Original Article

Arrangement of the Autonomic Nerves Around the Pulmonary Vein-Left Atrial Junctions —Histologic and Immunohistochemical Analyses—

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Introduction: Imbalanced autonomic activity in the area of the pulmonary veins (PVs) can result in spontaneous atrial fibrillation (AF). Histologic characteristics of the PV sleeve musculature and associated autonomic nerve are not fully understood. We investigated the arrangement of autonomic nerve fibers around PV-left atrium (LA) junctional musculature.

Methods: Thirteen autopsied adult hearts (9 men and 4 women; mean age at death, 66.2 years) were studied. The atria were removed from each heart, along with all PV stalks, and cut longitudinally to each PV myocardial sleeve. After treatment with azan-Mallory stain and immunohistochemical staining for S-100 and tyrosine hydroxylase (TH), autonomic nerve distribution was assessed by counting the numbers of TH-positive (adrenergic) and -negative (non-adrenergic) fibers within S-100-positive fibers (>50 µm in diameter) in the anterior, posterior, and septal junctions.

Results: TH-positive adrenergic fibers, consisting of sympathetic nerves, were most predominant in the anterior and septal junctions. In the anterior junction, these fibers were packed tightly among myocardial sleeve fascicles. In the posterior junction, the numbers of adrenergic and non-adrenergic fibers were fewer. In the septal junction, the number of TH-negative non-adrenergic fibers (predominantly parasympathetic nerves) was greater, concomitant diffuse ganglionic nodule distribution in the interatrial fat pad.

Conclusions: In each PV-LA junction, autonomic nerves were localized on the anterior and septal walls. Heterogeneous distribution of TH-positive and TH-negative fibers and ganglion nodules around each PV opening appears to represent the major histologic characteristic in these areas.
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Key words: Atrial fibrillation, Histology, Myocardial sleeve, Nerve distribution, Tyrosine hydroxylase

Introduction

Most spontaneous ectopic beats triggering paroxysmal atrial fibrillation (AF) are recorded from the pulmonary vein (PV) musculature.[1)] Therefore, radiofrequency ablation for drug-resistant AF is

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frequently performed in this region.[2] Previous clinical studies have suggested that an autonomic nerve imbalance is associated with the initiation of AF.[3,4] A recent report suggested that parasympathetic changes associated with the vagal reflex decreased the recurrence of AF.[5] Although several degrees of myocardial extension have been observed histologically from the human left atrium (LA) to the PV[6] and the distribution of autonomic nerves and ganglionic plexuses around the PV-LA junctional area has been documented,[7,8] the characteristics of the PV sleeve musculature and autonomic nerve distribution are not fully understood histologically. In this study we investigated the arrangement of autonomic nerve fibers around the PV-LA junctional musculature.

Methods

Study population

Thirteen autopsied human hearts from individuals who were 43 to 83 years of age at death (mean, 66.2 years; 9 men and 4 women) were studied. None of the individuals showed conspicuous supraventricular tachyarrhythmia on surface electrocardiograms. Seven died of malignant disease, and the others died of pneumonia, cerebral hemorrhage, or sepsis. Scar tissue from non-lethal myocardial infarction was found in two hearts.

Tissue preparation

All hearts were fixed with 10% formalin. After fixation, both atria were removed, along with all PV stalks, the left auricular appendage, and the openings of the superior vena cava and the inferior vena cava. According to lines from the upper to the lower poles of each PV opening, we divided the PV-LA junction into anterior, posterior, and septal areas (**Figure 1**) comprising eight segments, i.e., anterior and posterior junctions of the left superior and inferior PVs (LSPV, LIPV) and posterior and septal junctions of the right superior and inferior PVs (RSPV, RIPV).[9] Histologic specimens were cut longitudinally to each PV to obtain horizontal sections of the junction and sleeve musculature (**Figure 2**). From each segment, we obtained 3-μm-thick paraffin-embedded sections at three levels (superior, middle, and inferior). The sections were treated with hematoxylin-eosin and azan-Mallory stains.

