Quantitative study of nerves of the human left atrium

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OBJECTIVES To quantify and study the distribution of innervation of the left atrium and the pulmonary veins in humans.

BACKGROUND Damage to cardiac nerves has been hypothesized as the explanation for successful radiofrequency ablation of atrial fibrillation.

METHODS From January 2003 to September 2003, histologic and quantitative studies of innervation of the left atrium and the pulmonary veins was performed in 43 consecutive necropsied adult hearts (30 men and 3 women; mean age 45.5 ± 12.4 years). The left atrium was sectioned in 1-cm slices from left to right, with the plane of section perpendicular to the long axis of the heart. Sections of the pulmonary veins at their ostia and sections 1 cm away of this structure also were obtained. Nerve fiber density was counted manually for each case and expressed as the mean number per slice.

RESULTS Numerous epicardial nerve fibers and ganglia having distinct patterns of distribution in the left atrium were found. Nerve density was significantly higher at the ostia of the four pulmonary veins than in their distal part (7.1 ± 2.1 vs 5.2 ± 1.3 for left upper pulmonary vein; 6.3 ± 1.5 vs 5.2 ± 1.7 for right upper pulmonary vein; 7.4 ± 2 vs 5.9 ± 2 for left lower pulmonary vein; 6.7 ± 1.8 vs 3.9 ± 1.3 for right lower pulmonary vein). The left superior vein was significantly more innervated than the right inferior vein (12.3 ± 3 vs 10.6 ± 1.4). Gradients of innervation were found from right to left (9.8 ± 4.6 vs 18.5 ± 6.6, $P < .05$) and from the front to the rear of the atrium (17.2 ± 6.4 vs 20.7 ± 6.5, $P < .05$). The same heterogeneous distribution was observed at the myocardial level but with thinner nerve fibers, making quantification difficult. Only very thin nerve fibers were present in the endocardium.

CONCLUSIONS The human left atrium exhibits several gradients of innervation at discrete sites. These findings may have clinical implications for radiofrequency ablation of atrial fibrillation.

KEYWORDS Left atrium; Pulmonary veins; Innervation; Gross anatomy; Microscopy; Atrial fibrillation

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Introduction

Disturbance of the autonomic nervous system can, by itself or with other abnormalities, trigger or perpetuate atrial fibrillation (AF).[1,2] Either the adrenergic or vagal arm of the autonomic nervous system may be involved in atrial electrical instability. Vagal activation shortens atrial refractory periods and increases their dispersion, and adrenergic stimulation enhances atrial myocardial automaticity. Coumel,[3] who studied patients with paroxysmal AF and the relationship between this arrhythmia and the behavior of autonomic nervous system, was the first to identify two different clinical patterns: vagal and adrenergic AF. Several animal models of parasympathetic denervation have been shown to suppress vagal AF induction.[4–8] Evidence of autonomic dysfunction after surgical or endovascular ablation of AF

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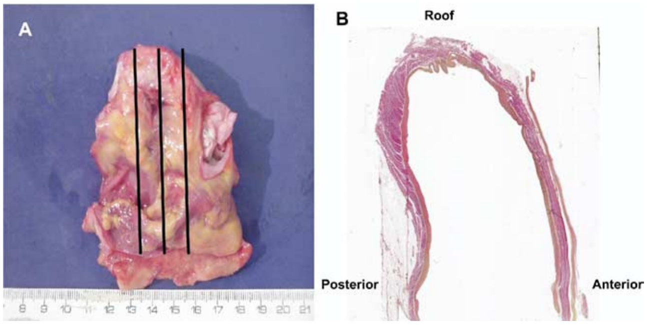

Figure 1 **A:** View of a left atrium divided into three regions, with the plane of section perpendicular to the mitral annulus. **B:** Overall view of the walls of the left atrium. Histopathologic analysis starts at the base of the anterior wall, then toward the roof and down to the posterior wall of the left atrium.

has been reported.[9,10] Denervation of the left atrium has been suggested as the explanation for successful radiofrequency ablation of AF.[11] However, no studies to date have quantitatively investigated innervation of the human left atrium. Therefore, we undertook this prospective histologic and quantitative study of the innervation of the left atrium and the pulmonary veins in 43 consecutive necropsied adult hearts.

