Neural substrate for atrial fibrillation: implications for targeted parasympathetic blockade in the posterior left atrium

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Atrial fibrillation (AF) occurs in a wide spectrum of patients, ranging from no anatomic structural heart disease to all varieties of heart disease with associated atrial pathology. It is therefore no surprise that the pathophysiology of AF is diverse. Importantly, the role of the autonomic nervous system in the pathogenesis of AF has been demonstrated in multiple settings. Hou et al. (10) suggested the presence of an intricate, interconnected neural network in the left atrium (LA) that may contribute to substrate for focal AF. A recent human study described heterogeneity of nerve distribution in the region of the pulmonary veins (PVs) and surrounding LA (3). Inasmuch as parasympathetic and sympathetic activation may have prominent effects on atrial conduction velocity and refractoriness, regional heterogeneities in nerve distribution could be responsible for the heterogeneities in conduction velocity and refractoriness that are necessary for the initiation and perpetuation of AF. Thus the distribution of autonomic nerves throughout the atrium could represent a substrate for AF, particularly in settings where there are no anatomic abnormalities.

In a recent study in normal canine hearts, we demonstrated that vagal responsiveness is more pronounced in the posterior left atrium (PLA) and PVs than in the rest of the LA (2). Clinical data suggest that focal “triggers” and “drivers” in the PV (even in structurally normal hearts) appear to be at least partially modified or regulated by the parasympathetic nervous system (11). In light of these prior studies, we hypothesized that the density of parasympathetic nerves and related muscarinic receptors is greater in the PLA than in the rest of the LA, with the parasympathetic innervation of the PLA playing an important role in the creation of AF substrate in structurally normal hearts. We also postulated that selective cholinergic blockade in this region can be attained by pharmacological methods, with a resulting decrease in vagal-induced AF. In this canine study, we have therefore compared the distribution and physiology of sympathetic and parasympathetic nerves among the PVs, PLA, and LA appendage (LAA). We have also attempted targeted parasympathetic blockade in the PLA with a muscarinic receptor blocker and have studied in detail the resulting electrophysiological response of the LA.

METHODS

The investigation conforms with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996). Approval for use of purpose-bred dogs (hounds) was obtained from the institutional Animal Use Committee.

Immunostaining

PVs were harvested from normal, healthy dogs. PVs were taken as the region extending from the antrum to the junction of the LA myocardium-PV smooth muscle. The adjoining PLA (defined as the confluence of the PVs) and the anterior LA (LAA) were also harvested. Regions containing fat were always incorporated in the tissue sections taken from the PLA. Control specimens were taken from the cervical vagus nerve and stellate ganglia. The tissue was frozen in liquid nitrogen. Serial circumferential cross sections were cut (proximal-to-distal) from the PVs (Fig. 1). Sections from the PLA and

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Sections were air-dried and fixed in acetic acid for 10 min and then washed in Tris-buffered saline (TBS). Hydrogen peroxide block (Dako, Carpinteria, CA) was placed on the sections for 10 min, and the slides were washed in TBS. Protein block was placed on the sections for 30 min. Primary antibodies were then incubated overnight at 4°C. Antibodies for dopamine β-hydroxylase (DBH; Chemicon, Temecula, CA) were used to stain sympathetic nerves, whereas antibodies for choline acetyltransferase (ChAT; Chemicon) were used to stain parasympathetic nerves. The specificity of DBH for sympathetic nerve fibers was confirmed by the use of a sympathetic nerve marker, i.e., tyrosine hydroxylase (TH; Chemicon), which stained the same nerve elements that were stained by DBH (Fig. 2, A and B). Similarly, the specificity of parasympathetic staining was confirmed using a second parasympathetic nerve marker, i.e., acetylcholinesterase (AChE; Chemicon), which stained the same nerve elements that were stained by ChAT (data not shown). Some sections were double-stained for ChAT and DBH. After incubation, the slides were washed in TBS, and the appropriate secondary antibody (Chemicon) was placed on the sections for 30 min. The sections were again washed in TBS, and the appropriate chromagen was added to each specimen. Sympathetic nerves were stained blue with 3,3′-diaminobenzidine (DAB). Cell nuclei were marked with 5-bromo-4-chloro-3-indolyl phosphate (BCIP), and parasympathetic nerves were stained brown with 3,3′-diaminobenzidine (DAB). The specimens were then dehydrated in alcohol, mounted, and examined by light microscopy. Similar techniques have previously been used to successfully characterize the autonomic innervation of other tissues (13, 26).

