Local Innervation and Atrial Fibrillation

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Atrial fibrillation (AF) is the most common human arrhythmia in the world and, depending on individual demographics, afflicts from 0.1% to 4% of the general population.1 With the steady rise of cardiovascular disease in the industrialized world, it is no surprise that the incidence of AF is also increasing.2,3 However, despite its ubiquitous presence in adult medicine, AF remains an enigmatic disease whose pathophysiology is very complex and incompletely understood. Unlike traditional macroreentrant arrhythmias that arise from better understood electrophysiological substrates, AF is a more complex disease arising from multiple, mutually interacting mechanisms. In part, because of our incomplete understanding of the mechanisms underlying AF, therapeutic success rates in patients treated with either antiarrhythmic drug therapy, catheter-based ablation, or both remain highly variable.4

Emerging data from the basic science laboratory is starting to shed some light on the mechanistic basis of this disease, which may lead to the development of long-term therapies for patients with AF. One of the ideas that has persisted for several decades about AF, and is fast gaining traction, is that there may be a strong contribution from the autonomic nervous system in both the initiation and maintenance of this disease.5 The structural basis for this theory stems from the discovery of rich autonomic innervation and ganglionated plexi (GP) associated with the pulmonary veins and sites in the atria of rich autonomic innervation and ganglionated plexi (GP) associated with the pulmonary veins and sites in the atria from which AF triggers often originate.6 Although the exact causal relationship between these autonomic connections and the initiation of AF is not well understood, anecdotal clinical and laboratory findings suggest that such a link is plausible. In this review, we will explore our current understanding of the cardiac nervous system and its role in the pathogenesis of AF, with an eye toward future therapeutic strategies that could exploit this relationship.

Anatomic Considerations
The cardiac nervous system broadly encompasses all segments of the central and peripheral nervous system that innervate the heart. Although this definition is broad enough to include nonautonomic nerves, such as somatosensory nerves innervating the pericardium, in this review we will focus on the autonomic component of the cardiac nervous system. In keeping with this theme, the majority of neuronal structures in the cardiovascular system are components of either the sympathetic and parasympathetic autonomic nervous system. Anatomically, the cardiac nervous system can be subdivided into an intrinsic cardiac nervous system (ICNS) and an extrinsic cardiac nervous system (ECNS).7 The ECNS comprises fibers that mediate connections between the heart and the nervous system, whereas the ICNS consists of primarily autonomic nerve fibers once they enter the pericardial sac.

The sympathetic input to the ECNS is derived from 2 major autonomic ganglia located on the great vessels: the superior cervical ganglion and the stellate ganglion (cervicothoracic ganglion). Both of these ganglia house the cell bodies of most postganglionic sympathetic neurons whose axons terminate on the surface of the heart. These axons form heterogeneous tracts, organizing themselves into superior, middle, and inferior cardiac nerves as they travel from the cervical sympathetic trunk to innervate the heart.8 Moreover, the left-to-right distribution of these nerve pairs is not symmetrical, with at least 3 major left-sided cardiac nerves and 4 right-sided nerves that often possess significant interindividual variability. Remarkably, the pattern of target innervation by these cardiac nerves gives rise to several fascinating electrophysiological properties. For example, differences in the spatial distribution of refractoriness are observed in the anterior versus posterior ventricular myocardium based on whether sympathetic stimulation is achieved via the right versus left cardiac nerves.9–11 However, because of the individual variability in the innervation pattern of the right and left cardiac nerves, the outcome of sympathetic stimulation from these cardiac nerves is not uniformly predictable in a simple left/right to anterior/posterior paradigm. In addition to the unpredictable innervation pattern of the major cardiac nerves, there is also wide variation in how other areas of the heart are innervated by different nerves of the ECNS. These nuances pose some hurdles to the use of generalized nerve stimulation as a treatment strategy for various cardiac arrhythmias.

In contrast to the sympathetic nervous system, the parasympathetic component of the ECNS originates predominantly in the nucleus ambiguus and, to a lesser extent, in the dorsal motor nucleus of the medulla. The parasympathetic preganglionic fibers are carried almost entirely within the vagus nerve and descend to the heart. Unlike their sympathetic counterparts that form extracardiac synapses in cervical and thoracic ganglia, the efferent fibers of the cardiac parasympathetic system form synapses on the surface of the atria and send short postganglionic fibers into the sinus node, atrioventricular node, and the atrial myocardium.12

