Effects of Renal Artery Denervation on Ventricular Arrhythmias in a Postinfarct Model

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Background—The therapeutic potential of renal denervation (RDN) for arrhythmias has not been fully explored. Detailed mechanistic evaluation is in order. The objective of the present study was to determine the arrhythmic potential of RDN in a postinfarct animal model and to determine whether any benefits relate to RDN-induced reduction of sympathetic effectors on the myocardium.

Methods and Results—Pigs implanted with single-chamber implantable cardioverter defibrillators to record ventricular arrhythmias (VAs) were subjected to percutaneous coronary occlusion to induce myocardial infarction. Two weeks later, a sham or real RDN treatment was performed bilaterally using the St Jude EnligHTN basket catheter. Parameters of ventricular remodeling and modulation of cardiac—renal sympathetic axis were monitored for 3 weeks after myocardial infarction. Histological analysis of renal arteries yielded a mean neurofilament score of healthy nerves that was significantly lower in the real RDN group than in sham controls; damaged nerves were found only in the real RDN group. There was a 100% reduction in the rate of spontaneous VAs after real RDN and a 75% increase in the rate of spontaneous VAs after sham RDN (P=0.03). In the infarcted myocardium, presence of sympathetic nerves and tissue abundance of neuropeptide-Y, an indicator of sympathetic nerve activities, were significantly lower in the RDN group. Peak and mean sinus tachycardia rates were significantly reduced after RDN.

Conclusions—RDN in the infarcted pig model leads to reduction of postinfarction VAs and myocardial sympathetic effectors. This may form the basis for a potential therapeutic role of RDN in postinfarct VAs. (Circ Cardiovasc Interv. 2017;10:e004172. DOI: 10.1161/CIRCINTERVENTIONS.116.004172.)

Key Words: cardiac arrhythmia ■ coronary occlusion ■ myocardial infarction ■ neuropeptide Y ■ renal sympathetic denervation

The therapeutic potential of renal denervation (RDN) for cardiac arrhythmias was previously explored, yielding case reports and small case series that suggested RDN may be an effective adjunctive treatment in addition to medication and cardiac ablation for patients with ventricular tachycardia (VT) storm and atrial fibrillation.1-5 These promising results call for large-scale, well-controlled trials and detailed mechanistic evaluations. However, findings of the SYMPLECTICITY HTN-3 study (Renal Denervation in Patients With Uncontrolled Hypertension) have raised grave concerns over the benefits from RDN therapy for hypertension.4 Hence, although RDN-based treatments are being developed for arrhythmias, it is important to reassess and confirm the antiarrhythmic potential of RDN in experiments designed to clearly demonstrate the impacts on cardiac electrophysiology. More importantly, the mechanistic basis of any antiarrhythmic effects should also be determined.

See Editorial by Koruth and Dukkipati

RDN has been shown to attenuate elevations in left ventricular end-diastolic pressure, suppress spontaneous premature beats, and reduce the incidence of ventricular fibrillation (VF) immediately post-acute coronary occlusion in pigs.6 RDN was also shown to reduce the incidence of ventricular arrhythmias (VAs) immediately after coronary occlusion in a similar acute canine model, in which RDN prolonged the ventricular effective refractory period (ERP) and the ventricular action potential duration, as well as decreased action potential duration dispersion.7 Although RDN is known to reduce renal sympathetic activity...
WHAT IS KNOWN

• The pleotropic effects of renal sympathetic denervation range from a reduction in renal sympathetic output (reduced norepinephrine spillover) to attenuation of elevation in left ventricular end-diastolic pressure and reduced spontaneous ventricular fibrillation after acute myocardial infarction.
• Several trials without a sham control have suggested renal denervation has an antihypertensive effect; however, after the negative findings of the SYMPLECTICITY HTN-3 trial (Renal Denervation in Patients With Uncontrolled Hypertension), the clinical application for renal denervation is yet to be clearly defined.

WHAT THE STUDY ADDS

• This study shows that radiofrequency ablation within the renal artery damages nerve bundles and axons within and just beyond the adventitia of the artery.
• Renal denervation reduces sinus tachycardia rate and the incidence of spontaneous ventricular arrhythmias after myocardial infarction in a swine model.
• The changes in electrophysiology are seen concurrently with reduction of sympathetic nerves and tissue abundance of neuropeptide-Y in the infarct zone.

as assayed by norepinephrine spillover, its effects on cardiac sympathetic activity have not been studied in detail, especially beyond the acute phase of myocardial infarction (MI).