Immunohistochemistry

Immunohistochemical examinations were performed with the use of S-100 antibody (polyclonal; DAKO, Glostrup, Denmark) as a marker of the peripheral nervous system including autonomic ganglia[10] and tyrosine hydroxylase antibody (TH, monoclonal; Novocastra, Newcastle, United Kingdom) as a marker of adrenergic fibers. Histologically, we considered S-100-positive and TH-positive fibers as sympathetic and S-100-positive and TH-negative fibers as non-adrenergic and including parasympathetic and sensory nerves.[11]

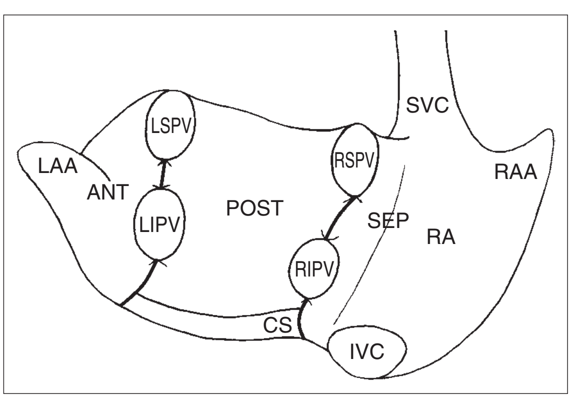

Figure 1 According to a line from the upper to the lower pole of each PV opening, each removed atrium was divided into three parts: anterior, posterior, and septal.
ANT, anterior area; POST, posterior area; SEP, septal area; CS, coronary sinus; IVC, inferior vena cava; LAA, left atrial appendage; LIPV, left inferior pulmonary vein; LSPV, left superior pulmonary vein; RA, right atrium; RAA, right atrial appendage; RIPV, right inferior pulmonary vein; RSPV, right superior pulmonary vein; SVC, superior vena cava.

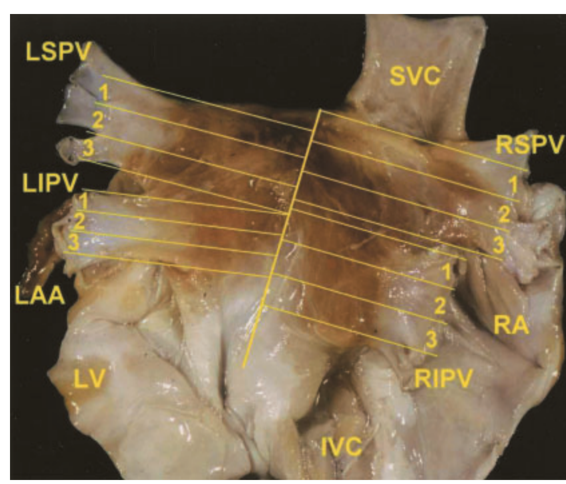

Figure 2 Macroscopic overview of both atria from the posterior aspect.
Each PV-LA junction was cut into three specimens (1, superior; 2, middle; 3, inferior). IVC, inferior vena cava; LAA, left atrial appendage; LIPV, left inferior pulmonary vein; LSPV, left superior pulmonary vein; LV, left ventricle; RA, right atrium; RIPV, right inferior pulmonary vein; RSPV, right superior pulmonary vein; SVC, superior vena cava.

