with apparently vagal-induced paroxysmal AF.\(^{11}\) It is possible that both the autonomic nerves and muscle sleeves in the PVs are important in triggering AF.

**Cellular Electrophysiology of Pulmonary Vein Cardiomyocytes**

Cheung\(^{12,13}\) demonstrated that ouabain infusion or norepinephrine infusion could trigger the onset of repetitive rapid activities from the isolated PVs of guinea pigs. Cellular determinants of the electrical activity of the PVs, especially transmembrane ion currents, have been studied extensively to understand the PV electrophysiological properties in genesis of AF. Chen et al.\(^{14,15}\) recorded transmembrane potentials of canine PVs by using standard glass microelectrodes, and illustrated several types of electrical activities within the PVs, including silent electrical activity, fast response action potentials (APs) driven by electrical stimulation, and spontaneous fast or slow response APs with or without early afterdepolarizations (EADs). The incidences of APs with an EAD and of spontaneous tachycardias were considerably greater in dogs with chronic rapid pacing than in normal dogs. Ehrlich et al.\(^{16}\) demonstrated that PV cardiomyocytes have distinct electrophysiological properties compared to left atrial (LA) cells. Differences included smaller phase-0 upstroke velocity \(V_{\text{max}}\), less negative resting membrane potential (Vm) and shorter action potential duration (APD) in PVs. Ionic current differences were noted between the PV and LA cardiomyocytes: in PV cardiomyocytes, the smaller \(I_{\text{K1}}\) is believed to contribute to the reduced resting Vm and enhances the development of delayed afterdepolarizations (DADs).\(^{17}\) and the larger \(I_{\text{Kr}}\), \(I_{\text{Kr}}\) and smaller \(I_{\text{Ca,L}}\) contribute to the shorter APD. But the intrinsic \(I_{\text{NCX}}\) and \(I_{\text{SCX}}\) protein expressions are similar in the PV and LA.

**Mechanisms Underlying Focal Discharge from the Pulmonary Veins**

Using high-density (1-mm resolution) computerized mapping techniques, we have demonstrated that rapid focal electrical activations are present in the PVs during sustained AF induced by LA pacing\(^{5,18,19}\) and non-sustained AF in heart failure dogs induced by rapid right ventricular pacing in vivo.\(^{20}\) Arora et al.\(^4\) were the first to use optical mapping techniques with voltage-sensitive dye Di-4-ANEPPS for Vm recording of canine PVs. They showed sustained focal discharges from the endocardial surface of PVs in the presence of isoproterenol; each focus was localized near the venous ostium. Honjo et al.\(^{21}\) showed that rapid pacing and low-concentration ryanodine shifted the leading pacemaker from the sinoatrial node to an ectopic focus near the right PV-LA junction in rabbit right atrial-right PV preparations. Both rapid pacing and low-concentration ryanodine increase intracellular calcium (Ca\(_i\)), which may cause voltage-independent Ca\(_{2+}\) release from the sarcoplasmic reticulum (SR) and activate \(I_{\text{NCX}}\). The pacing-induced activity was attenuated by either depletion of SR Ca\(_{2+}\) or blockade of the sarcolemmal \(I_{\text{NCX}}\) or Cl\(^{-}\) channels and potentiated by ß-adrenergic stimulation. Because PV cardiomyocytes have a less negative resting Vm than LA cardiomyocytes,\(^{16}\) the depolarizing currents may induce triggered activity and focal discharge in the PV but not LA. It was concluded that PV myocardial sleeves have the potential to generate spontaneous activity, and such arrhythmogenic activity is surfaced by modulation of Ca\(_i\) dynamics.