We, therefore, sought to determine the efficacy of RDN in preventing spontaneous VAs as monitored with an implantable cardioverter defibrillator (ICD) for ≤3 weeks after coronary occlusion and induced VAs in pigs. Concurrently, we explored RDN-induced modulation of sympathetic effectors on the myocardium. To test the hypothesis that RDN-induced reduction of myocardial sympathetic effectors leads to antiarrhythmic benefits in a postinfarct model, we performed percutaneous radiofrequency ablations along renal arteries to simulate RDN therapy in infarcted pigs. We quantified the neuronal damages inflicted in the renal arteries and analyzed spontaneous VAs continuously logged by ICDs, as well as induced arrhythmias brought on by programmed stimulation. We also assessed sympathetic nerve density in the infarcted hearts and tissue abundance of neuropeptide-Y (NPY) and nerve growth factor (NGF) in the myocardium. To confirm RDN-induced modulation of sympathetic effectors, we also evaluated sinus tachycardia rates logged by the ICDs.

Methods

This randomized, controlled study was performed according to the guiding principles of the Canadian Council on Animal Care and conforms to the Guide for the Care and Use of Laboratory Animals, US National Institutes of Health (revised 2011). This protocol was approved by the Animal Care Committee of St Michael’s Hospital. Randomization was performed at baseline before any of the experiments had been performed using a permuted block scheme generated by the website www.randomization.com.

Study Groups

The study was composed of 2 groups, totaling 14 pigs:

1. MI induction followed by sham RDN (n=8)
2. MI induction followed by real RDN (n=6)

Study Protocol

Each pig underwent 3 study sessions as part of this protocol:

Baseline Study

All pigs were evaluated with an echocardiogram and implanted with an ICD and were rested for at least 45 minutes before baseline measurements (arterial pressure recording, ECG, electrophysiological study) were taken. This was followed with MI by mid left anterior descending (LAD) coronary artery occlusion.

Midpoint Study (2 Weeks After Baseline Study)

After an echocardiogram, each pig had pre RDN measurements (arterial pressure recording, ECG, electrophysiological study) taken, followed by either a real or sham RDN, then rested for at least 30 minutes before post RDN acute measurements (same as pre RDN measurements except echocardiogram) were taken.

End Study (1 Week After Midpoint Study [3 Weeks After Baseline Study])

After an echocardiogram, end-study measurements of each pig (post RDN; arterial pressure recording, ECG, electrophysiological study) were taken. The pig was then euthanized for the harvesting of its heart and renal arteries.

A schematic illustration of the experimental protocol is shown in Figure 1. See below and Methods in the Data Supplement for methodological details.

Study Animal Preparation

Healthy 10- to 12-week-old Yorkshire pigs of either sex (26–51 kg) were housed in the animal facility at the Li Ka Shing Knowledge Institute of St Michael’s Hospital for at least 5 days before the baseline study. Pigs were fasted overnight and sedated with buprenorphine, ketamine, xylazine, and atropine (all intramuscular). Once anesthetized with isoflurane (5%), pigs were intubated for mechanical ventilation; anesthesia was maintained by continuous administration of isoflurane and oxygen. Because of limited access to chest leads in the pig model, 3 limb leads were placed for ECG recording. Two self-adhesive defibrillation pads placed on the left and right chest walls laterally were connected to an external biphasic defibrillator for emergency defibrillation.

Echocardiography

Echocardiography was performed as described in Methods in the Data Supplement.

ICD Implantation

Under sterile conditions, an incision was made in the lateral aspect of the neck over the left internal jugular vein, where a steroid-eluting, bipolar, IS-1 pacing/defibrillation lead (Durata; St Jude Medical, Minneapolis, MN) was inserted and then affixed to the right ventricular apex under fluoroscopic guidance. The lead was connected to a single-chamber ICD (Current or Fortify; St Jude Medical). Correct lead position was verified by fluoroscopy, as well as observing lead sensing (>5 mV) and lead impedance (500–1200 Ω). Each ICD was fixed in a cervical pocket and sutured in place. Programming consisted of a VF zone ≥270 bpm (20 intervals to detect) with antitachycardia pacing during charging, followed by 35 J shocks and a VT monitor zone ≥200 bpm (40 intervals to detect). These zones were the
highest allowable with the longest detections allowable by the ICDs to minimize therapies for sinus tachycardia. Bradycardia therapy was programmed at VVI 50 bpm. Because of limited data storage on the ICDs, we elected not to log any other arrhythmias, such as nonsustained VTs with <40 beats. Premature ventricular complex counts could not be obtained from these single-channel devices.

**Arterial Pressure, ECG Recording, and Electrophysiology Study**

Arterial pressure and ECG recordings and electrophysiological studies were performed as detailed in the Data Supplement.

**Induction of Myocardial Infarction**

Complete occlusion of the mid-LAD coronary artery was achieved by percutaneous angioplasty balloon inflation as detailed in the Data Supplement.

**Renal Sympathetic Denervation**

Through a femoral artery approach, the right and then the left renal arteries were identified and cannulated under fluoroscopic guidance. RDN was performed by delivering radiofrequency energy as described in the Data Supplement.

