New Concepts in Atrial Fibrillation: Neural Mechanisms and Calcium Dynamics

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KEYWORDS

• Atrium • Calcium • Fibrillation • Nervous system
• Autonomic

Atrial fibrillation (AF) is a complex disease with multiple possible mechanisms.[1] Many studies indicate 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. The exact mechanisms through which the arrhythmogenic foci are triggered remain elusive, however. One possible immediate trigger is the paroxysmal autonomic nervous system (ANS) discharge. In normal dogs, sympathetic nerve stimulation rarely triggers AF. In dogs that undergo chronic rapid atrial pacing, however, sympathetic stimulation can lead to rapid repetitive activations in the isolated canine pulmonary vein (PV) and vein of Marshall preparations.[2,3]

Sharifov and colleagues[4] reported that a combined isoproterenol and acetylcholine infusion is more effective than acetylcholine alone in inducing AF. Clinically, alterations of autonomic tone, involving the sympathetic and parasympathetic nervous systems, are implicated in initiating paroxysmal AF.[5] These results suggest that simultaneous sympathetic and parasympathetic (sympathovagal) discharge is particularly profibrillatory. Also, evidence shows heightened atrial sympathetic innervation in patients who have persistent AF,[6] suggesting that potential autonomic substrate modification may be part of a remodeled atrial substrate for AF maintenance.

PATTERNS OF ACTIVATION AT THE PULMONARY VEIN AND PULMONARY VEIN–LEFT ATRIAL JUNCTION DURING SUSTAINED ATRIAL FIBRILLATION

AF is characterized by the coexistence of multiple activation wavelets within the atria. The mechanisms through which multiple wavefronts occur have been debated for many years. The focal source hypothesis states that a single rapidly focal driver underlies the mechanisms of AF. Alternatively, 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 and Knope,[8] Spach and colleagues,[9] and Scherlag and colleagues[10] provided the first pieces of evidence supporting the importance of thoracic veins in the generation of electrical activity. The importance of these original works were proven by Haissaguerre and colleagues,[11] who showed the critical role of PV in the generation and maintenance of AF in humans.

Hamabe and colleagues[12] reported that the PV–left atrial (LA) junction has segmental muscle disconnection and differential muscle narrowing in dogs.

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Combined with the complex fiber orientations within the PV, these changes provide robust anatomic bases for generating conduction disturbances at the PV–LA junction and complex intra-PV conduction patterns, to facilitate reentry formation.

High-density (1-mm resolution) computerized mapping techniques have shown that rapid PV focal discharges[13–15] and PV–LA junction microreentry[15] are present during sustained AF induced by rapid LA pacing (**Fig. 1**). **Fig. 1**A shows the activation snapshots of right superior PV during sustained AF, showing three consecutive focal discharges (6081, 6203, and 6316 ms). The focal discharge wavefronts meet lines of functional conduction block (dotted lines) followed by the formation of complete reentry loops (6409–6595 ms). The wavefronts from LA also encountered a functional line of block, followed by the formation of reentry. After infusion of ibutilide (**Fig. 1**B), a typical class III antiarrhythmic drug that can prolong the effective refractory period of atria, focal discharges (6344 and 6582 ms) and reentrant wavefronts (6035–6296 ms) activated at slower rates. The conducted wavefronts between the PV and LA were reduced significantly by ibutilide. The overall incidence of focal discharge in the PVs was not suppressed, however. A high dose of ibutilide may terminate all reentrant activity completely, thereby converting AF to PV tachycardia before conversion to sinus rhythm. These findings suggest that sustained AF is the result of a combination of PV focal discharge and PV–LA reentrant activity.

A recent computational simulation study[16] showed that up-regulation of the L-type Ca^{2+} current steepened restitution curves of the action potential duration (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 PV ectopic foci than for right atrial foci. These findings imply that the ectopic beats originated from the PV are more likely to trigger AF than ectopic beats from elsewhere in the atria.

