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

In addition to atrial stretch and atrial structural alterations,1-3 changes in the autonomic innervation of the atria have been suggested to contribute to the development of atrial fibrillation (AF).4-6 Modulation of the autonomic nervous system by several strategies like ganglion stellatum ablation,7 high thoracic epidural anesthesia,8 low level vagal nerve stimulation,9 or ablation of ganglionated plexi10 has been shown to attenuate the development of electric, autonomic, and structural atrial remodeling during AF. Another strategy to influence sympathetic nervous system activity is renal denervation (RDN). Catheter-based RDN has been developed to interrupt afferent and efferent sympathetic signaling between the kidney and central sympathetic nervous system.11 The procedure results in a reduction of renal norepinephrine spillover measured with a radiochemical tracer methodology using 3H-norepinephrine,12,13 as well as in a reduction in firing of single sympathetic vasoconstrictor fibers, a measure of central sympathetic nerve outflow.14 Several studies have suggested an antihypertensive effect of RDN in patients with drug resistant hypertension.12,15 Surprisingly, a recently published randomized, sham-controlled, blinded trial did not confirm superiority of RDN in blood pressure reduction compared with an invasive sham procedure.15 However, modulation of the sympathetic nervous system by RDN may display effects beyond blood pressure control. In a pig model of sleep apnea, RDN reduced AF inducibility and occurrence of spontaneous AF.16,17 Additionally, in pigs with 30 minutes of AF, RDN reduced inducible AF duration. However, AF inducibility could not be reduced.18 The effect of RDN on the development of an atrial structural remodeling and AF has not been investigated sufficiently.19 In particular, the influence of RDN on the complexity of AF propagation patterns,20 representing the functional consequence of atrial structural remodeling,21,22 has not yet been studied. This study is aimed to determine the
WHAT IS KNOWN

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

WHAT THE STUDY ADDS

- Interventional neuronomodulation 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 antiaarrhythmic effects independent of changes in blood pressure.

Effect of sympathetic nervous system modulation by RDN on structural and neural remodeling in the atrium and AF complexity in a goat model of persistent AF without hypertension or structural heart disease.

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 Capsurex). 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 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 delivered in each renal artery. Reduction in renal tissue norepinephrine concentrations determined during the euthanasia experiments after 6-week AF was used as a proof of RDN-procedure effectiveness.

Induction of AF

Ten days after RDN and pacemaker implantation, AF was induced and maintained by repetitive 50 Hz burst pacing of the atrium at 3× threshold every other second. All goats were paced for 6 weeks.

Open Chest Experiments

Animals were anesthetized with sufentanyl (6 μg/kg per hour), propofol (10 mg/kg per hour), and pancuronium (0.3 mg/kg per hour). After left-sided thoracotomy, a high-density epicardial high-density mapping electrode (0.4 cm, 247 electrodes, interelectrode distance 2.4 mm) was positioned on the free right and left atrial (LA) walls. A silver plate served as indifferent electrode. Unipolar signals during AF were recorded using a custom-made 256 channel mapping amplifier (filtering bandwidth 0.1–408 Hz, sampling rate 1 kHz, A/D resolution 16 bits).

Analysis of Fibrillation Electrograms

Electrogram files of circa 30 seconds (≈225 cycle) were analyzed, to obtain AF cycle length (AFCL) and complexity parameters, using custom-made analysis software.24 Local deflections in the recorded electrograms were identified using a probabilistic electrogram algorithm.25 Median AFCL was calculated using all fibrillation intervals of all electrodes. Effective conduction velocity was determined by plane fitting the activation time points at each electrode and its direct neighbors in a 5×5 grid. The resulting plane then indicated the velocity (reciprocal value of the steepness of the plane) for each local activation point.24