**Neurofilament Score**

Specimens were fixed in 10% neutral buffered formalin and processed in a blinded fashion to produce 4-μm-thick sections for immunohistochemical staining. The neurofilament antibody (clone alpha F11; Dako) was used at a 1/1200 dilution with the MACH4 kit. Stained sections were viewed and analyzed in Image Scope (Leica) at 20× magnification. For image annotation and scoring, first the outer demarcation of the adventitia was drawn. Measurements of 500 μm to the outside of this line were then taken, and the 500 μm outer perimeter from the end of the adventitia was drawn. The inner demarcation of the adventitia (ie, delineation of adventitia from media) was also drawn. Darkly stained neurofilament-positive structures were counted. Structures identifiable as blood vessels were excluded from the analysis. To be included as positive, at least 2 axonal structures must be identified. Neurofilament-positive structures appearing as one and the same (ie, having 2 cross-sections but associated with the same blood vessel) were scored as 1. Nerve bundles were scored as damaged if they showed markedly distended axons or the presence of macrophages. This analysis was conducted by a neuropathologist (Dr Kiehl) who was blinded to whether the animal had received RDN.

**Spontaneous Arrhythmia Adjudication**

All device recordings were individually adjudicated by an electrophysiologist who was blinded to the intervention given to each animal. The rhythm was adjudicated to be VA if it had an abrupt onset and offset and was associated with a morphology change on the far-field electrogram. Because of programming and memory capacity of ICD logs, only VAs of at least 20 intervals at ≥270 bpm (VF zone) or at least 40 intervals at ≥2000 bpm (VT monitor zone) were recorded.

**Sinus Tachycardia Documentation**

Mean and peak sinus tachycardia rates were taken from ICD recordings as described in Methods in the Data Supplement.

**Quantification of Cardiac Sympathetic Nerve**

Presence of cardiac sympathetic nerve was analyzed as described in Methods in the Data Supplement.

**Western Blot of NGF and NPY**

Left ventricular tissues were analyzed for NGF and NPY by Western blotting as described in Methods in the Data Supplement. These analyses were conducted by a blinded technologist not aware of the treatments given to each animal.

**Statistical Methods**

The proportion of pigs with spontaneous VAs were compared categorically (any arrhythmias versus no arrhythmia) using McNemar’s test. Episodes of spontaneous VAs and the occurrence of appropriate and inappropriate shocks were compared quantitatively using negative binomial mixed effects regression models to account for both time and RDN/sham RDN as variables. Episodes of induced VAs at electrophysiology study and the effects of RDN on NGF and NPY expression were compared using the Mann–Whitney test and mean, and peak sinus tachycardia rates were compared using repeated measures analysis of variance. The density of sympathetic nerves as observed in histological slides was compared using the Mann–Whitney test. Echocardiographic parameters, ERP, and the effect of RDN on blood pressure were compared using 2-way repeated measures analysis of variance. Healthy neurofilament scores were compared using the Mann–Whitney test, and the quantity of healthy and damaged nerves were compared using 2-way analysis of variance. Statistical analyses were performed with Stata, Version 13/14, and GraphPad Prism 5. A P value under 0.05 was considered significant.

**Results**

A total of 14 pigs were randomized for this study. Two pigs in the sham RDN group died after MI and sham RDN without completing the 3-week protocol. One of them was found dead in its cage 2 days before the end study, and the other died during induction of anesthesia before the end study could begin; both of them were excluded from analysis. There were no significant differences in baseline body weight, sex, and age between the 2 groups.

**Verification of RDN and Renal Sympathetic Neuronal Damage**

To validate the efficacy of the ablation, each renal artery was carefully examined; lesions were found in each of the arteries in the RDN group, but not in the sham RDN group. We stained specifically for neurofilament and quantified healthy
Figure 2. Effects of renal denervation (RDN) on renal sympathetic nerve sprouting. A, Representative images (left; A1 and A2) showing the number of healthy nerve bundles (black arrows) in sham RDN (RDN−) and real RDN (RDN+). B, Magnified view showing a healthy nerve from image A1. C, Quantification of the healthy neurofilament score shows that the number of healthy neurofilaments is significantly reduced in the RDN (+) group (n=5) compared with RDN (−) group (n=6), **P=0.0043 (Mann–Whitney test). D, Representative images (left; D1 and D2) showing the healthy (black arrows) and damaged (red arrows) nerve bundle counts from sham RDN (RDN−) and real RDN (RDN+) animals. E, Magnified view of a damaged nerve bundle from image D2. F, Quantification of healthy and damaged nerves score, which shows that damaged nerves are significantly increased in the RDN(+) group (n=5) compared with those in the RDN(−) group (n=6), **P=0.0005 [2-way analysis of variance [ANOVA]].
and damaged nerve bundles (the neurofilament score). This score was calculated for the adventitia and 500 μm outside of the adventitia of each renal artery. The healthy neurofilament score within the adventitia and 500 μm outside of the adventitia of the renal arteries was significantly reduced in the RDN group compared with that in the sham RDN group (28.7±2.17 versus 97.05±17.80, respectively; P=0.004; Figure 2A through 2C). Compared with sham RDN animals, all animals receiving real RDN showed uniform medial cautery marks on the renal arteries, and axons within the adventitia were mostly lost, yielding healthy neurofilament score of 16.90±3.05 (real RDN) versus 67.94±11.31 (sham RDN; P<0.001). Axons in the outside of adventitia within 500 μm of the renal artery demonstrated variable damage, and healthy neurofilament score was not significantly different between the real and sham RDN groups (11.8±2.56 versus 29.11±7.03, respectively; P=0.06). The score for damaged or disrupted nerve was significantly higher at 19.0±3.4 for the real RDN group, compared with 0 for the sham RDN group (Figure 2D through 2F). These data together suggest that the RDN procedure had indeed affected renal sympathetic nerves and document the reduction of healthy nerves in addition to the presence of disrupted nerves in the RDN group. There was also a nonsignificant decrease in the healthy neurofilament scores outside the adventitia.

