Relationship Between Intrinsic Cardiac Autonomic Ganglionated Plexi and the Atrial Fibrillation Nest

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Background: Spectral analysis of the left atrium can identify high dominant frequency (DF) sites, which might play a role in the perpetuation of atrial fibrillation (AF). Furthermore, the role of the cardiac autonomic nervous system (CANS) in the genesis of AF has been demonstrated. The relationship between CANS and the high-DF sites (AF nest) was the aim of the investigation.

Methods and Results: In 12 dogs, high frequency stimulation was applied to locate 4 major left atrial (LA) ganglionated plexi (GPs). An Ensite Array and a mapping catheter were delivered into the left atrium for electroanatomical mapping. During sinus rhythm, spectral analysis was performed on the bipolar electrograms in the left atrium before and after epicardial GP ablation. The majority of AF nests were close to the GPs (52±18% of total AF nests). After GP ablation, the mean LA DF values decreased from 54±7 Hz to 49±4 Hz (P=0.023), and DF values of the AF nest decreased from 93±2 Hz to 87±4 Hz (P=0.001). Most of the previous AF nest sites close to the GPs disappeared (85±23%). The surface area of the AF nest decreased from 9±5 cm² to 3±2 cm² (P=0.001).

Conclusions: Catheter ablation of the GP decreased the DF values, AF nest areas and diminished the number of AF nests; particularly those close to the GPs, indicating that the CANS might play an important role in the mechanism of the AF nest. (Circ J 2014; 78: 922–928)

Key Words: Ablation; Atrial fibrillation; Autonomic; Ganglionated plexus
and characteristics of the AF nest.

**Methods**

**Animal Preparation**

The protocol for the animal preparation was approved by the Committee for Experiments on Animals at our institution. A total of 12 adult mongrel dogs (weighing 11.5–16.5 kg) were anesthetized with ketamine (10–20 mg/kg) and sodium pentobarbital (30 mg/kg intravenously). All animals received a warming blanket to maintain their core body temperature at 36.5±1.5°C. A suture was applied over the insertion site to prevent further bleeding.

**Cardiac Autonomic GPs and AF Nest**

The arterial blood gas was checked hourly to keep a balanced acid-base status (pH 7.35–7.45) and oxygenation (SaO₂ >90% without hypercapnia). All dogs were ventilated with room air by a positive pressure respirator. Oxygen was administered to maintain the SaO₂ >90%. Venous access was obtained by using the Seldinger technique with an 8-F sheath from the right femoral vein. Arterial access was setup at the right femoral artery for blood pressure and body temperature monitoring and blood sampling. The chest was opened via a mid-line thoracotomy and the heart was exposed after an incision in the pericardium. All electrograms recorded from the electrode catheter were amplified and digitally recorded by using a computer-based Bard Lab System (CR Bard Inc, Billerica, MA) filtered at 30–500 Hz. Raw data detected by the MEA is amplified and digitally recorded by using a computer-based Bard Lab System (CR Bard Inc, Billerica, MA) filtered at 30–500 Hz.

**Identification of the Autonomic System**

The detailed preparation procedures have been reported previously. After the thoracotomy, the GPs were identified by applying high-frequency stimulation using a bipolar electrode probe through a Grass stimulator (20 Hz, 0.1 ms duration, square waves, slowly increase from 0.6V to 8.0V) to the epicardial fat pad. If atrial myocardium was captured, the electrode was removed immediately, and further stimulation was delivered below the atrial capture threshold. A bradycardia response with a progressive slowing of the sinus rate by 50%, or the development of a second or third degree atrioventricular block resulting from incremental voltage levels applied to the fat pad, was defined as a marker for GP stimulation.

Four left atrial (LA) GPs were identified around the ostia of the 4 PVs: the anterior right GP (ARGP) was located between the caudal end of the sinoatrial node and the right superior PV-atrial junction; the inferior right GP (IRGP) was located at the junction of the inferior vena cava and both atria; the superior left GP (SLGP) was located between the LA appendage and left pulmonary artery, adjacent to the left superior PV-atrial junction; and the inferior left GP (ILGP) was located on the caudal side of the inferior left PV-atrial junction.

**Electroanatomic Mapping, Signal Recording and Analysis**

The use of the non-contact mapping system (EnSite 3000 with Precision Software, Endocardial Solutions, MN, USA) in our laboratory has previously been described in detail. In brief, the system consists of a 9-French catheter with a multi-electrode array (MEA) surrounding a 7.5-ml balloon mounted at the distal end. The MEA catheter and a 4-mm tip catheter were placed into the left atrium via the LA appendage through a small incision. A suture was applied over the insertion site to prevent further bleeding.

