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Background—Long-term rapid atrial pacing may result in atrial fibrillation (AF) in dogs. Whether there is histological evidence for neural remodeling is unclear.

Method and Results—We performed rapid right atrial pacing in 6 dogs for 111±76 days to induce sustained AF. Tissues from 6 healthy dogs were used as controls. Immunocytochemical staining of cardiac nerves was performed using anti–growth-associated protein 43 (GAP43) and anti–tyrosine hydroxylase (TH) antibodies. In dogs with AF, the density of GAP43-positive and TH-positive nerves in the right atrium was 470±406 and 231±126 per mm^2, respectively, which was significantly ($P<0.001$) higher than the nerve density in control tissues (25±32 and 88±40 per mm^2, respectively). The density of GAP43-positive and TH-positive nerves in the atrial septum was 317±36 and 155±85 per mm^2, respectively, and was significantly ($P<0.001$) higher than the nerve density in control tissues (9±13 and 30±7 per mm^2, respectively). Similarly, the density of GAP43-positive and TH-positive nerves in the left atrium of dogs with AF was 119±61 and 91±40 per mm^2, respectively, which was significantly ($P<0.001$) higher than the nerve density in control tissues (10±15 and 38±39 per mm^2, respectively). Furthermore, in dogs with AF, the right atrium had a significantly higher nerve density than the left atrium. Microscopic examinations revealed an inhomogeneous distribution of cardiac nerves within each sampling site.

Conclusions—Significant nerve sprouting and sympathetic hyperinnervation are present in a canine model of sustained AF produced by prolonged right atrial pacing. The magnitude of nerve sprouting and hyperinnervation was higher in the right atrium than in the left atrium. **(*Circulation*. 2001;103:22-25.)**

Key Words: remodeling ■ electrophysiology ■ nervous system, autonomic ■ tachyarrhythmias ■ catecholamines

Long-term rapid right atrial (RA) pacing causes sustained atrial fibrillation (AF), presumably due to pacing-induced electrical[1–5] and anatomical[5,6] remodeling. Jayachandran et al[7] recently demonstrated that prolonged rapid atrial pacing is associated with heterogeneous changes in atrial sympathetic innervation. [C-11]-Hydroxyephedrine retention was heterogeneous, and it was greater in the RA than in the left atrium (LA). These findings raised the possibility that neural remodeling might play an important role in the generation and maintenance of the AF induced by rapid pacing. However, no histological data were presented in that article to document the presence of increased sympathetic innervation. The purpose of the present study was to use immunocytochemical techniques to study atrial sympathetic nerve density in a canine model of sustained AF produced by long-term, rapid RA pacing. The results were used to test the hypothesis that prolonged, rapid RA pacing results in sympathetic nerve sprouting and heterogeneous atrial sympathetic hyperinnervation.

Methods

This study protocol was approved by the Institutional Animal Care and Use Committee and followed the guidelines of the American Heart Association.

Pacing-Induced Sustained AF

Mongrel dogs of either sex, weighing 18 to 28 kg, were studied (n=6). Sustained AF was induced by prolonged, rapid RA pacing according to a protocol published elsewhere.[8] Briefly, a bipolar endocardial pacing lead was advanced to the RA appendage. It was connected to a Medtronic Itrel neurostimulator to deliver rapid RA pacing. Digoxin (0.25 mg per day) was given to control ventricular rate. The dogs were examined periodically for the presence of sustained AF by turning off the pacemaker. The dogs were consid-

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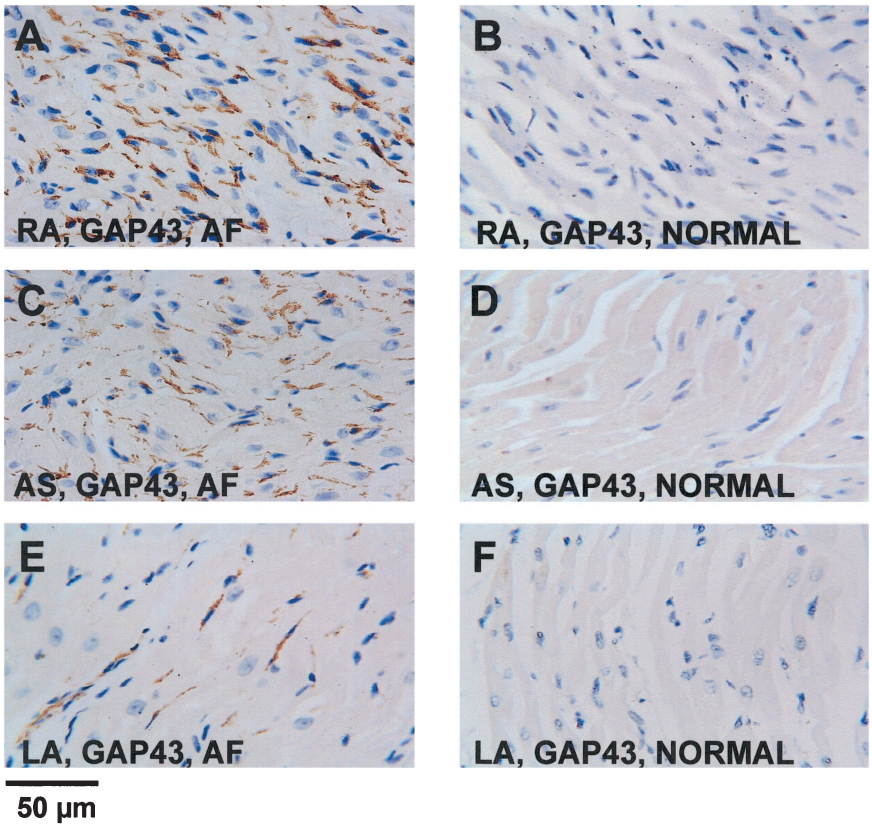