Spontaneous and Induced VAs

Figure 3 shows the incidence of spontaneous VAs recorded by ICDs during the 3-week protocol. In the real RDN group, 83% (5/6) of the pigs had spontaneous VAs in the period after MI, but none of these pigs (0/6) had spontaneous VAs after RDN (P=0.07, McNemar’s test; Figure 3A). In the sham RDN group, 50% (3/6) of the pigs had VAs after MI and prior to sham RDN treatment and during the ensuing week and 50% (3/6) of them had spontaneous VAs (P=0.61, McNemar’s test; Figure 3A). In the real RDN group, there were 14 spontaneous VAs in the 2 weeks post-MI (1.17 arrhythmias per pig/week) and 0 in the week after RDN (0 per pig/week). In the sham RDN group, there were 8 VAs in the 2 weeks post-MI (0.67 per pig/week) and 7 VAs in the 1 week after sham RDN (1.17 per pig/week). This represents a 100% reduction in the rate of spontaneous VAs per week in the real RDN group and a 75% increase in the rate of spontaneous VAs per week in the sham RDN group (P=0.03, negative binomial mixed effects regression model; Figure 3A through 3C).

The difference in the incidence of appropriate (P=0.31) ICD shocks was not statistically significant in this study. Nor was there any difference in inappropriate shocks between the 2 groups (P=0.96; Figure 4A through 4C). With anesthesia induction, we found that pigs would often have a profound sinus tachycardia response, which lead to several inappropriate shocks for sinus tachycardia at the time of induction. Total number of inappropriate shocks was accounted for by just 6 different pigs, primarily at the time of anesthesia. After this was discovered, atropine was then removed from the induction protocol, and this largely mitigated inappropriate shocks at this time.

Inducible VAs at electrophysiological study were seen in 5/6 pigs in the sham RDN group post-MI and in 5/6 pigs after sham RDN. In the real RDN group, inducible VAs were seen in 3/6 pigs post-MI and in 3/6 pigs after real RDN (P=1.0 for interaction).

Presence of Sympathetic Nerves in the Myocardium

Figure 5 shows the analysis of the sympathetic nerves present in the infarcted region of the hearts after MI and sham or real RDN. Histological analysis by pixel count showed greater nerve staining in the sham RDN group as compared with the real RDN.

Figure 3. Effect of renal denervation (RDN) on cardiac arrhythmias after myocardial infarction (MI). A, The proportion of spontaneous ventricular arrhythmias (VAs) recorded by implantable cardioverter defibrillator during the 2 weeks after MI but preceding real RDN/sham RDN (pre RDN) and during the 1 week after real RDN/sham RDN (post RDN). N=6 in each group, P=0.07 (McNemar’s test) in real RDN group and P=0.61 (McNemar’s test) in sham RDN group compared with pre RDN period. B, Total episodes of spontaneous VAs in individual pigs in sham RDN group compared with pre RDN period. C, Total episodes of spontaneous VAs in individual pigs in sham RDN group. N=6 in each group, P=0.03 (negative binomial mixed effects regression models) compared with sham RDN.
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group (3.55±0.71 mm² versus 1.39±0.37 mm²; \(P=0.009\)). When analyzed as a percentage of the infarcted myocardial area, the presence of sympathetic nerves was also less in the real RDN group (1.12±0.40 versus 2.84±0.73%; \(P=0.026\)).

Myocardial NPY and NGF Expression

Western blotting for NPY showed lower expression in the region of MI in the RDN group compared with that in the sham RDN (\(P=0.002\)) and also in noninfarcted tissue away from the lesion at the posterior myocardium (\(P=0.04\); Figure 6A and 6B). In contrast, NGF expression was higher at the infarcted sites (\(P=0.03\)) and at sites away from the infarct lesion in the posterior myocardium (\(P=0.03\)) in RDN-treated pigs, when compared with that in corresponding tissues from sham-treated pigs (Figure 6C and 6D).