Methods

From January 2003 to September 2003, quantitative histologic studies of innervation of the left atrium and the pulmonary veins were conducted in 43 consecutive necropsied adult hearts. Autopsies were performed when requested by judicial authorities after occurrence of sudden death without obvious explanation. Circumstances of death were obtained from police, forensic records, and relatives of the deceased person. Gross and microscopic examinations of heart-lung blocks were performed as previously described.[12,13]

After complete examination of the heart, the left atrium was sectioned at the level of the mitral annulus and the four pulmonary veins 2 cm from the ostium. The pocket-like left atria were fixed in distension with the pulmonary veins using an alcohol, formol, and acetic acid mixture for 1 week.

To analyze pulmonary vein innervation, two sections were obtained, one near the ostium and the other 1 cm distal from the ostium. To study the left atrium, parallel longitudinal slices every centimeter from the left toward the interatrial septum were obtained (Figure 1A). Histologic analysis was initiated at the foot of the anterior wall of the atrium up to the roof and then toward the posterior root (Figure 1B). The histology of all layers of the heart was examined at each site. Tissue samples were stained with hematein-phloxine-safran, which stains fibrous tissue golden yellow, cardiomyocytes red, and nuclei dark-blue. All nerve fibers that could be recognized by microscopy at 10 × 10 magnification were included and counted manually by one investigator (A.T.).

The left atrium was arbitrarily divided into nine regions: anterior, roof, and posterior walls, each divided into right, middle, and left segments. The total number of nerve fibers from one of three randomly chosen sections per region was counted and used to calculate the mean number of nerve fibers per slice for each of the nine different regions of the left atrium. Some sections were stained with antibodies to neurofilaments. Altogether, 15 to 20 samples were examined in each heart.

Statistical analysis

Continuous data are expressed as mean ± SD. The Chi-square test was used to assess the significance of differences between groups. $P < .05$ was considered significant.

Results

The study group consisted of 30 males (range 5–57 years, average 44 ± 12.4) and 13 females (range 10–65 years, average 35.5 ± 20.1). Mean heart weight was 391 ± 98g. Arrhythmogenic right ventricular dysplasia was found in 7 cases, cardiomyopathy in 11, and coronary artery disease in 12. Nerve fibers ranged from large trunks to thin fibrils. Ganglia sizes also were variable.

Pulmonary veins

Venous segments close to the pulmonary hilus appeared to be composed exclusively of vessel wall, with all the nerve filaments and ganglia located in the adventitia. As we advanced toward the ostium of the vein, myocardial sleeves were visualized as scarce and discontinuous muscular islands, with rare nerve elements. The closer the segment was to the ostium, the more the vein appeared externally doubled by atrial tissue. In segments with myocardial sleeves, a high density of nerve elements was seen in the external fibroadipose layer, with more sparse elements within the muscle layer. These nerve elements were isolated or associated with vessels. Nerve density was significantly higher at the ostium of the four pulmonary veins than distally (7.1 ± 2.1 vs 5.2 ± 1.3 fibers per slice for left upper pulmonary vein; 6.3 ± 1.5; vs 5.2 ± 1.7 for right upper pulmonary vein; 7.4 ± 2.0 vs 5.9 ± 2.0 for left lower pulmonary vein; 6.7 ± 1.8 vs 3.9 ± 1.3 for right lower pulmonary vein). The left superior vein was significantly more innervated than the right inferior vein (12.3 ± 3.0 vs 10.6 ± 1.4).