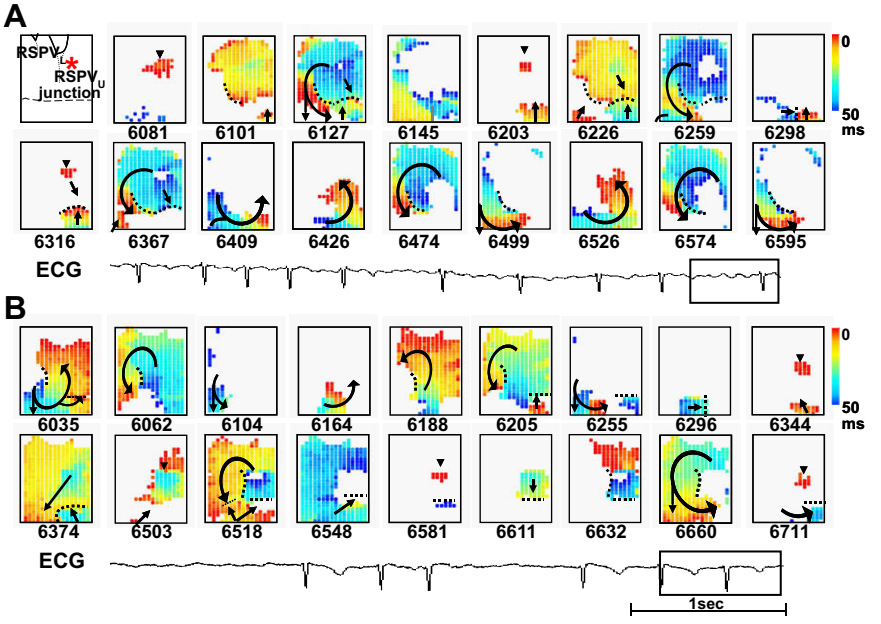

Fig. 1. Patterns of activation during sustained AF induced by chronic rapid atrial pacing. (*A*) Snapshots of focal discharge and reentrant activation patterns within right super PV at baseline AF. Asterisk in the left upper corner indicates the anatomic location of focal discharge at the proximal right superior PV. Black horizontal dotted line indicates the PV–LA junction. The number below each snapshot represents the time in milliseconds, 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; arrowhead, site of focal discharge. The color bar on the right shows the time scale (0–50 ms). (*B*) Snapshots of focal discharge and reentrant activation patterns within right superior PV after ibutilide infusion (0.02 mg/kg). A rectangle toward the end of the ECG tracings shows the period corresponding to the snapshots in (*A*) and (*B*). (*From* Chou CC, Zhou S, Tan AY, et al. High-density mapping of pulmonary veins and left atrium during ibutilide administration in a canine model of sustained atrial fibrillation. Am J Physiol Heart Circ Physiol 2005;289:H2704; with permission).

ANATOMIC AND NEURAL SUBSTRATES IN THE PULMONARY VEINS

Zipes and Knope[8] conclude that not only did atrial muscle extend for some distance into these the thoracic veins but also these muscle sleeves received vagal innervation. Both the autonomic nerves and atrial muscles in the PVs may have been important in triggering AF. Subsequent works showed significant heterogeneity of the cell types within the PV muscle sleeves. Masani and colleagues[17] 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 appeared singly or in small groups among ordinary myocardial cells.

Cheung[18] reported that isolated PVs were capable of independent pacemaking activity. Light and electron microscope studies suggest that cells morphologically akin to specialized conduction cells were present in human PVs.[19] Chou and colleagues[20] reported that canine PVs had a layer of large, pale, periodic acid-Schiff (PAS)–positive cells at the site of focal discharge, supporting the notion that Purkinje-like cells were present in the PVs.

A distinguishing feature of the sinus node, compared with other parts of the atria, is the presence of rich autonomic innervation.[21–23] In comparison, Tan and colleagues[24] identified abundant sympathetic nerve fibers within the PV using immunohistochemical staining techniques. These findings are consistent with those reported by Masani,[17] who observed that in PVs, nerve fibers containing small and large vesicles with and without dense cores were juxtaposed with 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

Kawashima[25] performed detailed anatomic studies of human cardiac autonomic innervation. The cardiac sympathetic ganglia include a superior cervical ganglion that communicates with C1 through C3, and the cervicothoracic (stellate) ganglion, which communicates with C7 through T2. In addition, the thoracic ganglia (as low as the seventh thoracic ganglion) also contribute 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. Alternatively, the thoracic cardiac nerves in the posterior mediastinum follow a complex course to reach the heart in the middle mediastinum. Parasympathetic innervation is supplied by the vagus nerve and is divided into superior, middle, and inferior branches. Although 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.