The three-dimensional position of the electrodes on the 4-mm tip catheter was determined using its navigation signal relative to the MEA. Navigation provides the means to define a model of the chamber anatomy and to track the position of the 4-mm tip catheter within the chamber relative to labeled points of interest. Raw data detected by the MEA is amplified and digitally transferred to a computer workstation.

After acquiring the LA geometry, the 4-mm-tip catheter was selected as the roving catheter for sequential contact mapping. The left atrium was divided into 8 parts (Figure 1): high anteroseptal wall, low anteroseptal wall, high posterior wall, low posterior wall, anterior roof, posterior roof, right PV-atrial junction and left PV-atrial junction. The 4-mm tip catheter was used to collect local bipolar signals evenly distributed in those parts while it was swiped across the left atrium during SR. The signal from the mapping catheter was used to build a sequential map. After completion of the sequential map, all bipolar signals were exported for further analysis by the Matlab computer program (MathWorks Inc. Natick, MA, USA). The mean LA peak-to-peak bipolar voltage was calculated from the average bipolar voltages of the 8 LA regions.

Spectral analysis was performed on the single discrete bipolar electrogram during SR (unrectified, Hanning window, 1 s in duration). Following this, data obtained from single discrete electrogams during SR, and data from baseline on both side of the electrogams were exported to an external computer program. A fast Fourier transform analysis was performed using...
a Hanning window function on each segment from all recording sites in the atrium. The dominant frequency (DF) was defined as the frequency with the maximum power in the frequency range. Higher frequencies were mapped toward the purple end of the color spectrum, as a real time built-in function. To ensure the reliability of the DF detection, the lowest noise signal was chosen for the analysis. According to the normal distribution curve of the DF values, the top 5% were higher than 80 Hz, and those sites were defined as high frequency sites (AF nest sites). The mean LA DF value was calculated from the average DF values of the 8 LA regions.

Catheter Ablation Procedure
A 6-French 4-mm ablation catheter (Blazer, Boston Scientific) was placed at the GP site using direct manipulation from the epicardial side. Radiofrequency ablation was performed using a power of 45–50 W, and aimed to achieve a target temperature of 55–60°C. Completeness of GP ablation was verified by elimination of vagal responses induced by applying the same high-frequency stimulation at the same regions that had elicited responses before ablation. Identical signal recording and analyzing protocols for AF nest, as described above, were performed before and after ablation of GP.

Immunohistochemistry Study
Epicardial fat pad tissue and myocardium harvested from the study were used in immunostaining. A fixation procedure was performed when samples were acquired after the operations. Sections were cut parallel to the plane of mitral annulus to include the epicardial and endocardial aspects of the myocardium in each slice. The specimen was fixed in 20% formalin before staining. Primary antibodies were then incubated overnight at 37°C. Antibodies for tyrosine hydroxylase were used to stain sympathetic nerves, whereas antibodies for choline acetyltransferase were used to stain parasympathetic nerves. After incubation, the slides were washed in Tris-buffered saline, and the appropriate secondary antibody was placed on the sections for 30 min. The sections were again washed in Tris-buffered saline, and the appropriate chromagen was added to each specimen. The specimens were then dehydrated in alcohol, mounted, and examined under light microscopy. The tyrosine hydroxylase- and choline acetyltransferase-positive portions within GPs were localized and the cross-sectional areas of these positive portions inside the GPs were being measured.

Statistical Analysis
Quantitative data are expressed as the mean value ± standard deviation. The paired-samples t-test was used to make comparisons between the continuous data, and the Chi-squared test with a Pearson or Fisher’s exact test were used to make comparisons between the categorical data before and after GP ablation. Multiple-group comparisons were obtained with the ANOVA test. Statistical analysis was performed using SPSS Statistics 17.0 software (Chicago, IL, USA). All values were considered statistically significant at a two-tailed P value less than 0.05.

Figure 2. Regional distributions of the peak-to-peak bipolar voltage (A) and the dominant frequency value (B) of the left atrium. HA, high anteroseptal wall; LA, low anteroseptal wall; HP, high posterior wall; LP, low posterior wall; AR, anterior roof; PR, posterior roof; RPV-LA, right pulmonary vein-left atrial junction; LPV-LA, left pulmonary vein-left atrial junction. *P<0.001 before and after ablation, #P<0.001 compared to the DF values of non-PV sites.

Results
A total of 12 adult mongrel dogs (13.5 ± 1.5 kg, 5 male dogs) were included in the study. By applying high-frequency stimulation, ARGPs could be identified in all 12 dogs. The IRGP, SLGP, and ILGP could be identified in 10 (83%), 11 (92%) and 10 (83%) dogs, respectively. The MEA catheter was delivered in the left atrium via a LA appendage approach in all dogs.