Figure 4. Effects of renal denervation (RDN) on implantable cardioverter defibrillator shocks. A. An episode of rapid ventricular tachycardia (VT) that was successfully treated by a single shock from the pig implantable defibrillator. The mean cycle length of the VT was 185 ms (324 bpm), and the far-field signal (leadless ECG) shows a regular and broad complex rhythm consistent with ventricular tachycardia. B. Total number of appropriate shocks. There was no significant difference in the number of appropriate shocks after RDN (\(P=0.31\), negative binomial mixed effects regression models). C. Total number of inappropriate shocks after RDN. There was no significant difference in the number of inappropriate shocks after RDN (\(P=0.96\), negative binomial mixed effects regression models), \(n=6\) in each group. EGM indicates electrogram; F, ventricular fibrillation interval sensed; HV, high voltage shock; and VP, ventricular pace.
Mean and Peak Sinus Tachycardia Rates
The mean sinus tachycardia rates in the sham RDN group rose from 224±2 to 234±4 bpm, and in the real RDN group, they went from 225±7 to 219±5 bpm from pre RDN to post RDN, respectively (P=0.04 for the interaction). The peak sinus tachycardia rate for each pig was also significantly less in the real RDN group (261±7 to 276±13 bpm in the sham RDN group compared with 271±10 to 254±13 bpm in the real RDN group) from pre RDN to post RDN, respectively, P=0.03 for the interaction (Figure 7).

Discussion
In this randomized study, with blinded evaluation of end points, we tested the hypothesis that reduction of sympathetic activity induced by RDN would lead to antiarrhythmic benefits in a postinfarct pig model. We demonstrated that RDN reduces spontaneous VAs in this model ≤3 weeks after MI induction. Uniform loss of sympathetic nerves within and along renal arteries was confirmed in animals receiving RDN. This is an important aspect of this study given that in most instances, effective ablation of renal nerves was not confirmed with histology. We also demonstrated reduction in cardiac sympathetic activity on the basis of a reduction in the density of cardiac sympathetic nerves, reduction of myocardial NPY, and reduced peak and mean sinus tachycardia rates. These findings provide a mechanistic basis for alteration in myocardial substrate and triggers that maintain and give rise to VAs ≤3 weeks after MI.

Effect of RDN on Spontaneous and Induced VAs
Previous studies of the effects of RDN on VAs were limited to the early period of acute ischemia. Huang et al7 reported that incidences of PVCs and episodes of spontaneous VT/VF during LAD occlusion were reduced by RDN. Similarly, Linz et al6 demonstrated that RDN decreased episodes of VF during LAD occlusion and the number of PVCs during the first 10 minutes of ischemia, but not the occurrence of VF during reperfusion. They also demonstrated that the reduction in PVCs and VF during ischemia after RDN was similar to the reduction caused by atenolol administration.6 During the early stages of coronary occlusion, VAs occur predominantly because of reentry within the ischemic myocardium where depressed transmembrane potentials lead to slow conduction and block.9 PVCs that initiate reentry may be reentrants themselves or may be because of triggered activity from Purkinje fibers in ischemic regions in response to increased catecholamine levels.10 In the subacute period after acute MI, rapid VT may result from abnormal automaticity in surviving Purkinje fibers and possibly triggered activity.9 These delayed arrhythmias may be promoted by increased cardiac sympathetic nerve activity11 or by increased sensitivity of Purkinje fibers to catecholamines.9 In the current study, where infarcts were relatively small (mean left ventricular ejection fraction at 55%) and sustained VAs were often difficult to induce at electrophysiological study or terminate with antitachycardia pacing, abnormal automaticity may have been a prominent mechanism.9 By reducing cardiac sympathetic activity, RDN may be particularly effective in treating abnormal automaticity, providing one explanation as to why no pigs had further VAs after real RDN in this study. As beyond the subacute period after acute MI, spontaneous VAs are more likely to result from reentry involving slowly conducting surviving myocardial fibers,9 and inducible arrhythmias are mostly related to scar formation; this could explain the discrepancy of the effects of
RDN on spontaneous and inducible VAs in the current experimental model. Thus, the mechanism of benefit comes in the form of trigger reduction as opposed to substrate-based reduction of VAs. Through reducing sympathetic effectors, RDN may reduce the triggering of ectopic beats that initiate reentry at this stage or modify the electrophysiological properties of these reentry circuits.

**Effect of RDN on Ventricular ERP**

In a study by Huang et al., RDN in animals with a normal healthy heart and in a study by Gu et al. of stellate ganglion blockade, sympathetic denervation resulted in prolonged ventricular ERP. In the current study, ventricular ERP in the real RDN group increased from 196±23 ms at baseline to 247±23 ms after RDN. This was a greater increase than what was reported by Huang et al. (≈15 ms on average); however, ERP was prolonged by a similar magnitude in the sham RDN and the RDN groups, suggesting that any possible ERP prolongation after RDN was masked by progressive fibrosis at the interface between the ICD lead and the myocardium in this study. Resterilized active ICD leads in this study would have lacked any remaining steroid at the tip and, hence, unlikely to prevent fibrosis.