Histology

Part of the anterior LA, posterior LA, and right atrium (RA) was fixed in buffered 4% para-formaldehyde for 24 hours and embedded in paraffin for histological evaluation. Atrial sections (5 μm) were cut, deparaffinized, and rehydrated. Picro-sirius red staining was used to visualize fibrosis. Overall, intramyocardial fibrosis was calculated as the ratio of picro-sirius red positively stained area over total tissue area, excluding epicardial and endocardial fibrous layers and perivascular fibrosis. Endomyocardial fibrosis was determined after exclusion of fibrosis between the bundles. An additional set of atrial sections were stained with hematoxylin and eosin. Ninety to hundred transversely sectioned cells per animal were measured to determine cardiomyocyte diameter in the plane of the nucleus. Immunochemical staining of cardiac nerves was performed using anti-growth-associated protein 43 (GAP43, Millipore; MAB347), anti-tyroside hydroxylase (TH, Abcam; ab112), and anti-TH phosphorylated at Ser40 (Abcam; ab51206) antibodies. The ratio of positively stained area over total perivascular area was determined. Fifty to Sixty transversely sectioned vessels per animal were measured. The ratio between the phosphorylated form of TH at Ser40 and total TH (pTH/TH-ratio) was used to describe TH activity.26 To quantify special heterogeneity in atrial TH distribution, we determined the SD of all measurements in the posterior LA, anterior LA, and RA region in each animal. Leica Qwin version 3 morphometry software (Leica, Cambridge, UK) was used for analysis.

Biochemical Analysis

Plasma and renal tissue levels of norepinephrine were determined by high-performance liquid chromatography. Transcardiac levels (ng/mL) were defined as coronary sinus minus aortic concentrations. Superoxide production from atrial samples was measured by use of 2-hydroxyethidium detection by high-performance liquid chromatography.26 For Western blot analysis, goat atrial tissue was homogenized in 5 volumes of homogenization buffer (in mmol/L): Na2EDTA 5.0, NaF 25.0, saponin 0.1, chondroitinase ABC 0.5, PMSF 1.0, benzamidine 1.0, and KH2PO4 30.0 (pH 7.0). The resulting homogenate was centrifuged at 16,000 g for 20 minutes. Fifty microgram of protein was separated on a 8% to 10% SDS-PAGE and electrophoretically transferred to nitrocellulose membrane (0.2 μm pore size, Schleicher and Schuell, Dassel, Germany). Membranes were blocked in Tris-buffered saline containing 5% nonfat dry milk for 120 minutes at room temperature and exposed to anti-gp91-phox (Nox2; sc-5826, Santa Cruz Biotechnology, CA, USA; dilution: 1:1000), anti-NFβB (nerve growth factor-β; #04-1119, Millipore; dilution: 1:1000), anti-Connexin43 (Sigma-Aldrich, #C6219; 1:1000), antiphosphoSer368 Connexin43 (Cell Signaling, #3511; 1:1000), anti-beta Tubulin (Santa Cruz; sc-9104, 1:500), anti-Choline Acetyltransferase (Santa Cruz; sc-20672), and mouse monoclonal IgG anti-GAPDH (Santa Cruz, sc-32233, 1:5000). Secondary antibodies goat anti-mouse (Santa Cruz, 1:5000), and rabbit anti-goat (#172–1034, Bio-Rad, Germany; 1:5000) were incubated for 60 minutes at room temperature. Proteins were detected using a silver stain (Kodak, Biomax-MS). 1:2500) and rabbit anti-goat (Amersham, #04-1119, Millipore; dilution: 1:1000), anti-Connexin43 (Cell Signaling, #3511; 1:1000), anti-beta Tubulin (Santa Cruz; sc-9104, 1:500), anti-Choline Acetyltransferase (Santa Cruz; sc-20672), and mouse monoclonal IgG anti-GAPDH (Santa Cruz, sc-32233, 1:5000). Secondary antibodies goat anti-mouse (Santa Cruz, sc-9104, 1:500), anti-Choline Acetyltransferase (Santa Cruz; sc-20672), and mouse monoclonal IgG anti-GAPDH (Santa Cruz, sc-32233, 1:5000). Secondary antibodies goat anti-mouse (Santa Cruz, sc-9104, 1:500), anti-Choline Acetyltransferase (Santa Cruz; sc-20672), and mouse monoclonal IgG anti-GAPDH (Santa Cruz, sc-32233, 1:5000). Secondary antibodies goat anti-mouse (Santa Cruz, sc-9104, 1:500), anti-Choline Acetyltransferase (Santa Cruz; sc-20672), and mouse monoclonal IgG anti-GAPDH (Santa Cruz, sc-32233, 1:5000). Secondary antibodies goat anti-mouse (Santa Cruz, sc-9104, 1:500), anti-Choline Acetyltransferase (Santa Cruz; sc-20672), and mouse monoclonal IgG anti-GAPDH (Santa Cruz, sc-32233, 1:5000).
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 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 (L Apost), 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 in all analyzed areas (Figure I in the Data Supplement).