Left atrium

Numerous large epicardial nerve fibers and ganglia having distinct patterns of distribution were found. Nerve size from epicardium to endocardium, with large trunks seen mainly

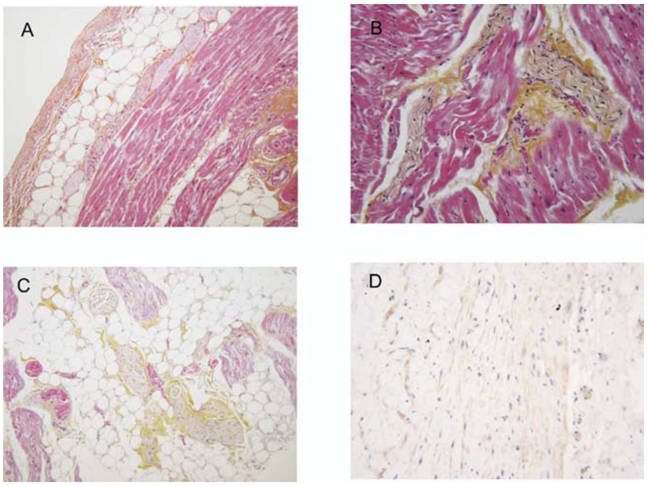

Figure 2 **A:** Nerve fiber overlying the atrial epicardium (magnification ×10, Hematein-Eosin-Safran (HPS) stain). **B:** Intramyocardial nerve fibers in intimate contact with muscle fibers (magnification ×25, HPS stain). **C:** Numerous large myocardial nerve fibers associated with muscular fibers within fatty tissue (magnification ×10, HPS stain). **D:** Fibrils seen in the atrial endocardium (magnification ×20, antibodies to neurofilament).

in the epicardium (Figure 2A). In the myocardium, fascicles of nerve fibers and sinuous fibrils extended between and parallel to the myocardial fibers (Figure 2B). Both these structures were in close contact. Nerve elements sometimes were associated with the vessels forming neurovascular bundles. Foci of rarefied myocardium with fatty tissue and nerve fibers were seen (Figure 2C). Gradients of innervation were present from right to left atrium and from front to rear of the atrium (Figure 3). The same heterogeneous distribution was observed at the myocardial level but with thinner nerves fibers, making quantification difficult. Only very thin fibrils were present in the endocardium (Figure 2D).

Discussion

In the present study using quantitative histologic evaluation, we identified gradients of innervation inside the left atrium and within the pulmonary veins. The most densely innervated sites were the left and posterior parts of the atrium and the antrum of the pulmonary veins, mainly the left veins. These findings may have implications in the era of percutaneous catheter ablation of AF.

Studies of innervation of the heart

To the best of our knowledge, no data on quantitative innervation of the human left atrium and pulmonary veins have been reported. Innervation of the human atrium depends on both the parasympathetic and sympathetic limbs, but cholinergic innervation appears predominant, as op-