Cardiac ganglia were defined as nerve bundles containing one or more neuronal cell bodies (26). The presence of neuronal cell bodies within nerve bundles was confirmed by staining these bundles for hematoxylin; all neuronal cell bodies stained positive for hematoxylin (data not shown), thereby excluding the presence of fat cells within these bundles.

Specimens taken from the cervical vagus nerve served as a positive control for parasympathetic nerve fibers but a negative control for sympathetic nerves. Specimens from the stellate ganglia served as a positive control for sympathetic fibers but a negative control for parasympathetic nerve fibers (control data not shown). The specimens were then dehydrated in alcohol, mounted, and examined by placement of the specimens in methyl green (Dako) for 10 min.

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one animal. Food coloring dye was mixed in normal saline and applied to the PLA in this dog. The color remained confined to the PLA, even after several hours.

For assessment of systemic absorption of tropicamide, the sinus rate was assessed in nine animals before and 5 and 30 min after tropicamide application. Vagal-induced sinus slowing was also measured at these time points. As a surrogate of systemic absorption of tropicamide, in two animals, we also examined the pupillary diameter before and 5 and 30 min after tropicamide application to the heart.

**Data Analysis**

*Nerve count estimation.* Nerve bundles, as well as individual nerve fibrils, were manually counted at \( \times 10 \) magnification for the entire section. Nerve bundles or trunks were defined as large collections of individual nerve fibers/fibrils with \( \geq 0.025 \) cm diameter. Nerve fibrils were defined as thin nerve fibers with \( < 20 \mu m \) diameter (26). Nerve bundle density was calculated as the number of nerve bundles per square centimeter. Since individual nerve fibrils were too numerous to be counted for the entire section, parasympathetic, as well as sympa-
thetic, nerve fibrils were counted in five, evenly spaced grid (10 × 10 mm) fields within each section; the average of these five fields was taken as a measure of the nerve fibril density (per cm²) for the section. Quantification was separately performed for the epicardial vs. endocardial half of the section.

In addition, the number of cholinergic and adrenergic fibers was counted manually within several, randomly selected nerve bundles that demonstrated colocalization of parasympathetic and sympathetic fibers; at least five bundles were selected from each region. To account for variation in nerve/nerve bundle size, the ratio of cholinergic to adrenergic nerve fibers (averaged for the randomly selected bundles from each region) was taken as an estimate of the relative distribution of cholinergic vs. adrenergic nerve fibers in each region.

$M_2$ receptors. To quantify $M_2$ receptor distribution, the number of tissue elements (myocytes, nerves, and blood vessels) stained for $M_2$ receptors at ×40 magnification was manually counted. The number of positively stained elements in each section was averaged for five grid fields (each at ×40 magnification).

Electrophysiological studies. For each location, the average ERP for all sites was determined. In each region, ERP changes in response to VS were compared with baseline ERP. Similarly, ERP changes in response to VS were compared within each region in the presence and absence of tropicamide. ERP changes with VS (with and without tropicamide) were also compared between the different regions, i.e., PV, PLA, and LAA.