LA Mapping Before GP Ablation
LA mapping points were 539 ± 240 points. The mean LA peak-to-peak bipolar voltage before GP ablation was 3.1 ± 0.9 mV, and the mean LA DF value was 54.3 ± 6.5 Hz. The regional bipolar voltage analysis of different LA sites is shown in Figure 2A, and the regional DF analysis of different LA sites is shown in Figure 2B. There were no differences among the bipolar voltages of the different LA sites. The DF value of the right PV-atrium junction was 61.0 ± 7.2 Hz and left PV-atrium junction was 60.7 ± 7.4 Hz. Both of them were significantly higher than the DF values of the other LA non-PV sites (52.1 ± 7.3 Hz, both P values < 0.001).

Overall, 105 mapping sites had a DF value higher than 80 Hz in all 12 dogs before GP ablation. The DF value of the AF nest was 92.8 ± 1.5 Hz. The highest LA DF value of each dog was 111.9 ± 2.8 Hz. The surface area of the AF nest was 9.1 ± 4.8 cm² (8.4 ± 5.4% of the total LA surface area). These high-DF sites
LA Mapping After GP Ablation

LA mapping points were 651±248 points. The mean LA peak-to-peak bipolar voltage after GP ablation was 2.9±1.3 mV, which was not significantly different to that before ablation (P=0.56). After GP ablation, the mean LA DF value decreased from 54.3±6.5 Hz to 48.9±3.6 Hz (P=0.023), the DF value of the AF nest decreased from 92.8±1.5 Hz to 86.6±4.4 Hz (P=0.001), and the surface area of the AF nest decreased from 9.1±4.8 cm² to 3.0±2.1 cm² (8.4±4.4% to 2.7±1.8% of the total LA surface area, P=0.001).

The regional bipolar voltage analysis of different LA sites is shown in Figure 2B. Compared to those before GP ablation, the DF values of the right and left PV-atrium junctions decreased significantly (both P values <0.001). The DF values of the other non-PV LA sites remained similar after epicardial GP ablation. After GP ablation, there were 38 mapping sites presenting

Figure 3. Examples of LA DF mapping before (A,C) and after (B,D) GP ablation. Before GP ablation, numerous high-DF sites were identified near the PV-LA junction, roof and posterior wall. After GP ablation, many high-DF sites disappeared, and these changes were prominently seen at the PV-LA junction. LA, left atrial; DF, dominant frequency; GP, ganglionated plexus; PV-LA, pulmonary veins-left atrial.

were found to be in specific regions and not equally distributed in the left atrium. There were 8.8±3.6 high-DF sites (range 3–14 sites) in the left atrium and PV region in each dog. The distribution of the high-DF sites was 5.3±2.3 sites (62.9±16.3%) near the PV region, including 4.3±1.9 sites (52.4±17.7%) close to the GPs, 0.9±0.8 sites (10.4±9.5%) in the LA anteroseptal wall, 1.4±1.2 sites (17.2±12.8%) in the LA posterior wall and 1.1±1.2 sites (10.0±9.5%) in the LA roof. The distribution of the high-DF sites was significantly higher in the PV-LA junction than those in the LA anteroseptal wall, posterior wall, and roof (all P values <0.001). The examples of LA DF mapping before GP ablation are shown in Figures 3A,C.

Catheter Ablation for Epicardial GPs

Catheter ablation procedures for GPs were performed in the order of SLGP, ARGP, ILGP and IRGP, and were verified by the elimination of the bradycardia response. Ablation time for each GP was 134±19s. A target temperature of 55–60°C could be achieved at all ablation sites. No AF or other tachycardia was induced during ablation. No steam pops happened during ablation.

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The main finding of this study was that the LA AF nest sites during SR were primarily located near the PV-LA junction close to the GPs before catheter ablation. Catheter ablation of the GP decreased the LA DF values, AF nest DF values, AF nest areas, and diminished the numbers of AF nest, especially at the PV-LA junction close to the GPs. These findings indicate that the cardiac autonomic nervous system might play an important role in the mechanism of LA AF nest sites.

Compared With Previous Studies

Pachon et al. showed that an abnormal atrial substrate could be differentiated from a normal one by using a fast Fourier Transform analysis during SR. According to the study, clusters of fibrillatory myocardium were characterized by multiple rapid deflections and fractionated electrograms in the time-domain signals, and high frequency peaks in the frequency-domain signals. Ablation of those high-frequency sites could eliminate the high-frequency peaks.