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In stark contrast to the relatively straightforward anatomy of the ECNS, the ICNS is more complex, and its precise anatomy is still not completely known (Figure 1). On entering the pericardial space, the majority of sympathetic afferents either directly innervate the myocardium or form synapses with neurons that constitute the intrinsic cardiac ganglia. Parasympathetic fibers, in contrast, almost entirely synapse with neurons within the cardiac ganglia located in a number of locations throughout the heart. Of note, each cardiac ganglion may contain anywhere from 200 to 1000 neurons,12,13 and the vast majority of these ganglia are organized into GPs on the surface of the atria and ventricles.13 Some of these atrial plexi have been mapped to the superior right atrium (adjacent to the sinoatrial node), superior left atrium and posterior right atrium (adjacent to the atrioventricular node), but a large number of formally unmapped smaller atrial plexi also exist.14 The pulmonary vein-left atrial junction in particular has a confluence of GPs and receives innervation from both limbs of the autonomic nervous system. A fair amount of data suggest that AF can be induced by directly stimulating the GPs at the PV-LA junction.15,16 Conversely, several studies in which AF can be induced by directly stimulating the GPs at the PV-LA junction and the PV ostia were ablated have achieved attenuation or extinction of preexisting AF.17–20 Some investigators, based on experimental evidence of this nature, have suggested that the atrial GPs serve as integration units that act cooperatively with components of the ECNS to manage cross talk between the heart and the autonomic nervous system. Atrial GPs also retain the ability to act independently and modulate numerous cardiac functions, such as pacemaker automaticity, myocardial contractility, and refractoriness. This body of growing evidence is helping to establish that GPs play an important role in the genesis and propagation of AF.

At the cellular level, the modulation of afferent autonomic function occurs through a myriad of receptors including mechanoreceptors, baroreceptors, and chemoreceptors located on the surface of the heart and great vessels. The afferents from these receptors communicate with the central nervous system, whose efferent output is received by the heart via adrenergic and muscarinic receptors in the myocardium. The interplay of this cast of receptors in the nervous system and heart integrate and facilitate the hemodynamic response to various cardiac stressors in a physiologically meaningful manner. For instance, the discharge pattern of atrial mechanosensors is a function of cardiac and respiratory cycles, with discharge rates increasing commensurate with rising wall tension that induces tachycardia and diuresis. This differs from ventricular mechanosensors, whose response to wall stress is one that results in bradycardia and hypotension.

**Pulmonary Vein Isolation**

The evolution of catheter-based ablation as an effective strategy for treating AF is rooted in our progressive understanding of its pathogenesis. Early work in this area suggested that AF was initiated by multiple wavelets generated within the atria, and sustained by wave breaks triggered by heterogeneous dispersion in repolarization.21 In 1972, several independent investigators provided evidence contrary to this view, contending that the thoracic veins were crucial to the genesis of AF. In fact, these authors were the first to notice rich vagal innervation entering the myocardial sleeve of atrial tissue that extended into the thoracic veins.22–24 However, one of the most important discoveries in the field of AF occurred in 1998 when Haissaguerre and colleagues5 proposed that focal activity in the pulmonary veins was critical for the initiation and maintenance of AF in humans. Indeed, this seminal work launched the technique of targeting pulmonary vein triggers as a therapeutic strategy for AF and provided the framework for the technique of pulmonary vein isolation (PVI) that is widely used to treat AF today.

Of note, focal firing of the PV appears to require autonomic influences to convert the focal firing into AF,25 and directly targeting focal sites within the PVs with radiofrequency (RF) energy was not effective, because this approach was plagued by recurrent AF and PV stenosis.26 The current approach of applying RF lesions outside the PV ostia was then adopted and has proven to be far more efficacious. This strategy uses circumferential RF lesions delivered between the focal source of PV firing and the atria in an attempt to electrically isolate the AF triggers to the PV from the substrate of AF propagation in the atria. Although this approach works reasonably well, it has also produced some interesting observations and insights into AF mechanisms. For example, it was realized early on that complete PVI was not crucial for successfully eliminating AF. In some cases, delivering RF lesions partially across the PV ostia was then adopted and has proven to be far more efficacious. This strategy uses circumferential RF lesions delivered between the focal source of PV firing and the atria in an attempt to electrically isolate the AF triggers to the PV from the substrate of AF propagation in the atria. Although this approach works reasonably well, it has also produced some interesting observations and insights into AF mechanisms. For example, it was realized early on that complete PVI was not crucial for successfully eliminating AF. In some cases, delivering RF lesions partially across the PV ostia was then adopted and has proven to be far more efficacious. This strategy uses circumferential RF lesions delivered between the focal source of PV firing and the atria in an attempt to electrically isolate the AF triggers to the PV from the substrate of AF propagation in the atria. Although this approach works reasonably well, it has also produced some interesting observations and insights into AF mechanisms. For example, it was realized early on that complete PVI was not crucial for successfully eliminating AF. In some cases, delivering RF lesions partially across the PV ostia was then adopted and has proven to be far more efficacious. This strategy uses circumferential RF lesions delivered between the focal source of PV firing and the atria in an attempt to electrically isolate the AF triggers to the PV from the substrate of AF propagation in the atria. Although this approach works reasonably well, it has also produced some interesting observations and insights into AF mechanisms. For example, it was realized early on that complete PVI was not crucial for successfully eliminating AF.