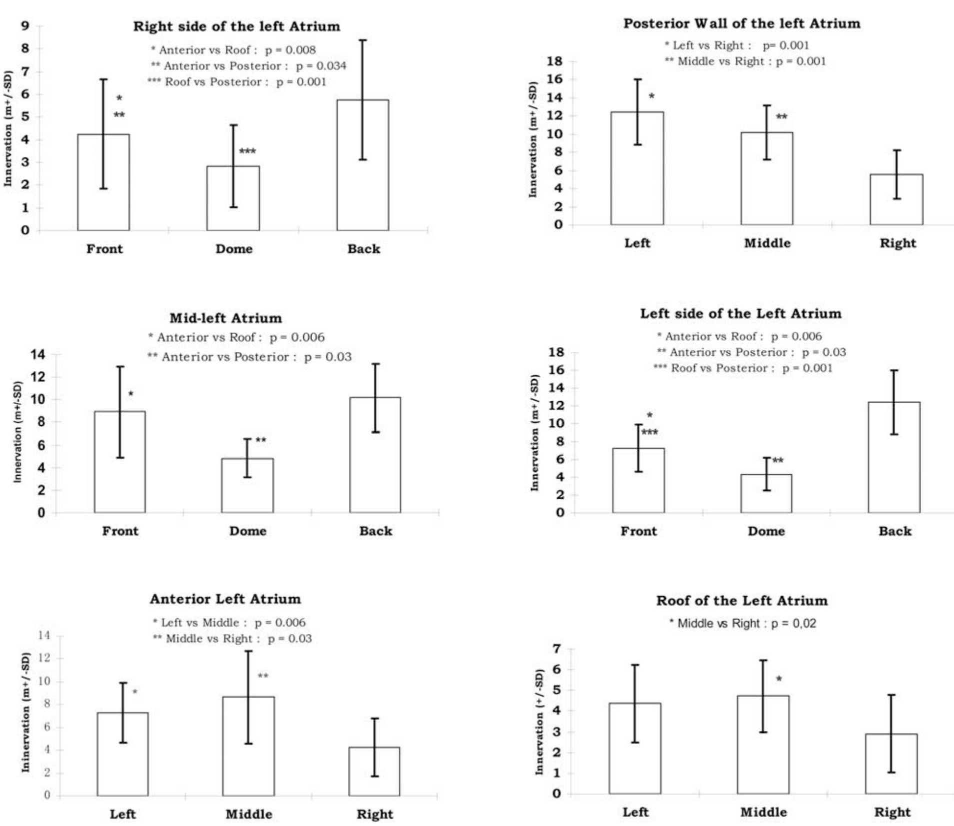

Figure 3 Nerve fiber density of the left atrium (mean number of fibers per slice). The total number of nerve fibers from one of three randomly chosen sections per region was counted and used to calculate mean values for each of nine different regions of the left atrium.

posed to the ventricle.[14–17] Chiou et al[4] demonstrated in dogs that most of the efferent vagal fibers to the atrium travel through a fat pad located between the medial superior vena cava and the aortic root. For the human left atrium, Armour et al[14] identified two atrial ganglionated plexuses: the superior left atrial ganglionated plexus between the pulmonary veins and the posterior medial plexus. Regionality in the distribution of the cardiac nerves has been recognized, and differential patterns of innervation have been observed in mammalian hearts.[4,16,18,19] Distribution of nerve terminal arborization at the endocardial and epicardial level in humans was described by Marron et al.[16] These authors found that the endocardium of the left atrium was less innervated than the endocardium of the other cardiac chambers. As for epicardial innervation, a ventricular-to-atrial gradient was observed.[20] At the myocardial level, the left atrium appears to be more innervated than the ventricles.[15] Smirnoff[21] was the first to suggest a histologic substrate for a myoneural connection in the human ventricle. According to Smirnoff, there is a neural connection to every muscle fiber. Our data also suggest that the proximity of nerve fibers to atrial myocytes permits rapid transmission of autonomic influences.

Innervation of the human pulmonary veins has never been described in detail. The junction of the atrium and of the pulmonary veins reflects a sheath-like arrangement between the vein and the atrial myocardium. The accompanying nerve elements that stem from the pulmonary side and from the epicardial aspect overlie the veins.

Autonomic nervous system and AF

The neurogenic theory of AF has received much attention and has been subject to numerous studies.[1,22,23] Spatial heterogeneity of nerve distribution throughout the left atrium shown in the present study could result in profound heterogeneity of myocardial excitability and refractoriness and thus influence the AF threshold.[24,25] Vagal nerve stimulation triggers marked increases in the heterogeneity of atrial repolarization.[22] Jayachandran et al[25] reported in a dog model of AF that increased spatial heterogeneity of hydroxyl ephedrine uptake was associated with electrical remodeling. In a canine model of AF produced by right atrial pacing, Chang et al,[26] using immunochemical studies, observed a significant increase of nerve fibers in the atrium and heterogeneity of innervation. Olgin et al[8] demonstrated in dogs histologic evidence of neural remodeling and increased propensity to AF in chemically induced heterogeneous atrial sympathetic innervation.