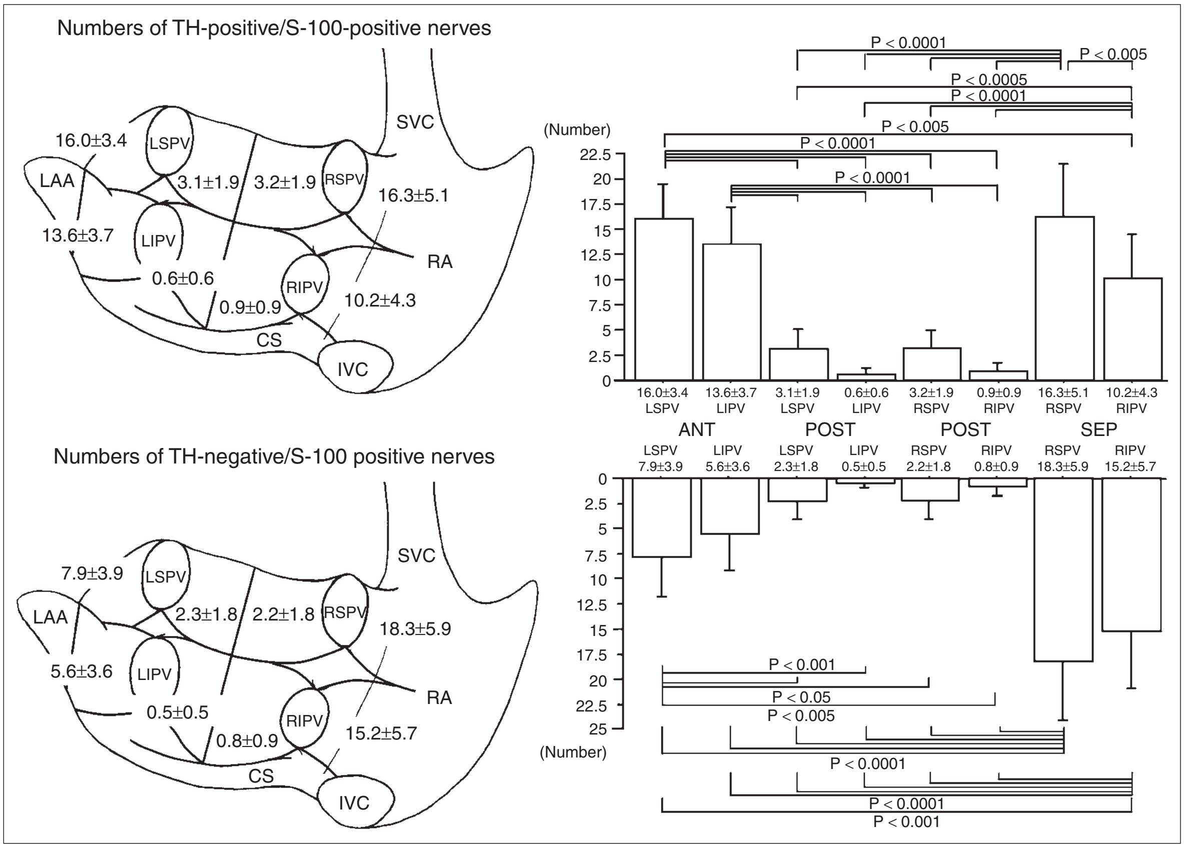

Figure 3 (Upper) Mean numbers of TH-positive/S-100-positive nerves distributed around the PV-LA junction area. TH-positive/S-100-positive (sympathetic) nerves were most abundant in the anterior LSPV and LIPV junctions and were also abundant in the septal RSPV junction. Although the number of sympathetic nerves was significantly less in the posterior junction, a slight predominance in both LSPV and RSPV posterior junctions was observed. (Lower) Mean numbers of TH-negative/S-100-positive nerves distributed around the PV-LA junction area. TH-negative/S-100-positive nerves, including predominantly parasympathetic nerves, were prevalent in the superior and inferior septal junction areas.

Quantification of autonomic nerve fibers

At the PV-LA junction area, we counted the numbers of TH-positive or TH-negative nerve fibers within S-100-positive fibers greater than 50 µm in diameter and the ganglionic nodules associated with the distribution of the efferent vagal pathways.[12] We compared these numbers between the eight segments. For each segment, the average numbers of nerves and ganglionic nodules (of the superior, middle, and inferior levels combined) were obtained.

Statistical analysis

Data are presented as the mean ± standard deviation. Differences in mean values were analyzed by Scheffe's post-hoc test. P values <0.05 were considered statistically significant.

Results

Histologic characteristics

The numbers of TH-positive/S-100-positive (sympathetic) nerves and TH-negative/S-100-positive (mainly parasympathetic) nerves (**Figure 3**), and ganglionic nodules (**Figure 4**) distributed in the eight segments of the PV-LA junction area are shown for all 13 cases. All of the ganglionic nodules were TH-negative/S-100-positive. We showed the average numbers of nerves and ganglionic nodules of the superior, middle, and inferior levels combined because there were no significant differences between levels.

