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New Concepts in Atrial Fibrillation: Mechanism and Remodeling

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Atrial fibrillation (AF) is a complex disease with multiple possible mechanisms.¹ Many studies have indicated that the arrhythmogenic foci within the thoracic veins are AF initiators. Once initiated, AF alters atrial electrical and structural properties (atrial remodeling) in a way that promotes its own maintenance and recurrences and may alter the response to antiarrhythmic drugs. However, the exact mechanisms by which the initiators trigger AF remained elusive. One possible immediate trigger is the paroxysmal autonomic nervous system (ANS) discharge. In normal dogs, sympathetic nerve stimulation rarely triggers AF. However, in dogs underwent chronic rapid atrial pacing, sympathetic stimulation can lead to rapid repetitive activations in the isolated canine pulmonary vein (PV) and vein of Marshall preparations.^{2,3} Sharifov et al reported that a combined isoproterenol and acetylcholine infusion is more effective than acetylcholine alone in the induction of AF.⁴ Clinically, alterations of autonomic tone, involving both the sympathetic and parasympathetic nervous system, have been implicated in initiating paroxysmal AF.⁵ These results suggest that simultaneous sympathetic and parasympathetic (sympathovagal) discharge is particularly pro-fibrillatory. Also, there is evidence for heightened atrial sympathetic innervation in patients with persistent AF,⁶ suggesting that potential autonomic substrate modification may serve as part of remodeled atrial substrate for AF maintenance.

Patterns of Activation at the PV and PV-left atrial Junction During Sustained AF

AF is characterized by the co-existence of multiple activation wavelets within the atria. The mechanisms by which multiple wavefronts occur have been actively debated for many years. The focal source hypothesis states that a single rapidly focal driver underlies the mechanisms of AF. On the other hand, the multiple wavelet hypothesis posits that heterogeneous dispersion of repolarization is responsible for wavebreaks and the generation of multiple wavelets that sustain AF.⁷ Zipes et al⁸, Spach et al⁹ and Scherlag et al¹⁰ provided the first pieces of evidence to support the importance of thoracic veins in the generation of electrical activity. The

importance of these original works were proven by Haissaguerre et al,¹¹ who demonstrated the critical role of PV in the generation and maintenance of AF in humans. Hamabe et al¹² reported that the PV- left atrial (LA) junction has segmental muscle disconnection and differential muscle narrowing in dogs. These changes combined with the complex fiber orientations within the PV provide robust anatomical bases for generating conduction disturbances at the PV-LA junction and complex intra-PV conduction patterns, to facilitate reentry formation. With high-density (1-mm resolution) computerized mapping techniques, we have demonstrated that rapid PV focal discharge¹³⁻¹⁵ and PV-LA junction microreentry¹⁵ are present during sustained AF induced by rapid LA pacing. Figure 1 shows an example. Panel A shows the activation snapshots of right superior PV during sustained AF, showing three consecutive focal discharges (6081 ms, 6203 ms and 6316 ms). The focal discharge wavefronts met lines of functional conduction block (dotted lines), followed by the formation of complete reentry loops (6409 ms to 6595 ms). The wavefronts from LA also encountered a functional line of block, followed by the formation of reentry. After infusion of ibutilide (Figure 1B), a typical class III antiarrhythmic drug that is effective in prolonging the effective refractory period of atria, focal discharges (6344 ms, 6582 ms) and reentrant wavefronts activated (6035 ms to 6296 ms) at slower rates during AF. The conducted wavefronts between the PV and LA were significantly reduced by ibutilide. However, the overall incidence of focal discharge in the PVs was not suppressed. High dose of ibutilide may completely terminate all reentrant activity, thereby converting AF to PV tachycardia prior to conversion to sinus rhythm. These findings suggest that sustained AF is due to a combination of PV focal discharge as well as PV-LA reentrant activity.