**Figure 1.** Ganglionated plexi in the human heart. Shown is an illustration of the posterior aspect of the human heart and major vessels that shows the locations of the posterior atrial and ventricular ganglionated plexi. Note that the mediastinal nerves run adjacent to the aortic root and join the 2 superior atrial ganglionated plexi. The positions of the superior vena cava (SVC), inferior vena cava (IVC), right ventricle (RV), left ventricle (LV), and right superior pulmonary vein (RSPV) are shown. GPs indicate ganglionated plexi; LIPV, left inferior pulmonary vein; LSPV, left superior pulmonary vein; RIPV, right inferior pulmonary vein; and PA, pulmonary artery. Adapted from Armour et al13 with permission of the publisher. Copyright ©1997, John Wiley & Sons. Authorization for this adaptation has been obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.
system with AF initiation and propagation, implicated the cardiac nervous system in the genesis of AF.

The Cardiac Nervous System in AF

More than 3 decades ago, Coumel et al published their classic account of the cardiac nervous system and first proposed that AF may have a nervous system trigger. Coumel’s neural hypothesis was supported by 2 key clinical observations. First, a distinct subtype of AF that developed with bradycardia was identified and termed vagotonic AF or nocturnal AF. A second group of patients was also described whose AF triggers were the exact opposite of those with vagal AF. In this latter group, arrhythmogenesis was driven by high sympathetic tone induced by stressors such as emotional excess and exercise. Patients with vagotonic AF are often young, healthy individuals with no overt cardiac pathology, whereas patients with sympathetic AF were usually older, with multiple comorbidities and structural heart disease. However, these 2 patient populations are often difficult to differentiate from each other just based on clinical characteristics, because there is often significant overlap between those with vagally mediated AF and adrenergically driven AF. In subsequent investigations, the question of whether AF was triggered by autonomic stimuli was intensely explored, and numerous reports and retrospective studies clearly supported Coumel’s theory. In fact, the clinical literature is full of case reports of patients whose AF was triggered by vagal events, adrenergic triggers, or a combination of both. The major shortcoming in most of these accounts, however, was the absence of a strong causal relationship between the arrhythmia and autonomic dysregulation. In many of these reported cases, autonomic tone was established by reporting heart rate variability, which is notoriously unreliable as the patient ages owing to the increasing prevalence of comorbidities and structural heart disease. This is particularly true in patients with concomitant heart failure, in whom heart rate variability attributable to altered autonomic tone could be misinterpreted because of underlying sinus node dysfunction or the compensatory effects of heart failure itself.

Large-Animal Studies

Because of the aforementioned problems with studying the contribution of the autonomic system to AF triggers in patients, some of the most compelling data supporting this theory come from the large-animal laboratory where data can be obtained in a precisely controlled setting. Sharifov and colleagues demonstrated that infusing the parasympathetic neurotransmitter acetylcholine into the sinoatrial nodal artery was associated with induction of AF in a dog model. Interestingly, these authors also found that coinfusion of the β-adrenergic agonist isoproterenol decreases the threshold for acetylcholine-induced AF in this model. This result suggests that, not only was AF easily induced by simultaneous sympatocholinergic stimulation, but also that the natural trigger was perhaps a balance between both limbs of the autonomic system. Indeed, experiments by Tan and colleagues with long-term implantable pacemakers and nerve recorders in dogs showed that simultaneous sympatocholinergic activity was the most frequent trigger of atrial ectopy. Similarly, cryoablation of bilateral stellate ganglia and cardiac branches of the vagus nerve (eliminating sympatocholinergic stimulation altogether) eliminated paroxysmal AF induced by rapid atrial pacing. Taking these experiments a step further, Shen et al demonstrated that the preexisting autonomic milieu dictates the propensity for AF induction in a large-animal model by demonstrating that a distinct baseline spatiotemporal pattern of sympatocholinergic activity is present in dogs that determines the vulnerability to AF induction by rapid atrial pacing. Specifically, these investigators showed that dogs had either a nonlinear (L-shaped) or linear sympatocholinergic correlation based on a scatter plot of activity of the stellate ganglion versus the vagus nerve. Dogs that possessed a linear sympatocholinergic correlation consistently had higher baseline vagal tone and a greater propensity for the onset of AF.