**Mean and Peak Sinus Tachycardia Rates**

In the present study, the rise in mean sinus tachycardia rates (>200/minute as recorded by the ICDs) in infarcted hearts was attenuated by RDN. Moreover, the peak sinus tachycardia rates in these infarcted hearts increased in the sham RDN group and decreased in the real RDN group. Linz et al. demonstrated that RDN reduced the mean ventricular rates during atrial fibrillation by 24% and reduced heart rates during sinus rhythm by a mean of 20 bpm, consistent with the concept highlighted in our model. In the resistant hypertension setting, RDN reduced mean sinus rates more modestly by 2 to 3 bpm and by 4 bpm in 2 different studies. Reduction in mean and peak heart rates in association with reduced spontaneous VAs after RDN may both relate to lowered whole-body sympathetic activity mediated by a reduction in renal afferent nerve activity. Conversely, faster heart beats may exacerbate the
rate-dependent recovery of ischemic cells and make reentrant VAs more likely.17

Presence of Cardiac Sympathetic Nerves and NPY
There was reduced staining for sympathetic nerves in infarcted hearts from the RDN group compared with that in the sham RDN group. Previous study suggested that acute MI results in nerve injury and subsequent sympathetic nerve sprouting with regional heterogeneous and hyper-innervated myocardium.18 Patients with an increased density of sympathetic nerves after acute MI have an increased incidence of VAs,18 and augmented sympathetic nerve sprouting by NGF infusion leads to a greater incidence of sudden cardiac death and VAs in dogs.18 This sympathetic nerve sprouting hypothesis is also consistent with the evidence for β-blockade in preventing sudden cardiac death after MI.19 Linz et al demonstrated that the reduction of atrial sympathetic nerve sprouting after RDN in goats is associated with decreased complexity of atrial fibrillation.20 In our experimental model, reduction of sympathetic nerves in infarcted hearts after RDN treatment may be a key mechanism for reduced spontaneous VAs. Treatment with carvedilol after MI also ameliorated cardiac sympathetic nerve sprouting.21

To explore the mechanism of the actions of RDN on cardiac sympathetic innervation, we assessed tissue abundance of NGF, a key regulator of nerve growth and maintenance in the myocardium. The presence of fewer sympathetic nerves in the myocardium after acute MI results in nerve injury and subsequent sympathetic nerve sprouting with regional heterogeneous and hyper-innervated myocardium.18 Patients with an increased density of sympathetic nerves after acute MI have an increased incidence of VAs,18 and augmented sympathetic nerve sprouting by NGF infusion leads to a greater incidence of sudden cardiac death and VAs in dogs.18 This sympathetic nerve sprouting hypothesis is also consistent with the evidence for β-blockade in preventing sudden cardiac death after MI.19 Linz et al demonstrated that the reduction of atrial sympathetic nerve sprouting after RDN in goats is associated with decreased complexity of atrial fibrillation.20 In our experimental model, reduction of sympathetic nerves in infarcted hearts after RDN treatment may be a key mechanism for reduced spontaneous VAs. Treatment with carvedilol after MI also ameliorated cardiac sympathetic nerve sprouting.21

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Increased tissue NPY abundance was previously associated with increased sympathetic activity.26,27 Our Western blot analysis showed decreased levels of NPY at sites of MI and at sites distant from MI in the RDN group. The neurotransmitter NPY, a marker of sympathetic activity, has been demonstrated to be decreased after RDN.25 Therefore, decreased NPY after RDN is consistent with the finding of reduced sympathetic nerve density and activity at the site of infarction and suggests that sympathetic nerve activity may also be reduced at noninfarcted sites as well.

Figure 7. Effect of renal denervation (RDN) on sinus tachycardia rates in individual pigs. Implantable cardioverter defibrillator interrogation for sinus tachycardia rates during the pre RDN period and the post RDN period. A. Mean sinus tachycardia rates in sham RDN group. B. Mean sinus tachycardia rates in real RDN group. n= 6 in each group, P=0.04 (repeated measures analysis of variance [ANOVA]). C. Peak sinus tachycardia rates in sham RDN group. D. Peak sinus tachycardia rates in real RDN group, n=6 in each group, P=0.03 (repeated measures analysis of variance [ANOVA]).
Effect of RDN on Renal Sympathetic Nerves

In the present study, the adventitia of renal arteries in the RDN group exhibited a significantly reduced healthy neurofilament score, with a nonsignificant decrease in the healthy neurofilament scores outside the adventitia (this study was likely underpowered to show a statistically significant difference in the healthy neurofilament scores outside the adventitia). As expected, damaged nerve bundles were only found in the RDN group, but not the sham RDN group, confirming that this was not because of instrument placement but RF treatment to the renal arteries. Seven days after RDN, axons are still in the process of degeneration. Degenerated axons and even the detached segment of axon have the ability to release neurotransmitters. Complete degeneration of the axon varies from animal to animal and species to species. In our study, we euthanized the animals 7 days after RDN, and thus, our histological analysis was limited to such a time period. This time factor could explain why we observed variable neurofilament scores outside the adventitia where large renal sympathetic nerves can be found. These immunohistological findings also demonstrate the association of renal artery sympathetic denervation with the observed decreased spontaneous arrhythmias in our experimental model.