In another study, Lin et al. performed spectral analysis in patients with paroxysmal AF during SR. The high-frequency sites within the PV ostia could be identified in 80% of the paroxysmal AF patients, and circumferential PV isolation could eliminate 69% of the high-frequency sites within the circumferential isolation line. Similar to previous studies, the majority of AF nest sites were located near the PV-LA junction in this animal study.

Histological Studies of the GP

Figures 5 and 6 compare the results of immunostaining in the GPs between the experimental dogs and the other 7 normal control dogs. The total surface area of the GP cross-section measured 0.35±0.26 mm² in experimental dogs and 0.46±0.29 mm² in control dogs (P=0.022). In experimental dogs, the choline acetyltransferase-positive nerves comprised 28.3±15.9% of the GP cross-sectional area, while the tyrosine hydroxylase-positive nerves comprised 11.2±9.8% of the GP cross-sectional area. The percentage of choline acetyltransferase-positive nerves and tyrosine hydroxylase-positive nerves were significantly less in experimental dogs compared with control dogs. (choline acetyltransferase-positive nerves 37.1±11.3%, P=0.019; tyrosine hydroxylase-positive nerves 20.4±10.2%, P<0.001; Figure 6). Except for the neuronal cellular injuries within the GPs, there was no significant damage of the underlying muscular cells (Figure 5G).

Discussion

Major Findings

The main finding of this study was that the LA AF nest sites during SR were primarily located near the PV-LA junction close to the GPs before catheter ablation. Catheter ablation of the GP decreased the LA DF values, AF nest DF values, AF nest areas, and diminished the numbers of AF nest, especially at the PV-LA junction close to the GPs. These findings indicate that the cardiac autonomic nervous system might play an important role in the mechanism of LA AF nest sites.

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Underlying Mechanisms of the AF Nest

Pachon et al. suggested that the AF nest might be caused by the non-uniform arrangement of atrial fibers, localized fibrosis and anisotrophy with discontinuous conduction. In contrast, it also might reflect a transitional area between neural, vascular and atrial myocardial tissue.

Increased parasympathetic nervous activity might also be associated with the AF nest. In patients who underwent catheter
Ablation for paroxysmal AF, Lellouche et al. found that the local fractionated electrogram was associated with parasympathetic responses during radiofrequency application. A recent study showed that patients with vagal-type AF had a higher LA DF value than those with sympathetic-type AF. The elevated vagal activity to the heart might shorten the regional refractory period, result in the fractionation of the electrogram and cause an increased DF values. However, that study did not analyze the areas and distributions of AF nests.

Unlike other studies mentioned above, catheter ablation of the endocardial high-frequency sites was not performed in this study. Rather, in the current study, catheter ablation was performed epicardially on the GP sites. Because the LA peak-to-peak bipolar voltage after GP ablation was not significantly different to that before ablation, the ablation seems to result in no or only minor damage to the endocardial atrial myocardium in the pathology results. Nevertheless, the GP ablation significantly decreased the numbers of AF nest sites, predominantly at the PV-LA junction close to the GPs. This finding indicated that the cardiac autonomic nervous system might play an important role in the mechanism of AF nest sites. In contrast, the damage to the epicardial myocardium could also contribute to the DF changes after epicardial ablation.
Interestingly, the GP ablation did not completely eliminate all the AF nests, even at the PV-LA junction. There are several possible explanations. First, in the current study, we ablated 4 major LA GPs. However, the other LA GPs, such as the Marshall tract GP, which might also contribute to the AF nest, were not identified and ablated. Second, the autonomic neural innervation and abnormal atrial substrate could be 2 independent mechanisms of the AF nest. Hence, ablation of the GPs alone could not totally eliminate the AF nest. In the current study, we found that the catheter ablation of GPs had less impact on LA posterior wall high-frequency sites. The muscular architecture and electrical properties of the LA posterior wall have been shown to play a crucial role in the initiation and maintenance of AF. It seems that the myofiber orientation with anisotropic electrical properties might be more important, and the autonomic nervous system might play a minor role in the mechanism of the posterior LA AF nest. However, further studies are warranted to clarify this hypothesis.

Study Limitations

Normal healthy animals were used in this experiment. Autonomic remodeling could proceed in the atrial substrate of AF, and previous studies showed an increase in atrial sympathetic nomic remodeling could proceed in the atrial substrate of AF, to clarify this hypothesis.

Conclusions

In this canine model, the high-DF sites were found to be located primarily near the PV-LA junction close to the GPs during SR. Catheter ablation of the GP decreases the LA DF values, AF nest DF values, AF nest surface areas and diminished the number of AF nests, especially at the PV-LA junction close to the GPs, indicating that the cardiac autonomic nervous system might play an important role in the mechanism of high-DF sites.

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Disclosures

None of the authors have any conflict of interest or financial relationship to declare.

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