**Dual Optical Mapping of Canine Pulmonary Veins**

To gain further insights into the mechanisms of the nonreentrant focal discharge, we used dual optical mapping techniques for simultaneous Vm and Ca\(_i\) mapping in isolated, Langendorff-perfused PV-LA preparations of normal dogs.\(^{22}\) The tissues were stained with the Ca\(_{2+}\) indicator Rhod-2 AM and the voltage-sensitive dye RH237. Epifluorescence was collected simultaneously through a 715-nm-long pass filter for the Vm image and a 580 ± 20-nm interference filter for the Ca\(_i\) image.
by using two charge-coupled device cameras. We used low-concentration ryanodine, isoproterenol and rapid pacing to facilitate the induction of focal discharge from the PVs. Burst pacing tested the inducibility of arrhythmia, then 0.5 \( \mu \text{mol/L} \) ryanodine was infused over a 15 min period. The same burst pacing protocol was subsequently applied. Next, isoproterenol was infused for 15 min and burst pacing protocol was repeated. Both spontaneous and pacing-induced arrhythmias were mapped to determine the source of the focal discharge, if any. The mapping results revealed no focal discharge was induced at baseline. After 0.5 \( \mu \text{mol/L} \) ryanodine administration, rapid atrial pacing induced 26 episodes of focal discharge from the proximal PVs. The cycle lengths were longer with ryanodine infusion (223 \( \pm \) 52 ms) than with combined ryanodine and isoproterenol infusion (133 \( \pm \) 59 ms). The simultaneous \( \text{Ca}_i \) and Vm mapping data demonstrated that there was a rise of \( \text{Ca}_i \) preceding Vm activation at the sites of PV focal discharge. The \( \text{Ca}_i \) prefluorescence at the focal site suggests that focal discharge was induced by the Vm-independent \( \text{Ca}^{2+} \) release from the SR. The process by which spontaneous \( \text{Ca}_i \) release induces nondriven electrical activity is known as reverse excitation-contraction coupling,\(^{23}\) and is responsible for inducing the triggered activity in the PVs.

**Autonomic Influence on Atrial Fibrillation Arrhythmogenesis**

Most patients with idiopathic paroxysmal AF appear to be vagally dependent, with a heightened susceptibility to vasovagal cardiovascular response. In contrast, paroxysmal AF in most patients with organic heart diseases appear more sympathetically dependent.\(^{24}\) Experimentally, sympathetic nerve stimulation rarely triggers AF in normal dogs; however, in dogs which underwent chronic rapid atrial pacing, sympathetic stimulation leads to rapid repetitive activations in the isolated canine PV and vein of Marshall preparations.\(^{14,25}\) Sharifov et al.\(^{26}\) reported that a combined isoproterenol and acetylcholine infusion is more effective than acetylcholine alone in the induction of AF in dogs. Clinically, Bettoni el al.\(^{27}\) reported that fluctuations of autonomic tone precede the occurrence of paroxysmal AF. Using heart rate variability, they showed that the low/high frequency ratio increased linearly until 10 minutes before paroxysmal AF, and then decreased sharply immediately before the onset of paroxysmal AF, suggesting a primary increase in the adrenergic tone followed by a marked modulation toward vagal predominance. Zimmermann et al.\(^{28}\) analyzed the dynamic changes in the autonomic tone preceding the onset of atrial arrhythmias in patients with paroxysmal AF and reported that the occurrence of paroxysmal AF was associated with variations in autonomic tone, with vagal predominance before the onset of AF. However, the autonomic nervous system activity in all these studies was indirectly evaluated by analyzing the heart rate variability parameters through continuous electrocardiographic recordings. Heart rate variability measures only changes in the relative degree of autonomic nervous system, but not the absolute level of sympathetic or parasympathetic discharge. To test the hypothesis that spontaneous autonomic nervous system discharges can serve as triggers of paroxysmal AF, Tan et al.\(^{29}\) implanted Data Sciences International Transmitters to directly and simultaneously record the left stellate ganglion nerve activity, left vagal nerve activity, and LA local bipolar electrograms or surface electrocardiograms in ambulatory dogs over several weeks. Intermittent rapid atrial pacing was performed, and autonomic nervous system activity was monitored when the pacemaker was turned off. Paroxysmal atrial tachycardia and paroxysmal AF were documented and simultaneous sympathovagal discharges were determined to be the most common triggers of paroxysmal atrial tachycardia and paroxysmal AF in this study.