TH was mainly expressed in the perivascular area of the atrium (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 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).

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-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)](image)

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).
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. Interestingly, comparable changes were observed in atrial tissue of humans with persistent AF. 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. TH is the rate-limiting enzyme in norepinephrine biosynthesis and is regulated by phosphorylation. 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, 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 cell proliferation.

Table. Electrophysiological Parameters During AF

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SHAM</th>
<th>RDN</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFCL, ms</td>
<td>149±30</td>
<td>135±26</td>
<td>0.03</td>
</tr>
<tr>
<td>P5, AFCL, ms</td>
<td>110±23</td>
<td>101±23</td>
<td>0.49</td>
</tr>
<tr>
<td>AFCL heterogeneity index</td>
<td>0.5±0.1</td>
<td>0.5±0.2</td>
<td>0.62</td>
</tr>
<tr>
<td>P50 conduction velocity, cm/s</td>
<td>65±10</td>
<td>75±9</td>
<td>0.02</td>
</tr>
<tr>
<td>CV heterogeneity index</td>
<td>2.6±0.4</td>
<td>3.6±1.7</td>
<td>0.12</td>
</tr>
<tr>
<td>Right atrium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AFCL, ms</td>
<td>137±31</td>
<td>131±25</td>
<td>0.38</td>
</tr>
<tr>
<td>P5, AFCL, ms</td>
<td>103±27</td>
<td>89±17</td>
<td>0.29</td>
</tr>
<tr>
<td>AFCL heterogeneity index</td>
<td>0.5±0.1</td>
<td>0.6±0.1</td>
<td>0.83</td>
</tr>
<tr>
<td>P50 conduction velocity, cm/s</td>
<td>65±12</td>
<td>75±14</td>
<td>0.19</td>
</tr>
<tr>
<td>CV heterogeneity index</td>
<td>2.8±1.0</td>
<td>3.3±0.6</td>
<td>0.25</td>
</tr>
</tbody>
</table>

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.\textsuperscript{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 al\textsuperscript{34} showed that not atrial myocytes but sympathetic neurons themselves 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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 epicardial breakthrough and thereby enhances the likelihood for transmural conduction and producing a 3-dimensional substrate for AF.\textsuperscript{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 structural alterations. To characterize the pattern of AF-RDN are probably not just because of a direct effect of reduced influence of norepinephrine but mainly represents the functional consequence of lower degrees of atrial remodeling characterized by better endo-epicardial electric connections.\textsuperscript{20} Although AF complexity was reduced, AF inducibility was not modulated by RDN in goats with 6 weeks of AF. This is in line with our previous findings in pigs with 30 minutes of AF, where RDN reduced inducible AF duration but did not influence AF inducibility by programmed atrial stimulation.\textsuperscript{18} Possibly, RDN also shows antiarrhythmic effects beyond prevention of structural remodeling processes. Interestingly, reduction in breakthrough events was significantly more pronounced compared with the reduction in peripheral waves. Reduced number of waves may be mainly because of less pronounced structural remodeling in the RDN-AF group. The reduced number of breakthroughs in the RDN group may be related to a reduced rate of ectopic focal discharges, which contribute to the overall rate in breakthroughs in the goat model of AF.\textsuperscript{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.\textsuperscript{16-18} Additionally, blood pressure at baseline is the only predictor of blood pressure response in resistant hypertension.\textsuperscript{11-13} The response in mild to moderate hypertension is detectable but substantially smaller.\textsuperscript{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, \(\beta\)-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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Linz et al Renal Denervation in Atrial Fibrillation 473


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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.