As with ERP, AF inducibility was measured before and after VS (in the absence, as well as the presence, of tropicamide). AF inducibility was measured as the number and duration of AF episodes after a single extrastimulus. The inducibility index was defined as the number of AF episodes lasting $>5$ s induced by a single atrial extrastimulus divided by the total number of single atrial extrastimuli delivered to measure each ERP ($≥3$ for each site). The inducibility index was compared for each maneuver.

Statistical analysis. Continuous data are presented as means ± SD. $P ≤ 0.05$ was considered significant. Paired comparisons were made by a $t$-test, multiple comparisons by ANOVA, and atrial inducibility index by $χ²$ test.

RESULTS

Tissue from six normal, healthy dogs was studied. Sections from PVs ($n = 22$), adjoining PLA ($n = 6$), and LAA ($n = 6$) were stained with AChE and DBH. Separate sections from the PV ($n = 5$), PLA ($n = 5$), and LAA ($n = 5$) were stained for $M_2$ receptors.

Large nerve bundles (trunks, ≥0.025 cm diameter) and thin nerve fibrils (<20 μm diameter) were seen in the PV and atrial myocardium (Fig. 2). Although nerve bundles were located predominantly in fibrofatty tissue overlying the epicardium (Fig. 2, see Fig. 4), 36% of all nerve bundles were located away from surface fibrofatty tissue in underlying and surrounding myocardium ($P = 0.001$; Fig. 2D). On the other hand, thin nerve fibrils were located almost entirely in the myocardium, extending between and parallel to myocardial fibers (Fig. 2E). Nerve bundles, as well as thin nerve fibrils branch out from larger nerve bundles, were close to the vasculature in all the regions that were studied (Fig. 2F).

Nerve Bundles

Significant differences in nerve bundle density were noted among the PV, PLA, and LAA, with the PLA having the greatest density of nerve bundles (PV $>>$ PLA $>>$ LAA, $P < 0.001$; Fig. 3A). Nerve bundles were predominantly localized to the epicardial half of the PVs, PLA, and LAA (Fig. 3B). The total number of nerve bundles was significantly greater in the superior than inferior veins (0.96 ± 0.9 cm² vs. 0.80 ± 0.8 cm², $P = 0.04$). The density of nerve bundles tended to be higher in the proximal than distal segments of the PVs (Table 1). The bundles tended to be larger in the PLA than the other regions (0.12 ± 0.15, 0.05 ± 0.06, and 0.025 ± 0.01 cm² in PLA, PV, and LAA, respectively, $P = 0.058$).

Sympathetic vs. Parasympathetic Nerve Distribution Within Nerve Bundles

Sympathetic and parasympathetic nerve fibers were colocalized within a large majority of nerve bundles in each region [67, 87, and 67% of bundles demonstrating colocalization in PV, PLA, and LAA, respectively, $P = 0.005$ (NS)]. The remainder of the nerve bundles was composed purely of parasympathetic fibers. No nerve bundles composed of sympathetic fibers alone were noted in any of the three regions. Figure 4, A–D, shows colocalization of sympathetic and parasympathetic fibers within an individual nerve bundle in the PV.

The proportion of sympathetic and parasympathetic nerves within the colocalized bundles did not significantly differ among regions, with a predominance of parasympathetic elements being noted in all the regions [parasympathetic (P) $>$ sympathetic (S) in PV, PLA, and LAA ($P < 0.005$ for each region) and P-to-S ratio = 4.4, 7.2, and 5.8 in PV, PLA, and LAA, respectively ($P = 0.005$); Fig. 4E]. Figures 2 and 4 demonstrate other examples of parasympathetic fiber predominance in individual nerve bundles.

Thin Nerve Fibrils

As opposed to nerve bundles, parasympathetic and sympathetic nerve fibrils appeared to be equally distributed within the epicardium and endocardium of the PLA and LAA (Table 2). This homogeneity was not observed within the PV, where the sympathetic nerve fibrils were more concentrated on the endocardium (Table 2).