Anterior PV-LA junction

Along with a relative lack of TH-negative/S-100-positive non-adrenergic fibers, TH-positive/S-100-positive sympathetic nerves were most conspicuous at both the anterior LSPV and LIPV junctions and at the septal RSPV junction (**Figure 3**). Typical findings in the anterior PV-LA junction area are shown in **Figure 5** (55-year-old man). The sympathetic nerves were rooted in the PV sleeve musculature in the

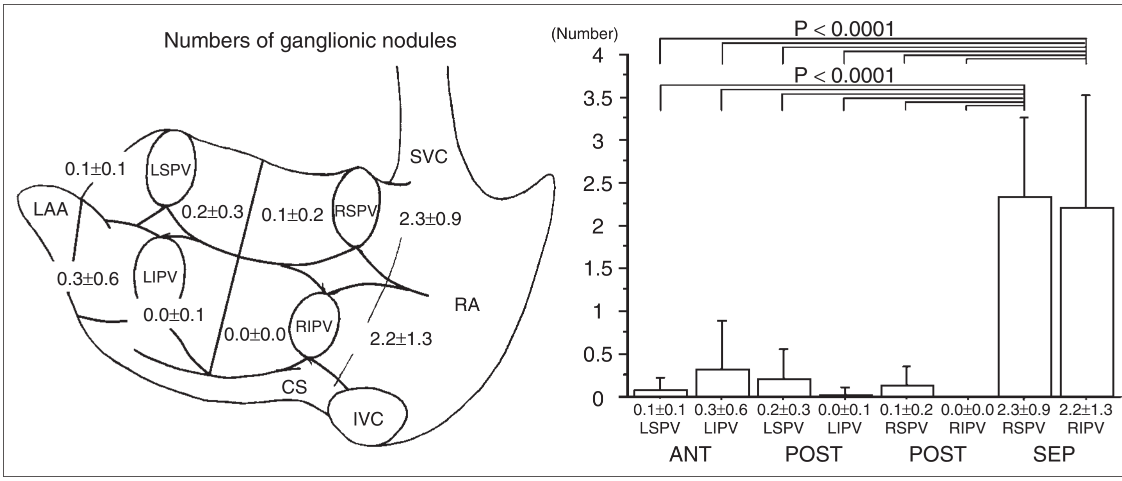

Figure 4 Mean numbers of ganglionic nodules distributed around the PV-LA junction area.
The distribution was similar to that of TH-negative/S-100-positive non-adrenergic nerves.

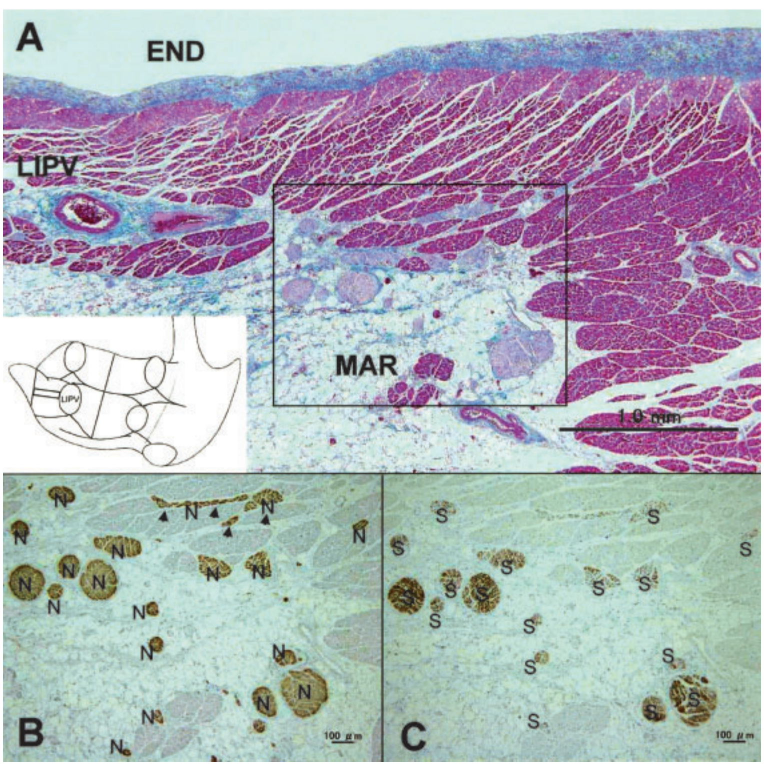

Figure 5 Histologic findings at the anterior LIPV-LA junction (55-year-old man).
(A) Nerves are rooted at the junction of the pulmonary vein sleeve musculature. Marshall bundles are closely intermingled with sympathetic nerves (Azan-Mallory stain). END, endocardium; LIPV, left inferior pulmonary vein; MAR, Marshall bundle. (B) High-power magnification of the inset in panel (A). Insertion of nerve fibers (arrowheads) into the left atrial musculature is observed (S-100 stain). N, nerve. (C) Most fibers are TH-positive/S-100-positive sympathetic nerves (tyrosine hydroxylase stain). S, sympathetic nerve.