Many investigators have studied the macroscopic and microscopic anatomy of cardiac autonomic nerves within the atria. Among those that focused on PV autonomic nerves, Armour and colleagues[26] 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 and colleagues[27] 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.

On a more microscopic scale, Chevalier and colleagues[28] 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 this junction can convert PV focal discharges into AF,[29] and radiofrequency ablation at these sites can potentially result in successful denervation and prevent AF inducibility.[30,31]

A recent experimental vagal AF study in canines[31] reported that ablation of the autonomic ganglia at the base of the PVs suppresses the effective refractory period–abbreviating and AF-promoting effects of cervical vagal stimulation, suggesting that ganglionic plexi ablation may contribute to the effectiveness of PV-directed ablation.

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,[32] pacemaker activity and atrioventricular conduction,[33] and induction of AF.[34] Cervical vagal stimulation shortens the atrial effective refractory period primarily in the high right atrium and facilitates induction of AF through single premature extrastimulus.[35] Coumel and colleagues[36]

reported that vagal activity might predispose patients to paroxysmal atrial arrhythmias. In 18 human cases (mostly middle-aged men, 30 ~ 50 years old), the investigators discovered sinus slowing often preceded the onset of atrial arrhythmias. The investigators proposed that vagal activation might induce shortening of the APD, facilitating reentrant atrial arrhythmias.

SYMPATHETIC ACTIVATION AND THE "CALCIUM-TRANSIENT TRIGGERING" HYPOTHESIS

Two recent works have enhanced understanding of the mechanisms through which sympathovagal activation facilitates the onset of paroxysmal AF. Burashnikov and Antzelevitch[37] infused acetylcholine to abbreviate atrial APD and permit rapid pacing in an isolated coronary-perfused canine right atrium, which led to intracellular calcium (Ca_i) accumulation. If this practice is coupled with a long pause (such as that occurred after AF), then a large Ca^{2+} release from the sarcoplasmic reticulum could induce the 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 and colleagues[38] 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 investigators termed this phenomenon *Ca_i transient triggering* and suggested that increased forward Na^+–Ca^{2+} exchanger current might contribute to the generation of EADs.

The muscle sleeves of thoracic veins are capable of developing automaticity and triggered activity during sympathetic stimulation.[39] Ryanodine at low concentrations (0.5–2 μmol/L) causes a calcium-independent Ca_i release and facilitates the development of pacemaker activity in rabbit PVs.[40] The importance of Ca_i transient in atrial arrhythmogenesis is 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 and low-dose ryanodine and isoproterenol infusion, and the rise of Ca_i preceded the action potential upstroke during focal discharge. A clustering of PAS-positive large cells was seen around the PV focal discharge sites.

To determine the interaction between sympathetic nerves and PAS-positive cells, Tan and colleagues[41] 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 results support the notion that sympathetic simulation induced PV focal discharge from sites, with juxtapositioning of specialized conducting cells and autonomic nerves.

STRUCTURAL ANATOMY OF THE ATRIAL AND PULMONARY VEIN AUTONOMIC NERVES

Pappone and colleagues[42] hypothesized that bradycardia induction was caused by vagal nerve stimulation, whereas its abolition with continued radiofrequency application suggests vagal denervation. The distribution of adrenergic and cholinergic nerves in this region were not delineated, however, and therefore whether sympathetic nerves were also eliminated during radiofrequency application is unclear.

Tan and colleagues[24] performed immunostaining of 192 PV–LA segments harvested from 32 veins of eight human autopsied hearts using antityrosine hydroxylase antibodies to label adrenergic nerves and anticholine 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 versus further distally in the PV or more proximally in the LA proper. Circumferentially, both nerve densities were higher in the superior aspect of the left superior PV, anterosuperior aspect of the right superior PV, and inferior aspects of both inferior PVs than diametrically opposite, and higher in the epicardium than endocardium.