Wijfels et al and Jayachandran et al expanded on the idea that preexisting autonomic tone dictates susceptibility to AF and asked if the converse may also be true. In other words, can the presence of sustained AF invoke changes in the autonomic environment? To address this question, they used rapid atrial pacing to induce AF and study changes in the electrophysiological and neuronal milieu in goats and dogs. Over time, they noted that rapid atrial pacing induced sustained electric and neural changes in the atria. Electrophysiological changes in the atria were mostly attributable to abnormal calcium handling and an abnormally shortened atrial action potential. Neural changes induced by rapid atrial pacing, which were also striking, involved the sprouting of new sympathetic axons associated with the PV and atria. Increased sympathetic innervation, in turn, was likely to increase autonomic stimulation with further electric and neural remodeling of the atrium (Figure 2). Taken together, this cyclic setup suggested a fascinating mechanism for sustained AF progression and propagation over time, leading to the playful aphorism “Afib begets Afib.”

Despite compelling data that support a role for the cardiac nervous system in the initiation and propagation of AF, it remains unclear which of the cardiac nervous system subdivisions may be involved in this process. For instance, it is not clear if both ECNS and ICNS activity is required for AF initiation, or if the ICNS functions independently of the ECNS to initiate AF. Choi and colleagues sought to address these questions by directly recording the electric activity of ECNS and ICNS neurons in dogs with induced AF. Intermittent rapid atrial pacing reliably induced AF in these animals and the majority of episodes of AF were centrally mediated with associated bursts of activity in ECNS nerves (stellate ganglion, thoracic vagal nerve). Surprisingly, they also noted episodes of AF that were induced by isolated activity in the GPs (and ligament of Marshall) without concomitant activity in the ECNS. These results suggest that although activity of the ECNS is required to induce most cases of AF, the arrhythmia could also be triggered autonomously from GPs of the ICNS without any higher input.

The possibility that autonomously firing GPs could trigger AF is exciting because it suggests a mechanism for increasing frequency of AF with advancing age. It is well demonstrated that increasing age drastically changes autonomic tone,
perhaps in part because of the loss of autonomic neurons, reduced responsiveness to autonomic stimuli, or a combination of the 2. Smith et al\textsuperscript{48} demonstrated that even weeks after denervation of the ECNS, GP neurons remain fully viable and retain their ability to increase neurotransmitter release. Thus, it is conceivable that, as a person ages, there may be an increased proclivity for AF owing to autonomously functioning GPs in the absence of strong autonomic tone. Lo and colleagues\textsuperscript{49} tested this hypothesis by ablating the nexus point between the ECNS and ICNS in dogs. Over a period of 10 weeks, the denervated dogs were monitored for the development of AF, and there was a gradual rise in the AF burden of ablated dogs in comparison with sham-operated dogs. In fact, in dogs without denervation there was virtually no AF. In human subjects, similar trends are seen in patients after cardiac transplantation. Noheria and colleagues showed post-operative AF in comparison with native heart patients and cardiac transplant patients have substantially lower rates of AF. Noheria and colleagues\textsuperscript{50} showed post-transplant candidates had additional autonomic denervation of the heart.

**Cellular Substrates**

GPs that innervate the atrium have pleiotropic effects both on the myocardium and, in turn, on the cardiac neurons themselves. But how the axonal termini in GP neurons communicate with cardiac tissue is not entirely known. Furthermore, it is not clear if there are specialized neurotransmitter receptors on cardiomyocytes, or if there are subpopulations of cells in the atria that either directly communicate with or modulate contact between these 2 cell types.