A recent computational simulation study¹⁶ showed that upregulation of the L-type Ca^{2+} current steepened restitution curves of both the APD and the conduction velocity. Spontaneous firing of ectopic foci, coupled with sinus activity produced dynamic spatial dispersion of repolarization, including discordant alternans, which facilitated unidirectional conduction block and initiated reentrant atrial flutter or AF. The size of vulnerable window was larger for PVs ectopic foci than the right atrial foci. These findings imply that the ectopic beats originated from PV are more likely to trigger AF than ectopic beats from elsewhere in the atria.

Anatomical and Neural Substrates in the Pulmonary Veins

Zipes and Knope⁸ concluded that not only did atrial muscle extended for some distance into the thoracic veins, but also that these muscle sleeves received vagal innervation. It was possible that both the autonomic nerves and atrial muscles in the PVs were important in triggering AF. Subsequent works demonstrated significant heterogeneity of the cell types within the PV muscle sleeves. Masani et al¹⁷ showed that node-like cells were present in the myocardial layer of the PV of rats. Among the ordinary myocardial cells resembling those of the atrial myocardium, clear cells with structural features similar to those of sinus node cells were identified. They either appeared singly or in small groups among ordinary myocardial cells. Cheung¹⁸ reported that isolated PVs were capable of independent pacemaking activity. Light and electron microscope studies suggested that cells morphologically akin to specialized conduction cells were present in human PVs.¹⁹ Chou et al²⁰ reported that canine PVs had a layer of large pale periodic-acid-Schiff (PAS)-positive cells at the site of focal discharge, further supporting the notion that Purkinje-like cells were present in the PVs. An important distinguishing feature of the sinus node, as compared with other parts of the atria, is the presence of rich autonomic innervation.²¹⁻²³ In comparison, Tan et al²⁴ identified abundant sympathetic nerve fibers within the PV using immunohistochemical staining techniques. These findings are consistent with that reported by Masani,¹⁷ who observed that in PVs, nerve fibers containing small and large vesicles with and without dense cores were juxtaposed to the node-like cells. The close interaction between the nerve structures and the specialized muscle cells might play a role in the generation of ectopic activities.

Cardiac Autonomic Innervation

Kawashima²⁵ performed detailed anatomical studies of human cardiac autonomic innervation. The cardiac sympathetic ganglia include a superior cervical ganglion which communicates with C₁₋₃, and the cervicothoracic (stellate) ganglion which communicates with C₇₋₈-T₁₋₂. In addition, the thoracic ganglia (as low as the 7th thoracic ganglion) also contributes to the sympathetic innervation to the heart. The superior, middle and inferior cardiac nerves from these ganglia innervate the heart by following a simple course along the brachiocephalic trunk, common carotid and subclavian arteries. On the other hand, the thoracic cardiac nerves in the posterior mediastinum have to follow a complex course to reach the heart in the middle mediastinum. The parasympathetic innervation comes from the vagus nerve and is divided into superior, middle and inferior branches. While both sides of the autonomic branches run through the ventral and dorsal aspects of the aortic arch, the right autonomic cardiac nerves tend to follow a ventral course.

Numerous investigators have studied the macroscopic and microscopic anatomy of cardiac autonomic nerves within the atria. Among those that focused on PV autonomic nerves, Armour et al²⁶ provided a detailed map of autonomic nerve distributions in human hearts. They found that autonomic nerves were concentrated in “ganglionic plexi” around great vessels such as the PVs. Chiou et al determined that these nerves converged functionally onto fat pads located around the superior vena cava-aortic junction, and that catheter ablation of this fat pad effectively denervated many regions of the atria but preserved innervation of the ventricle.²⁷ On a more microscopic scale, Chevalier et al²⁸ discovered several gradients of PV autonomic innervation, with nerves more abundant in the proximal PV than distal PV and more abundant in the epicardium than endocardium. The PV-LA junction is rich in autonomic innervation.²⁴ Stimulation of the ganglionic plexi at the PV-LA junction can convert PV focal discharge into AF²⁹ and radiofrequency ablation at these sites can potentially result in successful denervation and prevent AF inducibility.³⁰