**Late-Phase 3 Early Afterdepolarizations as Triggers of Paroxysmal Atrial Fibrillation**

Burashnikov et al.\(^{30}\) showed \( \text{Ca}_i \) overload after termination of vagally mediated AF. \( \text{Ca}_i \) may contribute to the development of late-phase 3 EADs. The late-phase 3 EADs was the possible mechanism responsible for the extrasystolic activity that reinitiated AF in an acetylcholine-induced canine right atrial model. Marked reduction in the APD, rapid rate of excitation and high SR \( \text{Ca}^{2+} \) release were required to elicit EADs in the initiation period following termination of AF. These extrasystolic activities were eliminated by 1 \( \mu \text{mol/L} \) ryanodine, further supporting the underlying intracellular and SR \( \text{Ca}^{2+} \) overload mechanism to reinitiate AF. Based on the time course of contraction, the levels of \( \text{Ca}_i \) would be expected to peak during the plateau phase of AP under control condition (Vm, approximately \(-5 \text{mV}\)), but during the late phase of repolarization in the presence of acetylcholine (Vm, approximately \(-70 \text{mV}\)). In the latter condition, \( \text{I}_{\text{NCX}} \) and \( \text{Cl}^- \) become strongly inward currents and are able to generate late-phase 3 EADs. The
rapid activation rates would cause an increase in the intracellular Na\(^+\) level, leading to an \(I_{\text{NCX}}\)-mediated cellular Ca\(^{2+}\) loading, which would generate an outward current. A long pause following the tachycardia results in augmented SR Ca\(^{2+}\) loading and release, then stimulates the extrusion of Ca\(^{2+}\) through \(I_{\text{NCX}}\) (inward current), responsible for the late-phase 3 EADs. This unique mechanism combining properties of both EADs and DADs, in which abbreviated repolarization permits “normal” rather than “spontaneous” SR Ca\(^{2+}\) release to induce an EAD-mediated closely coupled triggered response.\(^{31}\)

The late-phase 3 EADs mechanism has also been demonstrated in canine PV preparations. Patterson et al\(^{32}\) reported that autonomic nerve stimulation decreased the APD of the PV (\(\text{APD}_{90} = 160 \pm 17\) to \(92 \pm 24\) ms; \(P < .01\)) and initiated rapid (782 ± 158 beats/min) firings from EADs in 22 of 28 canine PV preparations. Failure to induce arrhythmias was associated with a failure to shorten \(\text{APD}_{90}\) (151 ± 18 to 142 ± 8 ms; \(P = .39\)). Muscarinic receptor blockade (3.2 \(\times\) 10\(^{-8}\) mol/L of atropine) prevented \(\text{APD}_{90}\) shortening in all 8 preparations and suppressed firing in 6 of 8 preparations, whereas \(\beta_1\)-adrenergic receptor blockade (3.2 \(\times\) 10\(^{-8}\) mol/L of atenolol) suppressed the firing in all 8 preparations. The suppression of the Ca\(^{2+}\) transient with ryanodine (10 \(\mu\)mol/L) completely suppressed the firing in all 6 preparations, while the inhibition of forward \(I_{\text{NCX}}\) by a transient increase in \([\text{Ca}^{2+}]_o\), suppressed the firing in 4 of 6 preparations. The authors termed this phenomenon “Ca\(^{2+}\) transient triggering” and suggested that the increased forward \(I_{\text{NCX}}\) current may contribute to the generation of EADs. Another study\(^{33}\) used extracellular bipolar and intracellular microelectrode recordings to investigate the electrophysiological basis underlying the trigger of rhythms by combined infusion of norepinephrine and acetylcholine in isolated superfused canine PVs. EADs were observed with pacing, catecholamine administration, and interventions increasing contractile force and \(I_{\text{NCX}}\). With further reduction in the APD of the PV after the infusion of norepinephrine plus acetylcholine, tachycardia-pause initiated focal arrhythmias (at the rate of 1,132 ± 53 beats/min) were observed originating within the PV sleeves. Ryanodine and the inhibition of \(I_{\text{NCX}}\) suppressed both EADs and pacing-induced firings initiated by norepinephrine plus acetylcholine. These data demonstrate simultaneous stimulation of both branches of the autonomic nervous system (causing both abbreviation of APD and enhancement of \(Ca^{2+}\) and \(I_{\text{NCX}}\)) triggered firings in canine PV myocardial sleeves.

Heart Failure and Atrial Arrhythmias

The Framingham Heart Study\(^{34}\) concluded that in heart failure subjects, late development of AF was associated with increased mortality. Heart failure-related atrial arrhythmias appear to arise from macroreentrant sources, primarily by increasing atrial size and promoting interstitial fibrosis.\(^{35}\) In addition to macroreentry, Okuyama et al\(^{20}\) demonstrated that some AF episodes were characterized by focal activations in the PVs and vein of Marshall, and by complex, fractionated wave fronts within the PVs in a canine heart failure model, suggesting the occurrence of significant proarrhythmic remodeling in the PVs during heart failure. Stambler et al reported that DADs-induced triggered activity may also be a mechanism of focal atrial tachycardias in pacing-induced heart failure dogs.\(^{36}\) A major arrhythmogenic mechanism in heart failure resulted from altered ryanodine receptor function.\(^{37}\) A combination of abnormal ryanodine receptor and increased sympathetic tone during exercise can cause triggered activity.\(^{38}\) Ryanodine at low concentrations locks the ryanodine receptor in a sub-conductance state mimicking the heart failure status. By simultaneous \(V_m\) and \(Ca_i\) mapping, we\(^{22}\) showed that sympathetic stimulation and low concentrations of ryanodine infusion induced spontaneous SR Ca\(^{2+}\) release, triggered activity and focal discharges from the PVs to perpetuate AF induction in normal dogs.