LIPV-LA junction area. This finding was in contrast to the significantly lesser number of TH-positive/S-100-positive fibers in the opposite left PV posterior junction. In 9 hearts (69.2%), bridge musculature adjacent to the Marshall bundle was observed in this area and was closely intermingled with the sympathetic nerve plexus within the epicardial fat pad (**Figure 5**).

Posterior PV-LA junction

Typical findings in the posterior PV-LA junction area are shown in **Figure 6** (71-year-old woman). The junctional musculature between the posterior LA and each of the four PV sleeves showed a smooth transition. Detailed observation of the posterior junction revealed a small number of nerve fibers scattered focally within a thin epicardial fat pad

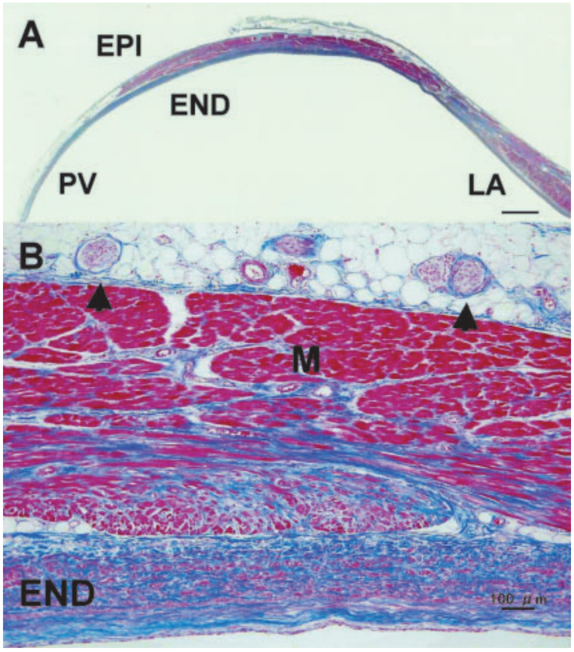

Figure 6 Histologic findings at the posterior PV-LA junction (71-year-old woman).
(A) The posterior junction between the LA posterior wall and the PV sleeve musculature shows a smooth transition (Azan-Mallory stain; Bar = 1 mm). EPI, epicardium; END, endocardium; LA, left atrium; PV, pulmonary vein. (B) Within the small epicardial fat pad, a fine group of nerve fibers (arrowheads) are focally distributed at the posterior junction (Azan-Mallory stain). END, endocardium; M, myocardium.

(**Figure 6B**). Although the total number of fibers was significantly fewer than that in the anterior or septal junctions, TH-positive/S-100-positive sympathetic nerve fibers were relatively prevalent in all four PVs (**Figure 3**). Numbers of TH-positive/S-100-positive and TH-negative/S-100-positive fibers did not differ significantly between the four PVs.

Septal PV-LA junction

Typical findings in the septal PV-LA junction area are shown in **Figure 7** (65-year-old man). The superior and inferior right PV septal junctions faced the interatrial groove fat pad. Within the voluminous adipose tissue, abundant ganglionic nodules and TH-negative/S-100-positive nerve fibers were diffusely distributed (**Figures 3**, **4**, **7**). Detailed observation revealed that ganglionic nodules were frequently located near the center of the fat pad (**Figure 7**). In addition to the abundant non-adrenergic distribution, including predominantly parasympathetic nerves, an equally dense sympathetic distribution characterized the RSPV septal junction. The scattered distribution pattern consisted of both sympathetic and parasympathetic nerve fibers, and the ganglionic nodule within the voluminous fat pad was in contrast to the proximity of sympathetic fibers to the anterior junctional musculature.