Significantly, the investigators noted no area of discrete adrenergic or cholinergic predominance.[24] Instead, 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 adrenergic and cholinergic nerves, more than 90% of ganglia contain adrenergic and cholinergic elements within the same ganglion, and up to 30% of ganglion cell bodies may express adrenergic and

cholinergic enzymes simultaneously within the neuroplasm. These data indicate that adrenergic and cholinergic nerves are highly colocated not only at tissue but also at cellular levels.

IMPLICATIONS OF NEURAL ANATOMY OF THE PULMONARY VEIN

If sympathetic and parasympathetic nerves are costimulated or ablated, why is bradycardia the dominant response elicited during ganglionic stimulation or ablation rather than tachycardia? Several explanations are proposed. First, complex extracardiac neural pathways[27,43] involved in the generation of bradycardic reflexes during stimulation or ablation around the PVs, project to vagal nuclei centrally but do not involve sympathetic tracts.[43] Second, a paracrine mechanism might be in operation, because ganglion cells predominantly are cholinergic[24] and release mostly acetylcholine when stimulated or ablated. Third, adrenergic nerves are distributed more widely than cholinergic nerves.[24,44] Hence, radiofrequency ablation is more likely to eliminate adrenergic nerves than cholinergic nerves, resulting in a heightened vagal tone and bradycardia. Clinical reports[30,42] show that autonomic reflexes are elicited most commonly within approximately 1 cm of the PV–LA junction. The anatomic colocalization of adrenergic and cholinergic innervations implies that it would be almost impossible to eliminate only sympathetic or parasympathetic nerves during catheter ablation of AF. The coexistence of adrenergic and cholinergic phenotypes within ganglionic cell neuroplasm also suggests that when ganglion cells are stimulated, adrenergic and cholinergic mediators may be released simultaneously, affecting cellular electrophysiology in ways that may predispose to triggered activity.[38]

AUTONOMIC NERVOUS SYSTEM AND ATRIAL FIBRILLATION IN HUMANS

Several observations suggest that the ANS plays an important role in the initiation and maintenance of AF in humans. Most patients who have idiopathic paroxysmal AF seem vagally dependent, with a heightened susceptibility to vasovagal cardiovascular response. In contrast, in most patients who have organic heart diseases, the paroxysmal AF episodes seem more sympathetically dependent.[45] A shift toward an increase in sympathetic tone or a loss of vagal tone has been observed before postoperative paroxysmal AF,[46] the onset of atrial flutter,[47] and paroxysmal AF occurring during sleep,[48] whereas a shift toward vagal predominance had been observed in young patients who have lone AF and nocturnal episodes of paroxysmal AF.[49] More recently, three studies reported a primary increase in adrenergic drive followed by marked modulation toward vagal predominance immediately before the onset of paroxysmal AF.[5,50,51] The ANS activity in all of these studies was evaluated indirectly, however, through 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. Therefore, sympathetic and vagal nerve activity must be recorded directly to prove or disprove these observations in ambulatory animals.

SYMPATHETIC NERVE RECORDINGS IN ANIMAL MODELS OF PAROXYSMAL ATRIAL FIBRILLATION

Barrett and colleagues[52] first reported successful continuous recording of renal sympathetic nerve activity in conscious rabbits for more than 7 days. The renal sympathetic nerve activity may not predict the cardiac sympathetic nerve activity, however.

To record cardiac sympathetic nerve activity, Jung and colleagues[53] used Data Sciences International transmitters to record stellate ganglion nerve activity, 24 h/d, 7 d/wk, for an average of 41.5 $\pm$ 16.6 days in normal ambulatory dogs. The results showed a circadian variation of sympathetic outflow. Normal dogs rarely develop paroxysmal AF, however. To test the hypothesis that spontaneous ANS discharges can serve as triggers of paroxysmal AF, an animal model of paroxysmal AF must be developed.

Wijffels and colleagues[54] showed 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 innervation[55] and extensive nerve sprouting.[56]

Tan and colleagues[57] 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 the investigators found that simultaneous sympathovagal discharges were the most common triggers of paroxysmal atrial tachycardia and paroxysmal AF. Cryoablation of the stellate ganglia and the superior cardiac branches of vagal

nerve eliminated all episodes of paroxysmal AF and atrial tachycardias.

