Catheter-Based Renal Denervation Reduces Atrial Nerve Sprouting and Complexity of Atrial Fibrillation in Goats

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Background—Atrial fibrillation (AF) leads to structural and neural remodeling in the atrium, which enhances AF complexity and perpetuation. Renal denervation (RDN) can reduce renal and whole-body sympathetic activity. Aim of this study was to determine the effect of sympathetic nervous system modulation by RDN on atrial arrhythmogenesis.

Methods and Result—Eighteen goats were instrumented with an atrial endocardial pacemaker lead and a burst pacemaker. Percutaneous catheter-based RDN was performed in 8 goats (RDN-AF). Ten goats undergoing a sham procedure served as control (SHAM-AF). AF was induced and maintained by burst pacing for 6 weeks. High-resolution mapping was used to record epicardial conduction patterns of the right and left atrium. RDN reduced tyrosine hydroxylase-positive sympathetic nerve staining and resulted in lower transcardiac norepinephrine levels. This was associated with reduced expression of nerve growth factor-β, indicating less atrial nerve sprouting. Atrial endomysial fibrosis content was lower and myocyte diameter was smaller in RDN-AF. Median conduction velocity was higher (75±9 versus 65±10 cm/s, P=0.02), and AF cycle length was shorter in RDN-AF compared with SHAM-AF. Left atrial AF complexity (4.8±0.8 fibrillation waves/AF cycle length versus 8.5±0.8 waves/AF cycle length, P=0.001) and incidence of breakthroughs (2.0±0.3 versus 4.3±0.5 waves/AF cycle length, P=0.059) were lower in RDN-AF compared with SHAM-AF. Blood pressure was normal and not significantly different between the groups.

Conclusions—RDN reduces atrial sympathetic nerve sprouting, structural alterations, and AF complexity in goats with persistent AF, independent of changes in blood pressure. (Circ Arrhythm Electrophysiol. 2015;8:466-474. DOI: 10.1161/CIRCEP.114.002453.)

Key Words: atrial fibrillation | autonomic nervous system | complexity | mapping | nerve sprouting | remodeling | renal denervation
WHAT IS KNOWN

- Atrial fibrillation is associated with increased sympathetic activation.
- Renal denervation can modulate autonomic nervous system activation and can display antiarrhythmic effects under certain conditions.

WHAT THE STUDY ADDS

- Interventional neuromodulation by renal denervation in goats with atrial fibrillation results in a reduction of atrial nerve sprouting and lower cardiac norepinephrine spillover.
- Attenuation of atrial neural remodeling by renal denervation lowers atrial fibrillation complexity with a lower number of breakthroughs.
- Renal denervation displays antiarrhythmic effects independent of changes in blood pressure.

Methods

In this study, the goat model of persistent AF was used.20–23 Animal procedures conformed to international standards on Research Animal Use and approved by the local ethical committee. All animals received a right atrial endocardial pacemaker lead (Medtronic Capsurefix). The lead was connected to a neurostimulator (Medtronic, Itrel2). In 8 animals, catheter-based RDN was performed (RDN-AF). In 10 animals, a sham procedure was performed (SHAM-AF). Ten days later, the pacemaker was switched on to induce AF in all animals for 6 weeks.