Vagal Influences on Cardiac Electrophysiology

It is well known that vagal nerve stimulation and acetylcholine infusion can result in significant changes of cardiac electrophysiology, including heterogeneous effects on atrial refractory period,³¹ on pacemaker activity and atrioventricular conduction,³² and on induction of AF.³³ Cervical vagal stimulation shortens the atrial effective refractory period primarily in the high right atrium and facilitates induction of AF by single premature extrastimulus.³⁴ Coumel et al³⁵ reported that vagal activity might predispose patients to develop paroxysmal atrial arrhythmias. In that report, the authors studied 18 human cases and discovered sinus slowing often preceded the onset of atrial arrhythmias in these mostly middle aged male. The authors proposed that vagal activation might induce shortening of the action potential duration (APD), which in turn facilitates reentrant atrial arrhythmias.

Sympathetic Activation and the “Ca_i-transient triggering” Hypothesis

Two recent works have further enhanced the understanding of the mechanisms by which sympathovagal activation facilitates the onset of paroxysmal AF. Burashnikov and Antzelevitch³⁶ infused acetylcholine to abbreviate atrial APD and permit rapid pacing in isolated coronary-perfused canine right atrium, which led to Ca_i accumulation. If this is coupled with a long pause (such as that occurred after AF), then a large Ca²⁺ release from the sarcoplasmic reticulum could induce late phase 3 early afterdepolarizations (EADs) and extrasystoles that initiated AF. This novel late phase 3 EAD mechanism is observed only in association with marked APD abbreviation. Patterson et al³⁷ showed that simultaneous infusion of norepinephrine and acetylcholine during rapid pacing facilitated the development of EADs and triggered atrial tachycardias. They also measured tension development and

discovered that the persistent diastolic elevation of tension was associated with EADs. Assuming that tension is a good measure of Ca_i , then diastolic Ca_i elevation underlies the mechanisms of EADs. The authors named this phenomenon “ Ca_i transient triggering” and suggested that increased forward Na-Ca exchanger current might contribute to the generation of EADs.

It is known that the muscle sleeves of thoracic veins are capable of developing automaticity and triggered activity during sympathetic stimulation.³⁸ Ryanodine at low concentrations (0.5–2 μ mol/L) causes a Ca^{2+} -independent Ca_i release and facilitates the development of pacemaker activity in rabbit PVs.³⁹ The importance of Ca_i transient in atrial arrhythmogenesis was further supported by a study that used isolated, Langendorff-perfused canine PV-LA preparations and two cameras to map membrane potential and Ca_i simultaneously.²⁰ Rapid PV firing was induced by rapid atrial pacing, low dose ryanodine and isoproterenol infusion; and the rise of Ca_i preceded the action potential upstroke during focal discharge. There was clustering of PAS-positive large cells around the PV focal discharge sites. To further determine the interaction between sympathetic nerves and the PAS-positive cells, Tan et al⁴⁰ performed a study in normal dogs. After sinus node crushing, left stellate ganglion stimulation caused PV tachycardias. The focus of tachycardia was determined by multichannel computerized mapping. PAS staining at the site of PV ectopy showed abundant pale-looking, glycogen-rich specialized conducting (Purkinje) cells. In addition, immunostaining showed abundant sympathetic (tyrosine hydroxylase positive) nerves at those sites. These preliminary results support the notion that sympathetic stimulation induced PV focal discharge from sites with juxtapositioning of specialized conducting cells and autonomic nerves.