Figure 1. GAP43 staining of cardiac nerves (brown twigs) in control dogs and dogs with AF. AS indicates atrial septum. Magnification, 40×.

ered to have developed sustained AF if AF persisted for ≥48 hours without pacing. The dogs were killed when sustained AF was documented.

To compare nerve density, cardiac tissues from 6 healthy mongrel dogs were used as controls.

Immunocytochemical Studies

Tissues were obtained from the RA appendage, RA free wall, atrial septum, LA appendage, and LA free wall for immunocytochemical studies. Details of the staining techniques have been published elsewhere.[9,10] Briefly, we used anti–growth-associated protein 43 (GAP43) antibody and anti–tyrosine hydroxylase (TH) antibody for immunocytochemical staining. The tissues from dogs with AF and control dogs were stained in the same session. We determined nerve density by a computer-assisted image analysis system (Image-Pro Plus 4.0). The slides were coded so that the investigator (C.-M.C.) who counted the nerves was blinded to the dog identification at the time of nerve count. Each slide was examined under a microscope with 20× objectives to select 3 fields with the highest density of nerves. The computer automatically detected the stained nerves in these fields by their brown color. It then applied a shape criterion to exclude round objects (such as the nuclei of muscle cells) and a size criterion to exclude any objects <2 pixels in size. The computer then calculated the number and area occupied by the nerves in the field. The nerve density was the nerve number or the nerve area divided by the total area examined. The mean density of nerves in these 3 selected fields was used to represent the nerve density of that slide.

Statistical Analysis

Data are presented as mean±SD. Because the SD was as large as the mean, we performed *t* tests using logarithmically transformed data. Non-paired *t* tests were used to compare the means of nerve density between dogs with AF and control dogs and among different locations. Because all data were used for 3 sets of comparisons, $P \leq 0.017$ was considered significant (Bonferroni correction). Pearson's correlation was used to compare the duration of pacing and the density of cardiac nerves. $P \leq 0.05$ was considered significant for these comparisons.

Results

Sustained AF was induced in all dogs in the experimental group after an average of 111±76 days. There was no correlation between the duration of pacing and the density of cardiac nerves in either the RA or LA.

Because the nerve density between the 2 RA sites and between the 2 LA sites was not significantly different, we combined the sites (RA free wall and RA appendage represented RA, and LA free wall and LA appendage represented LA). At all sites, nerves immunopositive to GAP43 and TH were more abundant in dogs with AF than in controls (Figures 1 and 2, Table). Among all sampling sites, the most robust increase was in GAP43-positive nerves in the RA; the least amount of nerves was found in the LA. The atrial septum usually had a nerve density between that of the RA and LA. In normal dogs, it seemed that the RA also tended to have a higher nerve density than the LA and septum. However, not all comparisons reached statistical significance. In addition to the asymmetry between the RA and LA, the distribution of nerves within the same microscopic field also showed significant inhomogeneity (Figures 1 and 2).

Discussion

This study showed significant nerve sprouting and sympathetic hyperinnervation in a canine model of sustained AF produced by prolonged RA pacing. The magnitude of nerve sprouting and hyperinnervation was higher in the RA than in the LA.