To address these questions, let us consider the cellular substrates that contribute to AF. The cellular substrates of AF have been an area of intense investigation in basic science. The earliest studies sought to better characterize the properties of the most obvious cellular target of the cardiac nervous system, the cardiomyocyte. The observation that β-adrenergic receptors (sympathetic) and M2-type muscarinic receptors (parasympathetic) are enriched in atrial and PV myocytes suggested that these cells may directly communicate with the ICNS.\textsuperscript{51,52} Although these observations suggest that cardiomyocytes interact with the ICNS, the actual role of cardiomyocytes in the genesis of AF is probably more complex than as just simple neurotransmitter receptor docks for the autonomic nervous system. Depending on the age and environment of the host, cardiomyocytes can respond in a myriad of different ways to the same neural stimulus.\textsuperscript{53} Part of this complexity at the cellular level may be the result of atrial remodeling that leads to atrial fibrosis and changes in cell signaling, such as calcium handling. For example, abnormal calcium release from the sarcoplasmic reticulum through the major calcium-release channel, known as the ryanodine receptor, results in the phenomenon of calcium alternans which is a pathological state in which there is beat-to-beat variation in calcium currents.\textsuperscript{54} Calcium alternans is a highly arrhythmogenic state that is mediated by the loss of functional integrity of the ryanodine receptor or the voltage-dependent L-type calcium channel in the cell membrane.\textsuperscript{55} In addition, there is mounting evidence that G-protein–coupled receptors, particularly G-stimulatory proteins, can induce phosphorylation of the ryanodine receptor through protein kinase A that results in increased sympathetic nerve sprouting which augments β-adrenergic tone leading to increased protein kinase A–mediated hyperphosphorylation of atrial ryanodine receptors (RyR2). Increased RyR2 phosphorylation promotes sarcoplasmic reticular calcium release that induces calcium-mediated inhibition of voltage-dependent L-type calcium currents, leading to shortening of atrial myocyte action potential duration (ADP) and effective refractory period (ERP). This electric remodeling promotes further AF and increased sympathetic nerve sprouting. In addition, increased atrial myocyte RyR2 calcium release induces myocyte apoptosis and fibrosis, which leads to atrial dilatation and structural remodeling that further increases the propensity for AF.
analogous cell populations in similar anatomic distributions in the human heart provides a rational basis for placing these discoveries in a clinically relevant context.

A few years after Cheung\textsuperscript{58} stated that isolated PV tissue possessed pacemaker activity, Fumiaki Masani\textsuperscript{59} described the first subpopulation of specialized cells in the human atrium, PV-LA junction, and PV tissue that could explain this autonomous activity. Ultrastructurally, these cells were nearly identical to sinus node cells and possessed many of the histological characteristics of cardiac pacemaker cells. Furthermore, each of these nodal-like cells was found to be juxtaposed with neurons from the ICNS. Chou and colleagues\textsuperscript{60} further advanced the characterization of these cells by invoking voltage-independent triggered ectopy in Langendorf-perfused canine hearts in the presence of ryanodine and isoproterenol. As atrial arrhythmias were provoked, these authors identified nodal-like clear cells clustered in the endocardium adjacent to sites from which frequent focal discharges were mapped. In addition, histological analysis of these cells revealed that they were enriched with glycogen that robustly labeled with periodic acid-Shiff stain, similar to Purkinje cells in the cardiac conduction system. Perez-Lugones et al\textsuperscript{61} found a compelling association between the presence of these cells and the incidence of AF by clinical histories associated with human autopsy specimens. Samples from 4 patients with a history of AF had the classic periodic acid-Shiff–positive sinus nodelike cells in their PV tissue. By comparison, no such cells were discovered in the PVs of 5 transplanted hearts from patients without a history of AF.

Recent work has uncovered an additional population of atrial cells that resemble the pacemaker cells of the gastrointestinal tract. Interstitial Cajal cells (ICCs) are the primary pacemaker cells that mediate peristalsis and the motility of the alimentary tract in response to autonomic input. These cells have a unique property of generating and propagating slow waves of electric activity that drives smooth muscle contraction in the gastrointestinal tract. Interestingly, a peculiar subpopulation of interstitial Cajal-like cells (ICLCs) localized to the PV-LA junction and PV that is histologically similar to ICCs has been identified. Morel et al\textsuperscript{62} described a correlation between the presence of ICLCs in the PV and clinical AF. A report by Gherghiceanu et al\textsuperscript{63} further strengthens the association between AF and ICLCs without any discrete evidence for whether these cells contribute to atrial arrhythmias. Although evidence that ICLCs directly contribute to AF is lacking, the fact that ICCs are well characterized in the gastrointestinal system and known to possess rhythmic pacemaker activity makes it reasonable to infer that ICLCs serve a similar role in the atrium. However, ICCs in the gut and ICLCs differ in many important electrophysiological feature, which is their response time. This distinction is important because the induction of AF requires response times that are several orders of magnitude faster than the response time of ICCs. Nevertheless, whether ICLCs possess electrophysiological properties that differ from their gastrointestinal brethren still remains to be determined. As new data emerge, insight into the role of these cells in the cardiovascular system and their connections to the autonomic nervous system will likely improve.