Structural Anatomy of the Atrial and PV Autonomic Nerves

Pappone et al⁴¹ hypothesized that the induction of bradycardia was due to vagal nerve stimulation whereas the abolition of bradycardia with continued RF application suggests vagal denervation. However, the distribution of adrenergic and cholinergic nerves in this region were not delineated, so it is unclear whether sympathetic nerves were also eliminated during RF application. Tan et al²⁴ performed immunostaining of 192 PV-LA segments harvested from 32 veins of 8 human autopsied hearts using anti-tyrosine hydroxylase antibodies to label adrenergic nerves and anti-choline acetyltransferase antibodies to label cholinergic nerves. Nerve densities were analyzed along the longitudinal and circumferential axes of the PV-LA junction. Longitudinally, adrenergic and cholinergic nerve densities were highest in the LA within 5 mm from the PV-LA junction, than further distally in the PV or more proximally in the LA proper. Circumferentially, both nerve densities were higher in the superior aspect of LSPV, anterosuperior aspect of RSPV and inferior aspects of the both inferior PVs than diametrically opposite; and higher in the epicardial than endocardial half of the tissue. Significantly, no area of discrete adrenergic or cholinergic predominance was noted. Rather both nerve types have similar macroscopic distributions in and around PVs. Additionally, confocal microscopy of dual-stained sections showed that at cellular levels, up to 25% of all nerve fiber bundles contained both adrenergic and cholinergic nerves, over 90% of ganglia contain both adrenergic and cholinergic elements within the same ganglion, and up to 30% of ganglion cell bodies may simultaneously express both adrenergic and cholinergic enzymes within its neuroplasm. These data indicate that adrenergic and cholinergic nerves are highly co-located not only at tissue but also at cellular levels.

Implications of Neural Anatomy of the Pulmonary Vein

If both sympathetic and parasympathetic nerves are co-stimulated/ablated, why is bradycardia the dominant response elicited during ganglionic stimulation/ablation rather than tachycardia? We propose several explanations. Firstly, complex extracardiac neural pathways^{27,42} that

involved in the generation of bradycardic reflexes during stimulation/ablation around the PVs project to vagal nuclei centrally, but do not involve sympathetic tracts generally.⁴² Secondly, a paracrine mechanism might be in operation as ganglion cells are predominantly cholinergic,²⁴ and would release mostly acetylcholine when stimulated/ablated. Thirdly, adrenergic nerves are more widely distributed than cholinergic nerves.^{24,43} Hence, radiofrequency ablation may eliminate a greater proportion of cholinergic nerves than that of adrenergic nerves, disrupting sympathovagal balance. Clinical reports^{41,30} show that autonomic reflexes are most commonly elicited within approximately 1cm of PV-LA junction. The anatomic co-localization of adrenergic and cholinergic innervations implies that it would be virtually impossible to selectively eliminate only sympathetic or parasympathetic nerves during catheter ablation of AF. The co-existence of adrenergic and cholinergic phenotypes within ganglionic cell neuroplasm also suggests that when ganglion cells are stimulated, both adrenergic and cholinergic mediators may be released simultaneously, affecting cellular electrophysiology in ways that may predispose to triggered activity.³⁷

Sympathetic Nerve Recordings in Animal Models of Paroxysmal AF

Barrett et al⁴⁴ first reported successful recording of renal sympathetic nerve activity in conscious rabbits continuously for > 7 days. However, the renal sympathetic nerve activity may not predict the cardiac sympathetic nerve activity. To record cardiac sympathetic nerve activity, Jung et al⁴⁵ used Data Sciences International transmitters to record stellate ganglion nerve activity 24 hr a day, 7 days a week for an average of 41.5 ± 16.6 days in normal ambulatory dogs. The results showed a circadian variation of sympathetic outflow. However, normal dogs rarely develop paroxysmal AF. To test the hypothesis that spontaneous ANS discharges can serve as triggers of paroxysmal AF, it is necessary to develop an animal model of paroxysmal AF. Wijffels et al⁴⁶ previously demonstrated that intermittent rapid pacing could induce progressively increased electrophysiological remodeling, leading to persistent AF. Rapid atrial pacing also causes significant neural remodeling characterized by heterogeneous increase of sympathetic innervation⁴⁷ and extensive nerve sprouting.⁴⁸ In a preliminary study, Tan et al⁴⁹ implanted Data Sciences International transmitters to directly record left stellate ganglion nerve activity, left vagal nerve activity and LA local bipolar electrograms or surface ECG simultaneously in ambulatory dogs over several weeks. Intermittent rapid atrial pacing was performed and ANS activity was monitored when the pacemaker was turned off. Paroxysmal atrial tachycardia and paroxysmal AF were documented and that simultaneous sympathovagal discharges were the most common triggers of paroxysmal atrial tachycardia and paroxysmal AF in this study. These preliminary results further support the hypothesis that ANS activity is important in the generation of paroxysmal AF.