Renal Sympathetic Denervation Procedure

The RDN procedure was performed via femoral access with a dedicated radiofrequency catheter (Symplicity Catheter System, Ardian/Medtronic Inc, CA, USA) inserted percutaneously and advanced to the distal segment of the renal artery under fluoroscopy using a guiding catheter. The goats received 5000 IU heparin intravenously before RDN procedure. The vessel wall was focally heated to 53±2 °C by means of high-frequency energy with a maximum of 8 Watts (W) for 120 seconds. Ablation delivery resulted in an impedance-drop of 18±5%. Between 6 and 8 ablations were performed via femoral access with a dedicated radiofrequency catheter (Symplicity Catheter System, Ardian/Medtronic Inc, CA, USA) inserted percutaneously and advanced to the distal segment of the renal artery under fluoroscopy using a guiding catheter. The goats received 5000 IU heparin intravenously before RDN procedure. The vessel wall was focally heated to 53±2 °C by means of high-frequency energy with a maximum of 8 Watts (W) for 120 seconds. Ablation delivery resulted in an impedance-drop of 18±5%. Between 6 and 8 ablations were performed via femoral access with a dedicated radiofrequency catheter (Symplicity Catheter System, Ardian/Medtronic Inc, CA, USA) inserted percutaneously and advanced to the distal segment of the renal artery under fluoroscopy using a guiding catheter. The goats received 5000 IU heparin intravenously before RDN procedure. The vessel wall was focally heated to 53±2 °C by means of high-frequency energy with a maximum of 8 Watts (W) for 120 seconds. Ablation delivery resulted in an impedance-drop of 18±5%. Between 6 and 8 ablations were performed via femoral access with a dedicated radiofrequency catheter (Symplicity Catheter System, Ardian/Medtronic Inc, CA, USA) inserted percutaneously and advanced to the distal segment of the renal artery under fluoroscopy using a guiding catheter. The goats received 5000 IU heparin intravenously before RDN procedure. The vessel wall was focally heated to 53±2 °C by means of high-frequency energy with a maximum of 8 Watts (W) for 120 seconds. Ablation delivery resulted in an impedance-drop of 18±5%. Between 6 and 8 ablations were performed via femoral access with a dedicated radiofrequency catheter (Symplicity Catheter System, Ardian/Medtronic Inc, CA, USA) inserted percutaneously and advanced to the distal segment of the renal artery under fluoroscopy using a guiding catheter. The goats received 5000 IU heparin intravenously before RDN procedure. The vessel wall was focally heated to 53±2 °C by means of high-frequency energy with a maximum of 8 Watts (W) for 120 seconds. Ablation delivery resulted in an impedance-drop of 18±5%. Between 6 and 8 ablations were performed via femoral access with a dedicated radiofrequency catheter (Symplicity Catheter System, Ardian/Medtronic Inc, CA, USA) inserted percutaneously and advanced to the distal segment of the renal artery under fluoroscopy using a guiding catheter. The goats received 5000 IU heparin intravenously before RDN procedure. The vessel wall was focally heated to 53±2 °C by means of high-frequency energy with a maximum of 8 Watts (W) for 120 seconds. Ablation delivery resulted in an impedance-drop of 18±5%. Between 6 and 8 ablations were performed via femoral access with a dedicated radiofrequency catheter (Symplicity Catheter System, Ardian/Medtronic Inc, CA, USA) inserted percutaneously and advanced to the distal segment of the renal artery under fluoroscopy using a guiding catheter. The goats received 5000 IU heparin intravenously before RDN procedure. The vessel wall was focally heated to 53±2 °C by means of high-frequency energy with a maximum of 8 Watts (W) for 120 seconds. Ablation delivery resulted in an impedance-drop of 18±5%. Between 6 and 8 ablations were performed via femoral access with a dedicated radiofrequency catheter (Symplicity Catheter System, Ardian/Medtronic Inc, CA, USA) inserted percutaneously and advanced to the distal segment of the renal artery under fluoroscopy using a guiding catheter. The goats received 5000 IU heparin intravenously before RDN procedure. The vessel wall was focally heated to 53±2 °C by means of high-frequency energy with a maximum of 8 Watts (W) for 120 seconds. Ablation delivery resulted in an impedance-drop of 18±5%. Between 6 and 8 ablations were performed via femoral access with a dedicated radiofrequency catheter (Symplicity Catheter System, Ardian/Medtronic Inc, CA, USA) inserted percutaneously and advanced to the distal segment of the renal artery under fluoroscopy using a guiding catheter. The goats received 5000 IU heparin intravenous...
visualized by enhanced chemiluminescence according to the manufacturer’s guidelines (Amersham Pharmacia Biotech, Freiburg, Germany). Autoradiographs were quantified by imaging densitometry and analyzed by the ImageQuant-TM b Software (Image Quant, Molecular Dynamics, Krefeld, Germany). Latest Western blots were quantified using the Fusion SL Detektion System (Peqlab; Germany) and analyzed by FusionCapture Advanced Software (Peqlab; Germany). Data are presented as intensity optical density.

Statistical Analysis
All data are expressed as mean±SEM. Renal norepinephrine levels as well as electrophysiological parameters during AF were compared using linear mixed effects model in SPSS with post hoc Sidak tests and confidence interval adjustment with animal ID as a random effect and the chamber and sham/RDN as fixed effects. All other analyses were done using Wilcoxon rank-sum test in SPSS. *P*<0.05 were considered significant.