Perhaps the newest addition to the growing family of specialized atrial cells comes from our own laboratory. We have described a unique pool of melanocyte-like cells localized in atrial tissue, the PV-LA junctions and the PVs that are sites from which AF triggers commonly originate. Similar to ICLCs and periodic acid-Shiff–positive nodal cells, melanocyte-like cells are electrically excitable and appear to be connected to the ICNS. However, more importantly, these cells can generate action potentials with the time course and speed sufficient to trigger AF. Immunohistochemically, melanocyte-like cells in the heart are notable for their expression of dopachrome tautomerase, a melanin synthesis enzyme that is involved in the regulation of intracellular calcium and scavenging of reactive oxygen species including melanin-synthesis by-products. Because these cells express melanin synthesis enzymes and morphologically resemble dermal melanocytes, we called this novel cell population cardiac melanocytes. Interestingly, cardiac melanocytes express both adrenergic and muscarinic receptors and are located in close apposition to autonomic nerve terminals of the ICNS (Figure 3). Mice harboring germline deletion of Dct (Dct\textsuperscript{−/−}) are more susceptible to atrial arrhythmias, and we showed that this property

![Figure 3. Dct-expressing cells in the atrium coexpress autonomic receptors and are near autonomic nerve terminals. Immunohistochemistry within the adult mouse atrium using an antibody to Dct demonstrates Dct-positive cells with characteristic morphology (A, D, G, J, and M). Sections were costained with antibodies to $\alpha_1$-adrenergic receptor ($\alpha_1$R; B), $\alpha_2$-adrenergic receptor ($\alpha_2$R; E), muscarinic receptor subtype 3 (M3R; H), tyrosine hydroxylase (T-OH; K), and choline acetyltransferase (ChAT; N). Merged images of Dct and each respective antibody are shown, demonstrating coexpression of Dct with $\alpha_1$R (C), $\alpha_2$R (F), and M3R (I) receptors (arrowheads). In addition, presumptive sympathetic nerve terminals that express tyrosine hydroxylase (L) and parasympathetic nerve terminals that express choline acetyltransferase (O) were seen in close proximity to Dct-expressing cells (arrows). Scale bars, 20 $\mu$m. Reproduced from Levin et al\textsuperscript{64} with permission of the publisher. Copyright ©2009, American Society for Clinical Investigation. Authorization for this adaptation has been obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.](http://circ.ahajournals.org/doi/10.1161/CIRCULATIONAHA.113.003901)
was cell autonomous. Mice with mutations of the tyrosine kinase receptor c-kit have migratory defects in melanocytes, and c-kit mutant mice actually lack cardiac melanocytes within their atria. Remarkably, Dcr−/− mice bred on a c-kit mutant background were resistant to atrial arrhythmias suggesting that it was the cardiac melanocyte itself, and not just the lack of dopachrome tautomerase, that was responsible for atrial arrhythmogenesis.64 This finding opens up a number of interesting questions, some of which include delineating how the ICNS influences the electric properties of these cells and how cardiac melanocytes communicate with atrial myocytes. Whether this unique melanocyte-like population works independently, or in concert with other subpopulations of specialized conduction tissues, also remains to be understood.

Therapeutic Strategies

Since the first description of PV isolation as a feasible therapeutic strategy for drug-refractory AF, there has been considerable interest in adjunctive therapies that may help enhance the success of this procedure. In addition to the circumferential lesions delivered during a standard PVI, linear lesions that traverse areas with high GP density have also been placed to improve procedural success rates.65 The natural inference from this association is that the success of PVI may be indirectly attributable to coincidental modification of nervous inputs to the atrium.66 Based on this provocative idea, a number of investigators have proposed that autonomic denervation might be a suitable strategy for AF ablation either in isolation, or in concert with standard PVI.67-70 Yet, investigations into this approach have yielded mixed results. Platt and colleagues were the first to attempt selective GP ablation as a therapeutic strategy for AF. These investigators delivered high-frequency stimuli to nerve clusters at the PV-LA junction and showed a marked slowing of the ventricular response in patients with AF. Complete ablation of these GPs terminated AF in nearly 90% of patients with persistent AF and resulted in 96% freedom from AF during a 6-month follow-up period.71 Scherlag et al similarly showed that ablation of GPs in addition to PVI increases ablation success from 70% to 91% in patients with paroxysmal or persistent AF up to 1 year later. However, other studies have been less optimistic regarding this approach. Lemery found that only 50% of patients who underwent combined GP ablation and PVI were free from AF during an 8-month follow-up period. These conflicting reports with regard to the success rate of GP ablation, with or without PVI, imply that interactions between the ICNS and AF are very complex. An obvious explanation for this variability is technical differences among the studies, or the heterogeneity of AF substrates themselves. For example, Scanavacca and colleagues used high-frequency stimulation induced vagal reflexes to identify atrial sites that may be amenable to GP ablation. Vagal responses were evoked in only 7 of 10 patients with presumed vagal AF after high-frequency stimulation, and, of these patients, only 2 remained free from AF after GP ablation. These findings highlight the complexity inherent in the genesis of AF among different patients and the difficulty in using a uniform strategy such as high-frequency stimulation to identify and treat even a single entity like vagal AF. In addition, there may be other technical differences such the method by which GPs were identified, either by RF stimulation or anatomic evaluation which could affect the outcome, as does the completeness of GP ablation, because plexi close to the phrenic nerve, for example, were not ablated in most studies.74-76