Autonomic Nervous System and AF in Human Patients

Several observations suggest the ANS plays an important role in both the initiation and/or the maintenance of AF in humans. Most patients with idiopathic paroxysmal AF appear to be vagally dependent, with a heightened susceptibility to vasovagal cardiovascular response. In contrast, in most patients with organic heart diseases, the paroxysmal AF episodes appear more sympathetically dependent.⁵⁰ A shift toward an increase in sympathetic tone or toward a loss of vagal tone has been observed before postoperative paroxysmal AF,⁵¹ before the onset of atrial flutter⁵² and before paroxysmal AF occurring during sleep⁵³; whereas a shift toward vagal predominance was observed in young patients with lone AF and nocturnal episodes of paroxysmal AF.⁵⁴ More recently, a primary increase in adrenergic drive followed by marked modulation toward vagal predominance immediately before the onset of paroxysmal AF was observed.^{5,55,56} However, the ANS activity in all these studies was indirectly evaluated by the analysis of heart rate variability parameters on continuous ECG recordings. Heart rate variability measures changes in the relative degree of ANS, not the absolute level of

sympathetic or parasympathetic discharges. It is therefore necessary to perform direct recording of sympathetic and vagal nerve activity to prove or disprove these observations in ambulatory animals.

Neural Modulation as a Potential Therapeutic Strategy

The effectiveness of autonomic modulation as an adjunctive therapeutic strategy to catheter ablation of AF has been inconsistent. While favorable results have been obtained by Pappone et al and Nakagawa et al,^{30;41} others have found no beneficial⁵⁷ or deleterious⁵⁸ outcomes between patients with denervation compared to those without, a finding also underlined by animal studies by Hirose et al³⁴, where partial vagal denervation of the high right atrium was found to increase inducibility of AF. These conflicting studies suggest that the interactions between the ANS and AF are more complex than are currently understood. Perhaps a degree of individual variability accounts for these discrepancies, with some patients having more pronounced autonomic triggers than others. As an illustration, Scanavacca et al⁵⁹ recently found that in a small number of patients with “autonomic” paroxysmal AF, denervation alone without substrate modification in the atria was effective in preventing AF recurrence in two out of eleven patients, these two patients having the most pronounced and persistent changes in heart rate variability. In summary, the evidence to date suggests that autonomic modulation does have an adjunctive role to play in catheter AF ablation, especially when applied selectively. Further mechanistic and clinical studies are warranted before a wider application can be recommended.

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Figure 1.

Patterns of activation during sustained AF in dog with chronic rapid atrial pacing. Panel A, snapshots of focal discharge and reentrant activation patterns within right super PV at baseline AF. Asterisk in the left upper corner indicates the anatomical location of focal discharge at the proximal right superior PV. Black horizontal dotted line indicates the PV-LA junction. The number below each snapshot represents the time in ms with the beginning of data acquisition as time zero. In snapshots, red color represents the wavefront; black arrows, the direction of wave propagation; black dotted line, line of block; arrow head, site of focal discharge. The color bar on the right shows the time scale (0 to 50 ms). Panel B, snapshots of focal discharge and reentrant activation patterns within right superior PV after 0.02 mg/kg ibutilide infusion. A rectangle towards the end of the ECG tracings shows the time period corresponding to the snapshots in Panels A and B. (permission from Chou *et al. Am J Physiol Heart Circ Physiol* 289: H2704, Figure 2)