Figure 1. Renal denervation (RDN) procedure: A, Representative x-ray images of the left (top) and the right (bottom) kidney. Locations of ablation delivery are indicated by superimposed small black points in the vessels. B, Renal tissue norepinephrine concentrations in the left and right kidney determined after the sacrifice experiments in SHAM and RDN goats after 6-week AF. There were no differences between right and left renal norepinephrine concentrations (*P*=0.11).

Figure 2. Atrial neural remodeling. A, Representative perivascular tyrosine hydroxylase (TH) staining (brown twigs) of cardiac sympathetic nerves in SHAM and renal denervated (RDN) goats with 6-week atrial fibrillation (AF; magnification, ×1000). B, Quantification of TH-positive (indicating sympathetic nerve structures), (C) growth-associated protein 43 (GAP43)-positive (indicating nerve sprouting) fraction of the perivascular area in the anterior left atrium (LAant), posterior left atrium (LAp), and right atrium (RA) in SHAM and RDN goats with 6-week AF. D, Transcardiac and (E) venous norepinephrine concentrations.
Results

RDN Effectiveness
Representative x-ray images of the RDN procedure are shown in Figure 1A. Renal tissue norepinephrine concentrations determined at the end of the study were significantly lower in both the left and right kidney of RDN-AF goats compared with SHAM-AF goats (Figure 1B). RDN did not significantly affect systolic blood pressure in goats with AF induced by atrial tachypacing for 6 weeks (115±7 versus 107±10 mm Hg, P=0.68).

RDN and Atrial Neural Remodeling
TH was mainly expressed in the perivascular area in the atrium of SHAM-AF (Figure 2A). Immunohistochemical staining showed less TH-positive nerve staining in the perivascular areas in RDN-AF compared with SHAM-AF. The phosphorylated form of TH was also lower in RDN-AF (not shown). Therefore, the pTH/TH ratio did not differ in RDN-AF and SHAM-AF goats. TH was expressed rarely outside perivascular areas. The spatial variation of sympathetic innervation, measured by the SD of the distribution of TH within 1 atrium, was lower in RDN-AF compared with SHAM-AF (Figure 2D). Venous norepinephrine concentration remained unchanged in the perivascular area of the atrium (Figure 2B), and decreased expression levels of NGFβ (Figure 3) suggesting reduced nerve sprouting in the atria of RDN-AF. Protein expression of choline acetyltransferase, a marker of the parasympathetic nervous system, was unaffected by RDN compared with SHAM in all analyzed areas (Figure I in the Data Supplement).

The transcardiac plasma norepinephrine concentration difference, sampled directly after termination of 6 weeks of AF, was lower in RDN-AF compared with SHAM-AF (Figure 2D). Venous norepinephrine concentration remained unchanged (Figure 2E). In the RDN-AF group, expression of β1- as well as β2-adrenergic receptors in the atrium was not altered by RDN (Figure 4).

RDN and Atrial Structural Remodeling
The total degree of atrial fibrosis in the RA or LA was not different between RDN-AF compared with SHAM-AF. To investigate further the distribution of fibrous tissue, intramyocardial fibrosis (fibrous tissue surrounding bundles and individual myocytes) and endomyocardial fibrosis (fibrous tissue separating individual myocytes within bundles) were distinguished. LA intramyocardial fibrosis was just reduced in the anterior LA, whereas endomyocardial fibrosis was significantly less pronounced in RDN-AF compared with SHAM-AF in all atrial regions investigated (Figure 5A). In the anterior LA, but not in the posterior LA or RA, the average myocyte diameter was significantly smaller in RDN-AF compared with SHAM-AF (Figure 5B). Total connexin expression, connexin phosphorylation, and connexin distribution were not altered by RDN (Figure II in the Data Supplement). Superoxide production and Nox2 protein expression were unchanged (Figure III in the Data Supplement).

Effect of RDN on Basic Electrophysiological Parameters During AF
The Table summarizes the effect of RDN on electrophysiological parameters during AF. In open chest experiments, the mean AFCL was shorter in the RDN-AF group than in the SHAM-AF group in the LA but not in the RA. The P5 of the AFCL, a surrogate parameter for the refractory period during AF, was not different between the groups. The median (P50) of conduction velocity during AF was significantly higher in RDN-AF compared with SHAM-AF in the LA but not in the RA. The heterogeneity index (P95-P5/P50) of conduction velocity or AFCL was not significantly influenced by RDN.