Parasympathetic fibrils were most abundant in the PLA, followed by the LAA and PV (PLA $> $ LAA $> $ PV, $P = 0.013$; Fig. 5). In contrast, sympathetic nerve fibril density was greatest in the LAA, followed by the PV and PLA (LAA $> $ PV $> $ PLA, $P < 0.001$; Fig. 5). The density of parasympathetic fibrils was significantly greater than the density of sympathetic fibrils in the PLA ($P > S$, $P = 0.034$; Fig. 5). No significant difference was noted between the density of parasympathetic and sympathetic fibrils in the LAA and PV (Fig. 5, Table 2).

The density of sympathetic, as well as parasympathetic, fibers tended to be greater in the proximal than distal segments of the PVs (Table 1). There were no significant differences among the PVs with respect to the density of sympathetic or parasympathetic nerve fibrils (Table 1).

Cardiac Ganglia

Cardiac ganglia containing parasympathetic neuronal cell bodies (Fig. 6) were found in the PLA and PV, but not the LAA (PV $> $ PLA $> $ LAA, $P = 0.04$; Fig. 6C). Ganglia consisting of neuronal cell bodies were juxtaposed with bundles of parasympathetic nerve fibers that appeared to arise from the neuronal cell bodies (Fig. 6A).

Even though the majority (86%) of cardiac ganglia containing parasympathetic neuronal cell bodies also contained sym-
pathetic nerve fibers, the proportion of neuronal cell bodies was significantly greater than the proportion of sympathetic fibers (P-to-S ratio = 1.65, \( P = 0.004 \)).

**M_2 Receptor Distribution**

Since cholinergic nerve fibers exert their downstream effects principally via M_2 receptors, M_2 receptor distribution in the LA was assessed. M_2 receptors were localized in and around the following structures: 1) nerve bundles and fibers, 2) perivascularly, and 3) in individual cardiomyocytes. Figure 7, A and B, shows M_2 receptors tracking along the distribution of autonomic nerve bundles and fibers in the PLA, as well as perivascular distribution of M_2 receptors. Figure 7, C and D, shows M_2 receptor localization at the level of single cardiomyocytes in the PLA and LAA, respectively; the number of M_2 receptor-stained cells is clearly greater in the PLA than LAA. M_2 receptor density was greatest in the PLA (17.8 \( \pm \) 8.3, 14.3 \( \pm \) 7.3, and 14.5 \( \pm \) 8 M_2 receptor-stained elements/cm\(^2\) in PLA, PV, and LAA, respectively, \( P = 0.012 \); Fig. 7E).

**Effect of Selective Cholinergic Blockade in the PLA on LA Electrophysiology and AF Inducibility**

VS led to ERP shortening in the PV, PLA, and LAA, with more pronounced ERP shortening in the PV and PLA than in the LAA (overall shortening 16\%, \( P = 0.009 \); Fig. 8A). Then 1\% tropicamide was applied to the PLA.

Table 1. *Nerve bundle and fiber distribution in PVs*

<table>
<thead>
<tr>
<th>PV Segment</th>
<th>LSPV*</th>
<th>LIPV</th>
<th>RSPV</th>
<th>RIPV</th>
<th>P</th>
<th>Proximal</th>
<th>Distal</th>
<th>P</th>
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<tr>
<td>Bundles</td>
<td>1.15±1</td>
<td>0.7±0.6</td>
<td>1.2±1.2</td>
<td>0.3±0.4</td>
<td>0.5</td>
<td>1.2±1</td>
<td>0.7±1</td>
<td>0.06</td>
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<tr>
<td>Fibers</td>
<td>S 35.1±13</td>
<td>31.8±16</td>
<td>30.6±17</td>
<td>41.7±21</td>
<td>0.6</td>
<td>40.9±14</td>
<td>30.5±19</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>P 33.1±19.5</td>
<td>41.5±26</td>
<td>43.7±18.1</td>
<td>51.1±17</td>
<td>0.51</td>
<td>54.9±31</td>
<td>41.2±21</td>
<td>0.07</td>
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</tbody>
</table>

Values are means ± SD. PV, pulmonary vein; LSPV, left superior PV; LIPV, left inferior PV; RSPV, right superior PV; RIPV, right inferior PV; S, sympathetic; P, parasympathetic.