Innervation gradients may contribute to the complex patterns of electrical propagation during AF. Small rotors located in the posterior left atrial wall and at the ostia of the pulmonary veins, where we found the highest density of innervation, may trigger and drive the fibrillatory process. Pulmonary veins are well innervated, and thus under autonomic nervous control, and have been shown to be arrhythmogenic. In patients with paroxysmal AF, most of the triggering foci originate in the pulmonary vein, especially in the left superior vein.[27] Furthermore, in dogs the left superior pulmonary vein (which is the most innervated) is the vein most sensitive to high-frequency stimulation for eliciting AF.[5] Stretch-related AF has been related to changes in the electrophysiology of the superior pulmonary vein.[28] Because neurons are coupled to the mechanoreceptors and modulated by these latter structures, hemodynamic changes may modify arrhythmia vulnerability via the intrinsic nervous system.

Implications for radiofrequency ablation of AF

Accumulated data suggest the efficiency of neural ablation by radiofrequency application as a potential treatment of AF.[4–8] Ablation of some atrial areas, particularly in the posterior wall, elicits bradycardia or AV block.[4,10] Thus, neural target sites can be identified during radiofrequency ablation due to vagal reflexes as demonstrated by Platt et al.[29]

Several animal studies have demonstrated the efficacy of neural ablation in preventing AF. Elvan et al[30] performed radiofrequency circumferential ablation of the pulmonary veins and abolished induction of sustained AF by burst stimulation of the atrium and cervical vagal stimulation. Schauerte et al[6] demonstrated that transvenous catheter ablation of cardiac nerves, along the right pulmonary artery, prevents vagal AF. We also demonstrated in an animal model of vagal AF that linear atrial myocardial lesions applied epicardially to the atria via thoracoscopy significantly decreased AF inducibility.[7] Evidence also exists that ablation of atrial nerves may cure patients with AF. Lonnerholm et al[9] demonstrated improvement of heart rate variability parameters in 17 patients after a maze procedure, suggesting that autonomic denervation participates in achieving surgical efficacy. Similarly, Pappone et al[11] demonstrated that denervation improves the success of ablation for AF. Interestingly, they found that target sites associated with vagal reflexes were located around the veins and in the posterior atrial wall. This latter finding is consistent with our histologic study, in which the highest density of nerves was found at these locations.

In our study, we found that nervous tissue at the ostia of the pulmonary veins was present mainly at the epicardial level. Therefore, radiofrequency applications of sufficient duration and energy likely are required to achieve successful transmural lesions in these deep structures. In agreement with this, Chiou et al[4] demonstrated in dogs that only transmural lesions were able to denervate the atria. Nevertheless, the presence of innervation within the myocardium suggests that less deep lesions also may have an impact on atrial innervation.

Study limitations

Only hearts of patients who died suddenly and without explanation were studied. We cannot exclude the possibility that atrial innervation is somewhat different in normal subjects. We used conventional histologic technique and not immunocytochemical technique, which would have allowed us to investigate parasympathetic and sympathetic nerve distributions in autopsied hearts. However, cholinergic innervation is recognized as predominant in the atria.[16] In addition, only one plane of section was obtained, which limits the information on the spatial arrangement of nerves inside the myocardium. Finally, similar work would be necessary in patients with AF to determine whether neural histology is comparable in these patients.

Conclusion

We found that the human left atrium is richly supplied with nerves and displays several gradients of innervation. Given the evidence of the role of innervation in AF initiation and maintenance, these findings may have clinical implications for improving ablation. Targeting specific sites with higher innervation densities may enhance radiofrequency ablation efficacy. Because innervation may vary on an individual basis, finding a means to accurately locate high nerve density areas likely is an important goal. Another active area of research is assessing the relationship between regional cardiac innervation and AF in a given patient.

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