An alternative approach to GP ablation is complete denervation by severing ECNS input to the heart. In large-animal studies, partial vagal denervation of the high right atrium decreased AF inducibility,77 and sympathectomy via denervation of the stellate ganglion was shown to reduce atrial tachyarrhythmias.42 Based on these and other studies, autonomic denervation has the potential to be a more effective strategy for treating AF than GP ablation, which can be challenging owing to the poor anatomic localization of the ICNS. Despite its promising outlook, autonomic denervation is neither simple nor uniformly sustainable. The challenges inherent in this approach are primarily attributable to the adaptive mechanisms of the nervous system that counteract most attempts at inducing denervation. Most of the autonomic nerves that innervate the heart are highly resilient peripheral nerves that can rapidly regrow in response to denervation. In vivo these nerves do so under the influence of target-derived trophic signals such as nerve growth factor, which when overexpressed leads to sustained survival and rapid axonal sprouting of sympathetic and sensory neurons.77-78 It has been shown that RF ablation in the vicinity of the GPs can stimulate massive neuronal hyperinnervation; and, building on this observation, Kangavari et al demonstrated that there is a concomitant elevation in the expression of nerve growth factor in the peripheral veins after RF ablation. Thus, in this situation, denervation is only a temporary solution with rapid hyperinnervation leading to stronger autonomic neurotransmitter release that promotes atrial arrhythmogenesis. In fact, vagal tone can be restored to near-normal range within just 4 weeks of ablation.80

As an alternative therapy for PVI, targeting sites with complex fractionated atrial electrograms (CFAEs) was first suggested by Nademanee and colleagues.31 CFAEs are regions of complex atrial electric activity that are thought to correspond to areas where pivot points for reentrant wavelets or areas of transient conduction block occur. Based on observational experimental data, these complex regions are assumed to represent AF substrate.82 Verma et al described an algorithm for automated CFAE identification and showed that ablating CFAEs alone terminated AF in 55% of patients and that using CFAE ablation as an adjunctive, rather than as an alternative therapy to PVI, provides an incremental increase in procedural success rates. Other studies have further provided proof for this hypothesis with increasing frequency.83,84 Still, the mechanisms underlying the origin of CFAEs and what role they play in the genesis of AF is not precisely known. One compelling thought is that CFAEs occur in the vicinity of anatomic GPs, and that the neural communication from the autonomic nervous system directly impacts the electric activity of the atria. Work by Katritsis and colleagues has lent credence to this hypothesis. Not only do anatomic GP sites cluster at locations where CFAEs occur with increased frequency, but also patients in whom CFAEs were undetectable at GP sites did not generate CFAEs elsewhere in the atria. In
addition to the data supporting CFAEs localizing to regions of atrial innervation, there are other elegant electrophysiologic strategies to identify anatomic substrates of AF. Pachon et al90 used Fast Fourier Transform to identify 2 distinct types of atrial myocardium based on frequency-domain analysis of atrial potentials. These authors identified a compact myocardium that consisted of normal in-phase conduction, suggesting highly compact cellular connections, and fibillar myocardium consisting of anisotropic out-of-phase conduction inferring a mixture of muscle and fibillar tissue including neural, vascular, and pathological tissue. Interestingly, during AF episodes there are clusters of fibillar myocardium that form AF nests that have higher frequencies than the surrounding atrium tissue. Hypothesizing that targeting these AF nests in the fibillar myocardium with RF ablation could terminate AF, these authors treated 35 patients and eliminated AF in all but 2 of them over a follow-up period of 9 months. Although these findings are interesting, the actual data that link CFAEs or the fibillar myocardium to nervous system activity and the autonomic basis of AF is still sparse.