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Fig. 3. Regional distribution of nerve bundles. A: relative predominance of nerve bundles in the PLA. Density of nerve bundles was significantly higher in the PLA than PV or LA appendage (LAA). B: epicardial vs. endocardial distribution of nerve bundles. Note epicardial predominance of nerve bundles in PV, PLA, and LAA.
The sinus rate did not change significantly from before to 5 and 30 min after tropicamide application (sinus cycle length = 618 ± 56, 608 ± 67, and 606 ± 77 ms, respectively, P = NS). The sinus cycle length increased significantly during VS compared with baseline, from 611 ± 64 ms at baseline to 682 ± 91 ms with VS (P = 0.02). The sinus cycle length during VS did not change significantly from before to 5 and 30 min after tropicamide application (P = NS). No significant changes in papillary diameter were noted before and after tropicamide-induced constriction. Light-induced pupillary constriction (miosis) was the same before and after tropicamide application.

After tropicamide application, the VS-induced ERP shortening was attenuated not just in the PLA but also in the PV and LAA (3.9% overall shortening, P = NS; Fig. 8A).

At baseline, AF was rarely induced using single atrial extrastimuli (inducibility index = 0.018). However, AF was more easily inducible in the presence of VS; 22 episodes of >5-s duration were induced in the presence of VS with single atrial extrastimuli (inducibility index = 0.12, i.e., 6.6 times the baseline ratio, P < 0.01; Fig. 8B). After tropicamide application, AF inducibility was significantly decreased in the presence of VS. Only one episode lasting >5 s could be induced; the inducibility index was 0.02, which represents a 92% reduction in AF inducibility (tropicamide + VS vs. VS alone, P < 0.001; Fig. 8B).

Table 2. Relative distribution of sympathetic and parasympathetic fibers in PV, PLA, and LAA

<table>
<thead>
<tr>
<th></th>
<th>Epicardial</th>
<th>Endocardial</th>
<th>P</th>
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<tr>
<td>PV</td>
<td>S 28.0±10.0</td>
<td>42.1±16.1</td>
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<tr>
<td></td>
<td>P 40.4±18.3</td>
<td>41.8±21.0</td>
<td>0.69</td>
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<tr>
<td>PLA</td>
<td>S 41.6±20.6</td>
<td>26.1±7.8</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>P 109.6±85.3</td>
<td>51±9</td>
<td>0.21</td>
</tr>
<tr>
<td>LAA</td>
<td>S 57.1±15.1</td>
<td>63.3±17.1</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>P 87.5±52</td>
<td>43.6±12.7</td>
<td>0.11</td>
</tr>
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</table>

Values are means ± SD. PLA, posterior left atrium; LAA, left atrial appendage.
DISCUSSION

The major findings of this study are as follows: 1) parasympathetic nerve trunks and fibers in the LA are preferentially located in the PLA, with nerve trunks being located in the fat pads as well as in surrounding myocardium, 2) M2 receptor density is also significantly more pronounced in the PLA than in the rest of the LA, and 3) since the majority of parasympathetic fibers and M2 receptors in the LA are located in the PLA, selective parasympathetic blockade in the PLA significantly alters vagal responsiveness in the entire LA, with a near-complete elimination of vagal-induced AF. These results indicate a potentially important role for the parasympathetic innervation of the PLA in the creation of substrate for AF (at least in structurally normal hearts). Furthermore, our results suggest that a targeted disruption of parasympathetic elements in the PLA may help successfully modify this substrate.