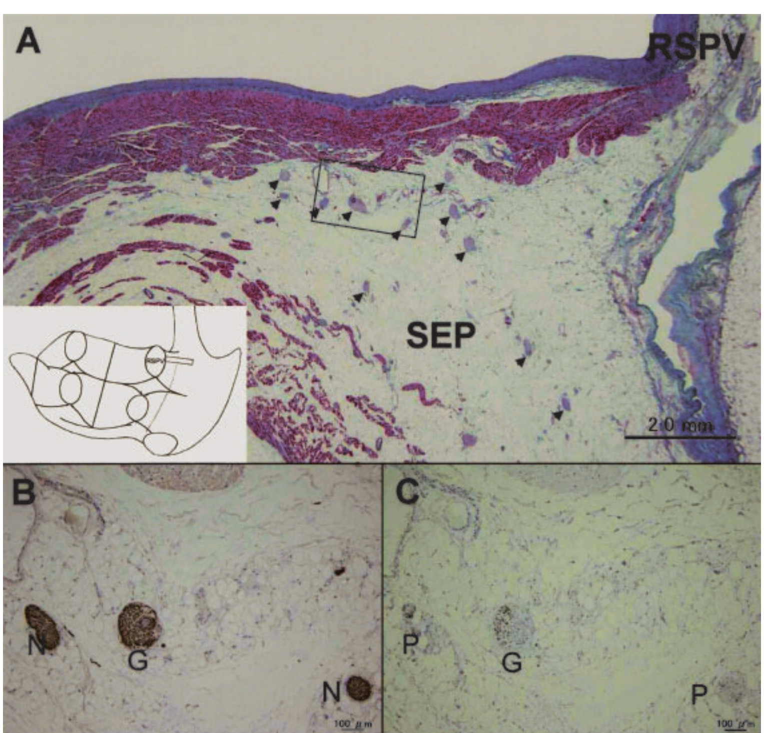

Figure 7 Histologic findings at the septal RSPV-LA junction (65-year-old man).
(A) Numerous nerve fibers (arrowheads) are diffusely scattered within the voluminous interatrial groove fat pad (Azan-Mallory stain). RSPV, right superior pulmonary vein; SEP, septum. (B) High-power magnification of the inset in (A). All nerve fibers and ganglionic nodules are S-100-positive (S-100 stain). G, ganglionic nodule; N, nerve. (C) With the relatively fewer numbers of TH-positive/S-100-positive (sympathetic) nerves, ganglionic nodules and TH-negative/S-100-positive that may contain mainly predominantly parasympathetic nerve fibers are conspicuous (tyrosine hydroxylase stain). G, ganglionic nodule; P, parasympathetic nerve.

Discussion

Previous studies have revealed the effect of adrenergic or vagal activity on the induction of AF and the benefit of denervation concomitant with a pulmonary vein ablation procedure.[3,5] Although recent anatomic studies clarified the distribution of the autonomic nerve plexus in the LA and showed that there is a considerably high density of fibers in the left PV-LA junction,[13] Tan et al.[8] reported the highest density of adrenergic fibers in the right superior PV and the highest density of cholinergic fiber in the left superior PV. Contrary to the marked decrease of nerve fibers in the posterior wall observed in the present study, Tan et al.[8] showed a relatively large number of cholinergic fibers in the posterior wall, particularly in the left and right inferior PVs. This discrepancy may be due to differences between the incision lines of the specimens. We used a horizontal–to-valvular annulus, whereas Tan et al.[8] used a radial line from the PV opening. This methodologic difference may have influenced the area of coverage around the PV. We focused on the polarity of sympathetic and parasympathetic distributions and arrangement with the myocardium at the anterior, posterior, and septal junctions.