\(Ca^{2+}\) Dynamics and Vagal Atrial Fibrillation in Heart Failure

Direct autonomic nerve recordings in a canine heart failure model showed that not only sympathetic but also vagal nerves discharges were increased in heart failure dogs, and simultaneous sympathovagal discharges were common triggers of atrial arrhythmias.\(^{39}\) It is well known that vagal nerve stimulation and acetylcholine infusion can cause significant changes in cardiac electrophysiology, including heterogeneous effects on atrial refractory period,\(^{40}\) on pacemaker activity and atrioventricular conduction,\(^{41}\) and on induction of AF.\(^{42}\) Cervical vagal stimulation shortens the atrial effective refractory period primarily in the high right atrium and facilitates the induction of AF by single premature extrastimulus.\(^{43}\) Counsel et al\(^{44}\) reported that vagal activity might predispose patients to develop paroxysmal atrial arrhythmias. They studied 18 middle-aged men and found that sinus slowing often

67
preceeded the onset of atrial arrhythmias in most subjects. The authors proposed that vagal activation might induce APD shortening, which in turn facilitates reentrant atrial arrhythmias. A computer simulation study has suggested that vagal AF may arise from acetylcholine-induced stabilization of the primary spiral-wave generator and disorganization of propagation by repolarization gradient that causes fibrillatory dynamics. As $I_{K_{ACH}}$ activation shortens APD and hyperpolarizes the cell membrane, Atienza et al. reported that adenosine activates $I_{K_{ACH}}$ and accelerates AF by promoting reentry rather than triggered activity in human. However, our recent study demonstrated the coexistence of PV focal discharge and PV-LA microreentry and suggested that both triggered and reentrant activities are important during vagal AF in a canine heart failure model. By simultaneous Vm and Ca$^{2+}$ mapping, we documented that pause-related large Ca$^{2+}$ elevation is associated with focal discharges in the PVs. A long preceding pause increases the Ca$^{2+}$ accumulation, leading to a greater SR Ca$^{2+}$ release at the first beat after the pause. Because the APD was reduced by acetylcholine, this large rise of Ca$^{2+}$ resulted in persistent Ca$^{2+}$ elevation into late phase 3 to induce late phase 3 EADs and PV focal discharges. These triggered beats followed by sustained PV-LA microreentry can induced atrial tachycardia and AF. Failing hearts have increased $I_{NCX}$ current, which renders them more susceptible to the late phase 3 EADs. Acetylcholine may increase Na$^{+}$ conductance and intracellular Na$^{+}$ activity, leading to altered $I_{NCX}$, reduced Ca$^{2+}$ efflux, and further enhanced Ca$^{2+}$ accumulation. The hypothesis is also supported by the suppression of late phase 3 EADs by ryanodine and thapsigargin infusion. Parasympathetic activation and acetylcholine release could be important mechanisms in the pathophysiology and atrial arrhythmogenesis in the heart failure status. Livinas et al. reported that neurally mediated mechanisms may be implicated in the pathophysiology of syncope in patients with dilated cardiomyopathy. In that study, both sympathetic and parasympathetic heart rate parameters were markedly stimulated.

**Conclusion**

There is increasing awareness that abnormal Ca$^{2+}$ handling contributes to both the initiation and maintenance of AF. Simultaneous sympathovagal activation increases Ca$^{2+}$ and abbreviates APD at the same time, resulting in triggered activity in the PVs, leading to initiation of AF. Also, there is evidence of heightened atrial sympathetic innervation in patients with persistent AF, suggesting that modification of autonomic substrate may in part serve as the atrial substrate for AF maintenance. The evidence to date has suggested that autonomic modulation could play an adjunctive role in catheter ablation of AF, although the effectiveness has not been consistent and the endpoint for assessment of autonomic modulation lacking. However, interactions between autonomic nervous system and AF are more complex than currently understood. Further studies are warranted before therapeutic application in clinical AF.

**References**