This study is unique for its therapeutic implications. Since the PLA contains a larger number of parasympathetic fibers and M2 receptors than the rest of the LA, our results indicate that a topical strategy targeted to the PLA can be successfully employed to achieve functional parasympathetic blockade in the entire LA.

Role of Parasympathetic Innervation of the PLA in the Genesis of AF

The role of the autonomic nervous system in the genesis of atrial arrhythmias has been extensively studied (12, 17). VS and/or administration of ACh have also been shown to decrease refractoriness in the LA and enhance inducibility of AF (15).

Recent clinical studies have shown that several cases of AF can be caused by a rapidly firing focus in the atrium (5–7, 11, 24). Clinical and animal data suggest that these PV triggers and drivers appear to be at least partially modulated by the autonomic nervous system (11, 24), with different studies implicating the sympathetic or parasympathetic nervous system in...
the genesis and/or maintenance of focal AF. More recent studies postulate a dominant role for the parasympathetic nervous system (in focal AF) (25), in large part on account of the Bezold-Jarisch-like reflexes noted during radio-frequency ablation of the PV tissues in patients with paroxysmal focal AF. Pappone et al. (19) suggested that altering vagal input to the LA and PVs may improve efficacy of ablation procedures for AF.

Despite these initial studies, the complete autonomic profile of the PVs and the precise role of the autonomic nervous system in the genesis of focal AF are just beginning to be systematically investigated in clinical and animal models. Recent data from our laboratory suggest a differential electrophysiological response of the PVs/PLA from that in the rest of the LA in response to autonomic maneuvers (2). In our physiological study (2), we demonstrated a greater decrease in refractory periods in the PVs and PLA than in the rest of the LA in response to VS. The heterogeneity of vagal responses in the LA in this study correlated with differences in the pattern of distribution of ACh-activated K⁺ current in the PV, PLA, and LAA. The structure and distribution of sympathetic and parasympathetic nerves within the PV and PLA, as well as their contribution to the electrophysiological profile of these regions, were not examined in our earlier study.

In a recent clinical study, Tan et al. (26) demonstrated colocalization of sympathetic and parasympathetic nerve fibers in nerve trunks in the LA. Although Tan et al. showed a relative predominance of sympathetic (compared with parasympathetic) fibers, electrophysiological studies were not performed to assess the physiological significance of this nerve distribution pattern. In addition, a relative comparison of nerve distribution was not performed between different regions of the LA; more specifically, the autonomic innervation of the PLA and PVs was not compared with the rest of the LA. The present study confirms the findings of Tan et al., in that colocalization of parasym pathetic and sympathetic nerve fibers was noted in the LA. In contrast, however, our study demonstrates a predominance of parasympathetic fibers in the LA, with the PLA having the highest nerve concentration within the LA. To our knowledge, systematic studies examining differences in

![Muscarinic (M2) receptor distribution in the PLA, PV, and LAA. A–D: immunostaining for M2 receptors (brown). A: M2 receptor localization along autonomic nerve bundles and perivascularly (arrows). Magnification ×10. B: individual nerve bundle in the PLA stained for M2 receptor (arrow). Magnification ×40. C and D: M2 receptor localization within single cardiomyocytes in the PLA and LAA, respectively (arrows). Magnification ×40. Number of M2 receptor-stained cells is clearly greater in the PLA than LAA. E: predominance of M2 receptors in the PLA. Error bars, SE.](http://ajpheart.physiology.org/)

Fig. 7. Muscarinic (M2) receptor distribution in the PLA, PV, and LAA.
parasympathetic vs. sympathetic innervation/tone have not been performed in other species, e.g., in animals such as horses, that are known to have frequent AF. Regardless of the difference in P-to-S ratio between the two studies (which may be accounted for by interspecies differences in neural innervation between humans and dogs or by the sparser sampling in the study by Tan et al.), the parasympathetic nervous system appears to be a major player in clinical, as well as canine, AF (19, 25).