These results further support the hypothesis that ANS activity is important in the generation of paroxysmal AF. Histologic examinations of cryoablated dogs showed cardiac nerve sprouting and sympathetic hyperinnervation in the atria. These findings suggest decentralization rather than denervation of the sympathovagal nerves underlies the antiarrhythmic mechanism of stellate ganglion and vagal nerve ablation.

CA_i DYNAMICS AND VAGAL ATRIAL FIBRILLATION IN HEART FAILURE

The Framingham Heart Study[58] concluded that in subjects experiencing heart failure, late development of AF was associated with increased mortality. Heart failure–related atrial arrhythmias seem to arise from macroreentrant sources, primarily by increasing atrial size and promoting interstitial fibrosis.[59] In addition to macroreentry, Stambler and colleagues[60] reported that triggered activity induced by delayed afterdepolarizations may also be a mechanism of focal atrial tachycardias in pacing-induced dogs with heart failure.

Okuyama and colleagues[61] showed that some AF episodes were characterized by focal activations in the PVs and vein of Marshall, and by complex fractionated wavefronts within the PVs in a canine heart failure model, suggesting the occurrence of significant proarrhythmic remodeling in the PVs during heart failure. A major arrhythmogenic mechanism in heart failure results from altered ryanodine receptor function.[62] A combination of abnormal ryanodine receptor and increased sympathetic tone during exercise can cause triggered activity.[63]

Alternatively, direct autonomic nerve recordings in a canine heart failure model showed that not only sympathetic but also vagal nerve discharges were increased in dogs with heart failure, and simultaneous sympathovagal discharges were common triggers of atrial arrhythmias.[64] A computer simulation study[65] suggested that vagal AF may arise from acetylcholine-induced stabilization of the primary spiralwave generator and disorganization of propagation by repolarization gradient that causes fibrillatory dynamics.

Because acetylcholine-dependent potassium channel (I_{KACh}) activation shortens APD and hyperpolarizes the cell membrane, Atienza and colleagues[66] reported that adenosine activates I_{KACh} and accelerates AF through promoting reentry rather than triggered activity in human. However, Chou and colleagues[67] reported that acetylcholine facilitates both PV focal discharges and PV–LA microreentry during vagal AF in a canine heart failure model.

Using isolated, Langendorff-perfused canine PV–LA preparations and two cameras to map membrane potential and Ca_i simultaneously, investigators showed that pause-related large Ca_i elevation is associated with focal discharges in the PVs.[37] A long preceding pause increases the Ca_i accumulation, causing a greater release of Ca^{2+} from the sarcoplasmic reticulum at the first beat after the pause. Because the APD was reduced by acetylcholine, this large rise of Ca_i resulted in persistent Ca_i elevation into late phase 3, to induce late phase 3 EADs and PV focal discharges.[37,38,68] These triggered beats, followed by sustained PV–LA microreentry, can induce atrial tachycardia and AF, suggesting that both triggered and reentrant activities are important during vagal AF.

Failing hearts have increased sodium-calcium exchanger channel current (I_{NCX}),[69] 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_i efflux[70,71] and further enhanced Ca_i 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 of heart failure. Livanis and colleagues[72] reported that neurally mediated mechanisms may be implicated in the pathophysiology of syncope in patients who have dilated cardiomyopathy. In that study, sympathetic and parasympathetic heart rate parameters were markedly stimulated.

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. Although favorable results have been reported by Nakagawa and colleagues[30] and Pappone and colleagues,[42] others found no beneficial[73] or deleterious[74] outcomes in patients who had denervation compared with those who did not, a finding also underlined by animal studies by Hirose and colleagues,[35] in which 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 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 and colleagues[75] recently found that in a small number of patients who had ''autonomic'' paroxysmal AF, denervation alone without substrate modification in the atria effectively prevented AF recurrence in 2 of 11 patients, with these patients having the most pronounced and persistent changes in heart rate variability. In summary, current evidence suggests that autonomic modulation has 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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