**Novel Therapies**

New therapeutic strategies that may exploit some of the aforementioned principles of AF genesis have generated considerable excitement in electrophysiology. However, these strategies are still limited to the basic science laboratory and have not been studied in humans in any meaningful fashion. Foremost among these is the potential use of vagal nerve stimulators to control AF. The origin of this idea comes from observations that low-level vagal activation suppresses ectopy within the PV and LA and thereby inhibits AF initiation. Li and colleagues88 explored this phenomenon in anesthetized dogs and discovered that low-level vagosympathetic nerve stimulation at a voltage only 10% below the threshold for inducing bradycardia could efficiently suppress AF induced by rapid atrial pacing. Mechanistically, a gradual prolongation of the atrial effective refractory period is purported to induce this phenomenon, because rapid atrial pacing without concurrent vagosympathetic stimulation causes a progressive shortening of the atrial refractory period with an increase in AF susceptibility.20,89 Furthermore, these findings have been extended to demonstrate that nearly equivalent results could be achieved by placing the stimulating catheter in the superior vena cava without surgically exposing the nerve trunks.90 This strategy, notwithstanding its potential elegance, has not been validated beyond animal models but does demonstrate a novel nonpharmacological, nonablative therapy for AF that may be clinically useful someday.

The idea that implantable nerve stimulators could be the future of refractory AF treatment may seem far-fetched today, but pharmacological therapies that impinge on the nexus between the nervous system and the cardiovascular system are likely to be adopted with less resistance. Among these drugs is botulinum toxin, which, like a myriad of other medical specialties, may finally have found a niche in cardiology. The idea that local botulinum toxin injection can cause denervation is certainly not novel. Oh and colleagues91,92 performed local injection of botulinum toxin into the epicardial fat pads in an attempt to mitigate rapid atrial pacing induced AF in anesthetized dogs. In doing so, they were able to achieve transient autonomic denervation, thereby raising the threshold for AF induction. Although this approach may sound intriguing, the problem is that nerve termini denervated by botulinum toxin often rapidly regrow over time, which would necessitate frequent drug injections to attain any meaningful therapeutic effect.

Similarly, the cardiac peptide molecule vasostatin-1 has garnered interest as a candidate molecule for AF suppression by inducing chemical denervation. Vasostatin-1 is released by autonomic nerve stimulation and has potent antiadrenergic effects.93,94 Preliminary experiments by Stavrakis et al95 showed that vasostatin injection into the cardiac fat pads successfully diminished AF susceptibility. Further experiments are clearly needed before this molecule is ready for clinical studies, but the ability of this single peptide to achieve such a level of efficacy raises interesting questions about its mechanism of action. Whether vasostatin reduces AF by lengthening the atrial refractory period, suppressing early afterdepolarizations or both is currently unknown.

Last, there are a number of other potential therapeutic targets of AF that include the use of peptides that modulate signal transduction via adrenergic and muscarinic receptors. For instance, Aistrup and colleagues86,97 demonstrated that the attenuation of vagosympathetic signaling and AF suppression could be achieved by disrupting G-protein–coupled receptor signaling between the ICNS and the atrial myocardium. To show this, these investigators experimentally injected an inhibitory G-protein alpha C-terminal peptide (believed to disrupt the interaction between cardiac M2-muscarinic receptors and activation of its downstream G-protein alpha) into the LA in a targeted fashion. This approach not only reproducibly diminished vagal responsiveness throughout the left atrium, but also achieved sustained inhibition of vagotonic AF.

**Conclusion**

In this review, we have sought to emphasize the mechanistic underpinnings of AF with respect to the autonomic nervous system and local innervation of the heart. A number of studies from both the basic science laboratory and clinical investigations have provided compelling evidence that implicate abnormal autonomic reactivity in the genesis and propagation of AF. Indeed, as highlighted in this article, local innervation-induced changes in the electrophysiological milieu of the atrial myocardium can itself act as an impetus for further propagation of sustained AF. Although the exact mechanism of this interaction is not known, emerging data suggest that neural communication with the cardiovascular system may be achieved via the autonomic nervous system. These effects may be induced by directly stimulating cardiac myocytes or key noncardiomyocyte intermediaries such as cardiac melanocytes, which in turn induce changes in signal transduction and calcium handling in cardiomyocytes. The disruption of the neural regulation of myocardial arrhythmogenesis may prove to be a compelling mechanism for treatment of refractory AF. A number of strategies are actively being explored at this time that include direct ablation of GP, pharmacological and surgical autonomic denervation, implantable stimulators, and small-molecule inhibitors. Whether any of these strategies...
will successfully transition from the laboratory to the bedside as clinically useful therapies for the treatment of refractory AF remains to be seen.

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