Detailed, in vivo electrophysiological experiments were also performed as part of the present study. Topical cholinergic blockade in the PLA resulted in a significant change in vagal responsiveness in the LA, confirming the importance of the parasympathetic innervation of the PLA in the genesis of vagal-induced AF.

The diminution of vagal reflexes in the entire LA in response to topical cholinergic blockade in the PLA alone suggests that the majority of the parasympathetic fibers innervating the LA originate or at least pass through the PLA before innervating the rest of the LA. In fact, studies recently performed by Hou et al. (9, 10) indicate the presence of interconnections between ganglionic plexi (GP) in the atria. It therefore appears likely that the remote effects of tropicamide in the rest of the LA are due to the functional disruption of an intricate and interactive neural network in the LA. This hypothesis is further supported by the following observations from the present study: 1) cardiac ganglia containing neuronal cell bodies are present only in the PLA and PVs but were completely absent in the rest of the atrium, 2) the majority of nerve trunks in the LA are located in the PLA, and 3) nerve trunks in the PLA are larger than in the rest of the LA. The remote effects of tropicamide on vagal responsiveness in the PVs and LA can therefore be best explained by a positive-feedback mechanism that involves muscarinic receptors on cholinergic neurons (18, 27), which are known to participate in negative- as well as positive-feedback mechanisms that modulate ACh release from neuronal cells (18, 27). We postulate that tropicamide, by inhibiting muscarinic receptors on ganglion cells and nerve trunks in the PLA, decreases ACh release more distally from neuronal synapses in the PVs and LAA.

Selective Parasympathetic Denervation of the PLA: A New Therapeutic Target for AF?

Elimination of vagal reflexes during ablation lesions has been shown in some studies to be correlated with improved PV ablation outcomes (19). However, other attempts at parasympathetic ablation for AF have been less successful (4, 8). Endocardial ablation strategies that target the parasympathetic nervous system of the LA are largely empirical, being guided by nonspecific electrophysiological responses such as sinus bradycardia and AV block (19, 23). More “anatomic” approaches that surgically target atrial fat pads have also met with somewhat mixed success (4, 28). An added disadvantage of an anatomic approach is that it inevitably causes transmural atrial tissue damage (16). In addition, the LA is innervated by several fat pads, all of which may not be responsible for the innervation of the PLA and PVs.

An ideal therapeutic strategy would be more precise in targeting the nerves involved in the genesis of AF without causing significant damage to surrounding tissue (14). Development of such a targeted approach to focal AF hinges on a better understanding of the complexities of neural innervation of the atria. Electrophysiological data reported by Hou et al. (10) suggest the presence of an intricate, interconnecting neural network in the LA that may contribute to substrate for focal AF. However, no attempt was made to correlate electrophysiological responses with the structure, function, and distribution of the underlying nerve fibers. A recent human study quantified autonomic nerve distribution in the PVs and surrounding LA and described heterogeneity of nerve distribution in this region (3), but the composition of these nerves, i.e., sympathetic vs. parasympathetic, was not reported.

In the present study, we have demonstrated that the PLA is the most richly innervated region of the LA, with parasympathetic fibers comprising a majority of the nerves supplying this area. Importantly, the present study shows that even though a majority of nerve bundles are located within the fat/fibrofatty tissue, up to one-third of nerve bundles in the PLA can be
located away from the fat in adjoining/underlying myocardium. This finding suggests that ablation strategies directed at atrial fat pads would not be expected to result in complete denervation of the PLA. Since vagal nerve fibers and M2 receptors are preferentially localized in the PLA, we postulated that topical, pharmacological disruption of cholinergic function in the PLA would alter vagal responsivity in the entire LA. Our experiments demonstrate that it is indeed feasible to attain selective cholinergic blockade in this region with a nonablative, pharmacological approach, with a resulting change in vagal-induced AF substrate.