To study the effect of RDN on AF complexity, the number of peripheral waves and breakthroughs was compared between groups. Representative fibrillation wave maps of the LA and RA are shown in Figure 6A. The total number of waves per AFCL was smaller in RDN-AF compared with SHAM-AF (Figure 6B). RDN-AF showed bigger waves compared with SHAM-AF (Figure 6C). The number of peripheral waves as well as breakthrough events per AFCL was significantly lower in RDN-AF (Figure 6D). Reduction in breakthrough events was significantly more pronounced compared with the reduction in peripheral waves (−38% versus −23%, P=0.01).

![Figure 3. Nerve growth factor-β (NGFβ)](http://circep.ahajournals.org/469)
Importantly, this reduction in complexity of AF pattern was observed in the LA but not in the RA. AF inducibility by programmed atrial stimulation was 100% in both groups, and inducible AF duration showed a high variability.

Discussion

In this study, we demonstrated that interruption of afferent and efferent sympathetic signaling between the kidney and central sympathetic nervous system by RDN in a normotensive goat...
model for persistent AF reduced atrial nerve sprouting and cardiac norepinephrine spillover measured directly after termination of AF. This was associated with a reduction in atrial structural remodeling and AF complexity.

RDN and Neural Remodeling in the Atrium

Atrial neural remodeling characterized by nerve sprouting, sympathetic hyperinnervation, and increased cardiac norepinephrine spillover has been described in canine models of AF induced by prolonged atrial pacing.28 Interestingly, comparable changes were observed in atrial tissue of humans with persistent AF.29 RDN resulted in a clear reduction in cardiac norepinephrine spillover, and plasma norepinephrine concentration was not affected. Expression of β-adrenergic receptors, the targets of norepinephrine, was unchanged in the anterior LA and RA and was just moderately increased in the posterior LA. This was associated with a reduction in TH-positive sympathetic nerve staining in the atria of RDN animals. Importantly, total TH positively nerve staining as well as the spatial variation of sympathetic innervation was lower in RDN-AF compared with SHAM-AF in all atrial regions investigated, which may contribute to less complex AF propagation pattern.26 TH is the rate-limiting enzyme in norepinephrine biosynthesis and is regulated by phosphorylation.25 The TH-p/Th ratio was not altered by RDN, indicating that the remaining TH-positive nerves were functional and had a normal activity level. Reduction in TH-positive nerves was associated with a reduction of GAP43-positive nerve staining in goats after RDN. GAP43, a protein expressed in the growth cones of sprouting axons, is a marker for nerve sprouting,30 indicative that reduced nerve sprouting may be responsible for the reduction in sympathetic innervation during AF in RDN goats. Interestingly, atrial expression of choline acetyltransferase, a marker of the parasympathetic nervous system, was not influenced by RDN.

Reduced expression of NGFβ in the atrium of RDN animals may be one mechanism involved in reduced nerve

<table>
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<tr>
<th>Parameters</th>
<th>SHAM</th>
<th>RDN</th>
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<tr>
<td>AFCL, ms</td>
<td>149±30</td>
<td>135±26</td>
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<td>P5 AFCL, ms</td>
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<td>Right atrium</td>
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There were no significant differences between right and left atrium. AF indicates atrial fibrillation; AFCL, AF cycle length; CV, conduction velocity; and RDN, renal denervation.

Figure 6. Quantification of atrial fibrillation (AF) complexity: A, Representative spatial and temporal distribution of AF waves during one AF cycle length of the right and left atrium in SHAM (top) and renal denervated (RDN; bottom) goats with 6 weeks. B, Number of waves per AF cycle length (AFCL), C, wave size, and D, number of breakthroughs (ie, de novo waves appearing within the recording area) per AFCL in the right (RA) and left atrium (LA) in SHAM and RDN goats with 6-week AF.
sprouting after RDN. NGFβ is a neurotrophine that stimulates the growth, maintenance, and survival of sympathetic neurons and enhances target innervation by regulation of TH.11–33 RDN inhibited the expression of atrial NGFβ, which was associated with a reduction of TH-positive nerves in the atrium. Accordingly, Saygili et al34 showed that not all sympathetic neurons are the source of NGFβ during high-frequency stimulation. NGFβ in turn activates tyrosine kinase A receptors in an autocrine/paracrine manner resulting in further nerve sprouting.34

Thus, RDN seems to inhibit this amplifying autoregulation. Alternatively, degeneration of nerves because of reduced NGFβ levels could also be a mechanism for a reduction in TH after RDN.