Sympathetic nerve distribution

In a study of the participation of autonomic tone in atrial arrhythmogenicity, Armour et al.[14] observed induction of atrial tachyarrhythmia with electrical stimulation of the sympathetic nerve running above the LSPV. Another recent study showed that AF can be induced by high-frequency electrical stimulation of the left PV.[15] These studies suggested that enhancement of the sympathetic tone of the left PVs can evoke adrenergic paroxysmal AF. Anatomic affinity between the left PVs and sympathetic nerve fibers was also shown by the left lateral cardiac nerve running along the anterior wall of the left PVs.[16] This area corresponds to free-wall insertion of the Marshall bundle intermingled with abundant sympathetic nerves.[17,18] The present study revealed that the branch of the left lateral cardiac nerve was tightly encased within the fascicle of the myocardial sleeve musculature at the anterior junction, and immunohistologic staining showed that this nerve was composed almost entirely of TH-positive/S-100-positive fibers. On the basis of these findings, the anterior wall of the left PV sleeve musculature was regarded as being under the influence of sympathetic fibers. Unexpectedly, the sympathetic nerves were predominant in the septal RSPV-LA junction, concomitant with abundant parasympathetic nerve distribution. In contrast to the anterior and septal junctions, a significantly fewer number of TH-positive/S-100-positive fibers was found in the posterior junction. The highly diverse distribution of adrenergic fibers between the two opposing faces of each PV opening characterized all four junctions.

Parasympathetic nerve distribution

A recent study reported vagal activity evoked by electrical stimulation of fat pads adjacent to the superior vena cava, right PV, and inferior vena cava.[12] Armour et al.[7] described the distribution of the ganglionated plexus in the interatrial groove fat pad (opposite the right PV) and its extension into the interatrial septum. In our observation of eight PV-LA segments, despite the fact that the numbers of sympathetic nerve fibers were similar in the RSPV and the LSPV-LA junction, localization of TH-negative/S-100-positive nerves containing predominantly parasympathetic nerves and ganglionic nodules in the interatrial groove fat pad covering the septal junction was conspicuous. Interestingly, the proximity between nerve fibers and the myocardial sleeve differed between the anterior and septal junctions. In the septal junctions, nerve fibers distributed diffusely within the voluminous interatrial fat pad showed loose contiguity with the myocardial sleeve. In contrast, in the anterior junction, nerve fibers were rooted tightly within the sleeve musculature.

Implications for PV isolation ablation

Pappone et al.[5] recently proposed a PV ablation line determined by vagal reflexes and noted that successful ablation resulted in decreased AF recurrence. According to their report, vagal reflexes were observed mainly in the vicinity of the border between the left atrial roof and the posterior wall, and this line appeared to correspond to the border between the left atrial body and the pulmonary venous component, as described by Anderson et al.[19] Concerning the vagal innervation of and the presence of arrhythmogenic foci around the left atrial posterior wall, Armour et al.[7] reported that ganglionic nodules encircled the pulmonary venous component and that this line appeared to meet the border of the left atrial arrhythmogenic area proposed by Saito et al.[6] Although the present study showed a prevalence of sympathetic nerve clusters in the left anterior junction, a small number of parasympathetic components contiguous with the interatrial fat pad may participate in the vagal reflex evoked by left PV stimulation. In adrenergic AF, as opposed to cholinergic AF, denervation of sympathetic nerves may be easily achieved because of the small epicardial fat pad and spatial proximity of sympathetic nerves in the anterior PV-LA junction. In contrast to the anterior junction, complete vagal denervation of the septal junction may require an additional strategy due to the considerable volume of the interatrial fat pad. These histologic characteristics of the right PV may be related to the decreased efficacy of PV ablation in patients with vagal AF.[20]

Study limitations

Our study included no cases of AF. Additional cases of sympathetic or vagal AF are needed for further histologic examination. Hamabe et al.[21] described the relation between differential myocardial thickness of the anterior junction and the occurrence of paroxysmal AF. Nerve sprouting and sympathetic hyperinnervation have also been reported in a canine model of AF.[22] Immunohistologic study of gap junction proteins may provide additional information regarding electrophysiologic phenomena of the PV-LA junction. Electron microscopic observation of nerve endings may also be useful. A recent clinical study reported enlargement of the PV diameter in patients with paroxysmal or chronic AF compared to that in patients without AF.[23] However, comparison of histologic changes of the PV-LA junction is difficult in human AF.

Conclusion

TH-positive sympathetic fibers were most predominant in the anterior PV-LA junctions. In all four posterior PV-LA junctions, the numbers of TH-positive fibers were significantly fewer. At the septal junctions, TH-negative, predominantly parasympathetic fibers were prevalent, concomitant with the presence of TH-positive fibers. Heterogeneous autonomic nerve distribution within diverse myocardial extensions characterized each PV opening.

Acknowledgments

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