It is important to realize that focal sources of AF been described elsewhere in the atria, e.g., in the superior vena cava and coronary sinus. In the present study, we chose to study/target the PLA, inasmuch as a large majority of ectopic foci have been found to be localized to the PVs and PLA. Although an approach targeted to the PLA would help attenuate/eliminate focal AF sources in the LA, it would not be expected to affect other, more distant focal sources in the heart.

Study Limitations

This was a canine study, the results of which cannot be directly extrapolated to the human LA. Moreover, the present study was conducted in normal dogs, and the extent to which autonomic remodeling occurs in the setting of AF is not known. Further studies are therefore needed to assess neural innervation in more “pathological” settings.

The nerve distribution in the LA and PVs is complex, as shown by previous anatomic studies (1, 3). The present study does not provide detailed functional-anatomical information of the distribution of bundles and ganglia into specifically localized plexuses. Armour et al. (1) demonstrated the presence of at least five major atrial fat pads, most of which were located on the posterior surface of the atria. Of these five fat pads, two were located on 1) the posterior medial surface of the LA and 2) the inferior and lateral aspect of the PLA, respectively. In addition, in the study of Armour et al., the posteromedial GP had one of the largest numbers of gangia among the atrial GPs. Although we did not perform a gross anatomic dissection in the present study, the site of tropicamide application (at the confluence of PVs) corresponds to the location of these two fat pads. We did see fat in the PLA, likely corresponding to at least one of the above-mentioned fat pads, even though formal confirmation was not obtained using gross anatomic dissection of these collections of fibrofatty tissue. The absence of a formal correlative study comparing the previously defined GP locations with our immunohistochemical-functional data is a limitation of the present study. However, our primary objective was to assess the underlying functional composition of the autonomic nervous system in each major region of the LA and to assess the significance of the PLA as a therapeutic target for selective vagal denervation.

Although our study demonstrates a substantial reduction of AF inducibility by attenuation of the electrophysiological effects of vagal stimulation on refractory periods, other potential mechanisms of AF as spontaneously triggered activity in the PVs may not be covered with this therapeutic approach.

In the present study, we did not assess the relative importance of ganglion cells vs. nerve fiber bundles/trunks in the creation of vagal AF substrate. Although it would seem appropriate that the preferential target for ablation should be ganglion cells, we discovered that neuronal cell bodies and nerve fibers were colocализed in several nerve bundles (Figs. 4 and 6). We also discovered that although large collections of neuronal cell bodies (i.e., ganglia) are relatively sparsely located, the nerve fiber bundles that arise from these ganglion cells are frequently seen. Despite their greater number, nerve fiber bundles/trunks were always found to be in the vicinity of (or found to arise from/juxtaposed to) the neuronal cell bodies. This suggests that large nerve bundles/trunks may be a good surrogate for the presence of nerve cell bodies and, therefore, provide a suitable ablation target. Nevertheless, more studies are needed to assess the relative contribution of nerve cell bodies vs. nerve bundle/trunks to the autonomic physiology of the LA.

The contribution of the sympathetic innervation of the PLA and PVs to AF substrate was not examined in the present study. Even though vagal innervation may be the more important “player” in the genesis of focal AF (20–22), the sympathetic nervous system may play an important “modulatory” role in the genesis of AF, helping provide a “catalyst” for the emergence of focal triggers/drivers in the presence of an increased vagal tone. In fact, even though the relative number of sympathetic fibers within nerve trunks/bundles was small and we did not find any bundles that were composed entirely of sympathetic fibers, parasympathetic and sympathetic fibrils were present in near-equal proportions in the PVs and LAA. It is therefore possible that several single sympathetic fibrils travel singly in the atria outside the bundles/nerve trunks. The detailed interactions between the sympathetic and parasympathetic nervous systems within the LA, especially as they relate to the genesis of AF, need to be addressed in more detail in future studies.

GRANTS

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REFERENCES