In conclusion, reduced spontaneous VAs after RDN may relate to reduced cardiac sympathetic activity after MI. These antiarrhythmic effects in the post-MI setting relate predominately to trigger reduction as opposed to substrate alteration. The outcome measures in this study were objectively quantified, using device logs without room for subjective bias. This work provides new insight into sympathetic nerve reduction by RDN in the postinfarct myocardium, pointing to a novel aspect of the renal–cardiac axis for VAs reduction and calls for randomized trials to confirm this benefit in the clinical setting.

Limitations

To ensure the survival of pigs beyond initial MI, coronary occlusion was performed in such a way that the resultant lesions were relatively small, with a modest decrease in left ventricular ejection fraction. This is still of clinical relevance as a significant number of patients with MI are treated early with PCI and end up with no or only a modest reduction in EF. As a significant number of patients with MI are treated early with PCI and end up with no or only a modest reduction in EF. This time factor could explain why we observed variable neurofilament scores outside the adventitia where large renal sympathetic nerves can be found. These immunohistological findings also demonstrate the association of renal artery sympathetic denervation with the observed decreased spontaneous arrhythmias in our experimental model.

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Disclosures

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Supplemental Methods

Echocardiography

Echocardiographic measurements, such as Standard 2D images and Doppler, were acquired using a Sonos 5500 ultrasound system for offline analysis. Averaged measurements made from three consecutive cardiac cycles were used for final calculations.

Arterial Pressure, ECG Recording and Electrophysiology Study

Heparin 40-60 μg/kg IV was administered to the pig to prevent arterial thrombosis during arterial catheterization. The left or right femoral artery was catheterized and five minutes simultaneous tracings of baseline ECG and femoral arterial pressure were amplified and recorded. At least 60 minutes were allowed to elapse between LAD occlusion and measurement of any electrophysiological variables. All stimulations were performed using the ICD. Ventricular effective refractory period (VERP) was determined using a pacing train of 8 beats (S1) at the
sinus cycle length–20ms followed by an extra stimulus (S2). The longest cycle length that failed to capture was recorded as the VERP. Up to three extra-stimuli were performed for the VT induction study. Inducible ventricular arrhythmias were defined as ≥30 seconds of VT, VT requiring treatment or VF. The VERP and VT study were then repeated with a pacing train at 400ms. The EP study was terminated if 2 defibrillations were required during this protocol.

**Induction of Myocardial Infarction**

Via a femoral arterial catheter sheath, a 6 Fr JR4 interventional angiography catheter was used to engage the left coronary system. With an angioplasty wire and balloon in place via the catheter, complete occlusion of the mid-LAD (immediately distal to the second diagonal) was then achieved by keeping the balloon inflated for 45 minutes. Lidocaine was administered IV (1 mg) before insertion of the guide system and amiodarone (1 mg/kg/min IV) was infused for the duration of the balloon inflation to attenuate arrhythmogenesis.

**Renal Sympathetic Denervation**

Through a femoral artery approach, the right and then the left renal artery were identified and cannulated under fluoroscopic guidance. RDN was performed by delivering radio frequency energy with the EnligHTN™ (St. Jude Medical) basket catheter according to a set protocol to produce a total of 8 ablation lesions at 2 positions per renal artery. For the first set of ablation lesions, the most distal electrode was placed 0.5 cm proximal to the bifurcation/trifurcation of the renal artery. With a 45° rotation of the catheter, a second ablation was performed to produce lesions proximal to the first set. Each ablation was performed for 90 seconds with a power of 6 watts and a maximum temperature limit of 75°C. This process was first performed on the right renal artery, and was promptly repeated on the left. For sham RDN, the renal arteries were visualized with at least 10 mL of contrast injected into both renal arteries with no ablation.
catheter positioned.

**Sinus Tachycardia Documentation**

Sinus tachycardia ($\geq 200$ bpm, for at least 40 beats) was frequently seen and this was characterized by a gradual onset and offset (occasionally with a change in morphology if an inappropriate shock had been delivered). The mean and peak ST rates were taken from the device recordings (the mean rate was calculated by the device from the detection period and the peak rate was the shortest RR interval during the entire episode). Recordings were excluded if they followed a VA or an appropriate/inappropriate shock.