**RDN and Atrial Structural Remodeling and Complexity of AF**

This is the first study directly correlating the influence of reduced neural innervation and remodeling of the atrium as a result of an interventional neuromodulation with functional electrophysiological measurements and complexity of AF. RDN reduced neural remodeling and preserved higher conduction velocities while atrial refractoriness during AF was not influenced. Temporal variation of AFCL or conduction velocity, reflected by the heterogeneity index, was also not influenced by RDN. We have previously reported that AF in goats with AF duration of 3 weeks was characterized by a higher number of simultaneous waves (both peripheral waves and epicardial breakthroughs) and a larger incidence of conduction block, leading to a more dissociated and complex fibrillation pattern compared with acute AF in goats.20,21 Importantly, AF complexity occurred independent from changes in AFCL and conduction velocity. AF complexity most likely represents the functional consequence of atrial structural remodeling.22 Structural atrial remodeling results in the progressive loss of endo-epicardial electric connections increasing atrial endo-epicardial dissociation and thereby enhances the likelihood for transmural conduction and producing a 3-dimensional substrate for AF.20 In the RDN group, LA AF complexity was lower than in SHAM-AF animals with the same AF duration. The complexity of AF activation patterns is determined by the incidence of conduction block, which contribute to the overall rate in breakthroughs in the goat model of AF.20,21

Surprisingly, the inhibitory effect of RDN on AF complexity was pronounced in LA, but almost absent in RA. One possible cause may be the site of pacing as the electrodes were implanted in the RA. Electric stimulation has been used to induce nerve sprouting in the brain and in the kindling model of seizure disorder. Accordingly, GAP43 was higher expressed in the RA compared with the LA. This continuous electric stimulation may inhibit the protective effects of RDN and may reduce the attenuation of myocyte hypertrophy as well as endomysial fibrous tissue amount after RDN in RA.

Importantly, all the observed effects on neural remodeling processes and AF complexity occurred in the absence of significant change in blood pressure. The lack in antihypertensive effect in normotensive goats is in line with our previous findings in normotensive pigs.16–18 Additionally, blood pressure at baseline is the only predictor of blood pressure response in resistant hypertension.11–13 The response in mild to moderate hypertension is detectable but substantially smaller.11

**Limitations**

Our study faces some limitations. The model of persistent AF induced by right atrial endocardial pacing is different from human AF. The effects of RDN on AF complexity in goats with untreated AF might be different from patients receiving antiarrhythmic drugs, β-blockers, and blockers of the renin–angiotensin system. The renal arteries in young goats may be different from human renal arteries in terms of calcification, length, and tortuosity. Therefore, RDN in humans may be more difficult to achieve and thus less effective. Mapping was restricted just to the atrial lateral walls. Compared with humans, goats hardly have any posterior wall of the LA because the chest is much deeper. In open chest experiments, it is difficult to access the posterior wall without causing severe bleeding and mechanical manipulation.

**Conclusions**

Interventional neuromodulation by RDN in goats with AF results in a reduction of NGFβ-mediated atrial nerve sprouting, lower cardiac norepinephrine spillover, and a lower AF.
complexity with a lower number of breakthroughs. These effects were independent of changes in blood pressure. Randomized SHAM-controlled studies are warranted to determine the effect of RDN on atrial arrhythmias in humans.

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Disclosures

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References


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Fig. S1: Protein-expression of choline acetyltransferase (CHAT) in homogenates of the anterior left atrium, posterior left atrium and right atrium in SHAM and RDN goats with 6 weeks of atrial fibrillation. There were no significant differences between the groups.
Fig. S2: Ratio of phosphorylated and total expression of connexin 43 (A). At the right, representative histological pictures for determination of special distribution of connexion 43 in RDN-AF and SHAM-AF (B).
Fig. S3  Superoxide production (A) and NOX2 protein expression (B) in homogenates of the anterior left atrium, posterior left atrium and right atrium in SHAM and RDN goats with 6 weeks of atrial fibrillation. There were no significant differences between the groups.