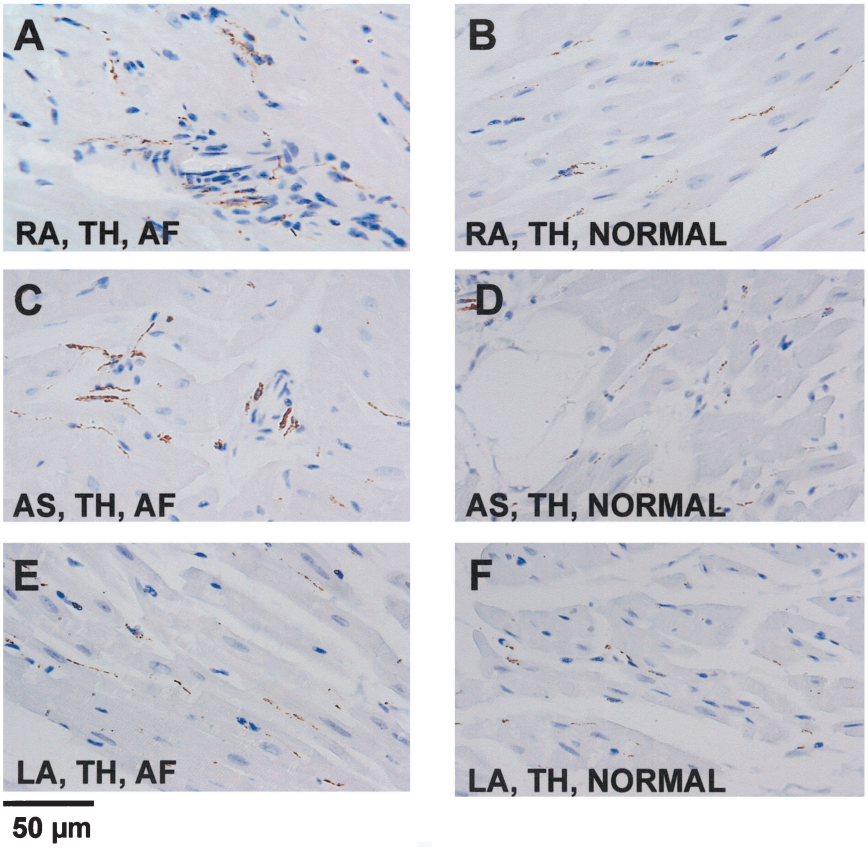

Figure 2. TH staining of cardiac nerves in control dogs and dogs with AF. AS indicates atrial septum. Magnification, 40×.

Neural Remodeling and Pathogenesis of AF

The generation and maintenance of rapid, pacing-induced, chronic AF were usually attributed to the electrical or anatomical remodeling induced by pacing.[2–6,11] Recently, Olgin et al[12] reported that sympathetic atrial denervation by phenol creates heterogeneous autonomic innervation, facilitating sustained AF. Jayachandran et al[7] used positron-emission tomography imaging to document that dogs with AF had inhomogeneous changes of atrial sympathetic innervation. The present study extended their observations by documenting inhomogeneous sympathetic hyperinnervation in the atria using immunocytochemical techniques. GAP43, a protein expressed in the growth cones of sprouting axons,[13] is a marker for nerve sprouting. A robust increase of GAP43-positive nerves in dogs with AF suggests that nerve sprouting is responsible for the sympathetic hyperinnervation in these dogs. We also found that the RA had a significantly higher density of sympathetic nerves than the LA. These findings are consistent with those reported by Jayachandran et al[7] and suggest that there is a correlation between the results of

Results of Immunocytochemical Studies at Different Atrial Regions

	GAP 43			TH		
	AF	Control	*P*	AF	Control	*P*
No. of nerves per mm^2						
RA	470±406	25±32	<0.001	231±126	88±40	<0.001
AS	317±36	9±13	<0.001	155±85	30±7	0.001
LA	119±61	10±15	<0.001	91±40	38±39	<0.001
P	<0.001 (RA vs LA and AS vs LA)	NS		<0.01 (RA vs LA)	<0.01 (RA vs AS and RA vs LA)	
Nerve area, μm^2/mm^2						
RA	12 534±18 290	650±1112	<0.001	4480±2378	2017±1349	0.007
AS	7 106±1 538	232±392	0.004	4570±1979	1360±494	0.003
LA	2 354±1 921	170±267	<0.001	2524±1274	1082±1171	0.009
P	≤0.01 (RA vs LA and AS vs LA)	NS		NS	NS	

Values are mean±SD. AS indicates atrial septum.

positron-emission tomography imaging and immunocytochemical staining.

One possible cause of nerve sprouting in this model is the electrical current, which has been used to induce nerve sprouting in the brain and in the kindling model of seizure disorder.[14] However, we do not have sufficient data from this study to test that hypothesis. It is also unclear whether neural remodeling is causally related to the pathogenesis of AF. Adrenergic stimulation in the electrically remodeled myocardium increases significant electrophysiological changes[15] and may be proarrhythmic.[8–10] Sympathetic nerve sprouting and hyperinnervation may strengthen this interaction and contribute to the generation and maintenance of AF.

Study Limitations

There was a density discrepancy between GAP43-immunopositive nerves and TH-immunopositive nerves in dogs with AF (Table). This discrepancy may be due either to a difference in the quantity between GAP43 and TH proteins or to a difference in the sensitivity to anti-GAP43 and anti-TH antibodies. A second possibility is that many GAP43-positive nerves may not yet be functional. A third possibility is that some of the GAP43-positive nerves were parasympathetic nerves. We attempted to stain with the anti-cholineacetyltransferase antibody. Although the parasympathetic nerve ganglion was well stained, no parasympathetic nerve twigs were identified. The mechanism by which more GAP43-positive nerves than TH-positive nerves were found remains to be explained. This is a limitation of the study.

A second limitation is that we did not perform functional electrophysiological measurements. Therefore, the refractory period and the dispersion of refractoriness are not available for comparison with the magnitude of nerve sprouting.

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