**Quantification of Cardiac Sympathetic Nerve**

At the end of the study, when the pig hearts were removed, transmural sections involving infarcted myocardium and adjacent healthy myocardium were taken for histology. Three stains were used, including Haematoxylin and Eosin, Picrosirius Red (to stain collagen in infarcted tissues) and Tyrosine Hydroxylase (to stain nerve fibres). Slides were digitized with 20× magnification and pixel counts were performed to quantify total tissue area, infarct area and nerve area for each pig. Nerve area was quantified in mm$^2$ and then calculated as a percentage of the infarct and total tissue areas. This analysis was conducted by a blinded operator who was not aware if the animal had received RDN.

**Western Blot of Nerve Growth Factor and Neuropeptide-Y**

Pig heart tissues were collected from the posterior LV wall and infarct area at the end of the experiment and snap frozen in liquid nitrogen. Proteins were extracted using a mechanical tissue homogenizer and lysis buffer composed of 30 mM KH$_2$PO$_4$, 0.3M sucrose, 0.5mM Na$_2$EDTA and Roche protease and phosphatase inhibitors. Protein concentration was measured using the QuantiPro BCA Assay Kit (Sigma). Extracted proteins were dissolved in SDS. Solubilised
proteins were separated by SDS-PAGE in 4-20% gradient gels. Separated proteins were transferred onto PVDF membrane. After blocking the membrane for non-specific protein interactions, the membranes were incubated with the primary antibodies for neuropeptide Y or NGF, followed by incubation with specific secondary antibodies. Protein bands were visualized on X-ray films using an enhanced chemiluminescence kit. Density of specific protein bands was quantified by using GS 800 Calibrated Densitometer (Bio-Rad) and Quantity One software (Bio-Rad). Specific protein band density was normalized to GAPDH band density in each sample. These molecular analyses were conducted by a blinded technologist who was not aware if the sample originated from animals that had received RDN.

Results

Left Ventricular Structure and Function

The mean LVEF decreased from 58±2% during the pre-MI period to 54±2% (p=0.28) during the post-MI period and the mean LVEDV increased from 40±4 ml to 49±5 ml (p=0.02) in the real RDN group. There was no significant difference between the sham RDN group and the real RDN group for LVEF (55±2% vs. 54±2%, p=0.31) or LVEDV (48.9±2 ml vs. 49±5 ml, p=0.44) following RDN, respectively (Supplementary Figure 1).

Systolic and Diastolic Blood Pressure

Two weeks after myocardial infarction, prior to sham or real RDN, there was no significant difference in pre-intervention systolic pressures between the two groups (105±6 vs. 110±8 mmHg respectively, p=0.06, for interaction by two way repeated measures ANOVA). Neither there was a significant difference in systolic pressure between the two groups immediately following sham (99±5 mmHg) and real RDN (82±14 mmHg, p=0.06, for interaction by two way repeated measures ANOVA) or 7 days following sham (105±2 mmHg) and real RDN (108±8
mmHg) (p=0.06, for interaction by two way repeated measures ANOVA) (Supplementary Figure 2).

**Myocardial Refractoriness**

At drive train cycle lengths of SCL-20ms and 400ms there was a consistent increase in ventricular ERP over time in both groups (p<0.001), but there was no significant difference in the ventricular ERP between the real RDN and sham RDN groups (p=0.86) (Supplementary Figure 3).

**Supplementary Figure 1.**

![Supplementary Figure 1](image)

**Supplementary Figure 1. Effect of RDN on LV Structure and Function**

Echocardiographic parameters are shown at baseline (prior to MI) and at the end of the study and 7 days after real or sham RDN (post RDN7). **a.** Left ventricular ejection fraction (%); n=6 in each group. **b.** Left ventricular end diastolic volume; n=6 in each group. There is no significant difference between the two groups, by two-way repeated measures ANOVA.
Supplementary Figure 2. Effect of RDN on Aortic Pressure

Invasive aortic pressure are shown at prior to real or sham RDN (pre RDN), immediately following real or sham RDN (post RDN acute) and at the end of the study following 7 days real or sham RDN (post RDN7). a. systolic blood pressure. b. diastolic blood pressure. n=5 in each group. There is no significant difference between the two groups, by two-way repeated measures ANOVA.
Supplementary Figure 3. Effect of RDN on ERP

The ventricular ERPs are shown at 3 time points. Baseline ERP was performed pre MI, midpoint ERP was performed just prior to real RDN/sham RDN (pre RDN) and the final ERP was performed seven days after real RDN/sham RDN before the sacking of the pig (post RDN). **a.** ERP with the drive train at sinus cycle length -20ms, n=6 in each group. **b.** ERP with the drive train at 400ms cycle length. n=6 in each cycle length. At both drive train cycle lengths there was no significant difference in the ventricular ERP following RDN (p=0.86, two-way repeated measure ANOVA). There was a consistent increase in the ventricular ERP over time in both randomization groups (p<0.001, two-way repeated measures ANOVA).