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 arrhythmogenic 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 cardio–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 activity, 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 cardia 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 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 SYMPLICITY 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.

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-chamber 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-mm-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 ≥200 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 (500 μm outside) of each renal artery 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 29.1±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.
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).
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.¹ These delayed arrhythmias may be promoted by increased cardiac sympathetic nerve activity¹³ or by increased sensitivity of Purkinje fibers to catecholamines.⁶ 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,⁸ 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 on 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.  

**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. Patients with an increased density of sympathetic nerves after acute MI have an increased incidence of VAs, and augmented sympathetic nerve sprouting by NGF infusion leads to a greater incidence of sudden cardiac death and VAs in dogs. This sympathetic nerve sprouting hypothesis is also consistent with the evidence for β-blockade in preventing sudden cardiac death after MI. Linz et al demonstrated that the reduction of atrial sympathetic nerve sprouting after RDN in goats is associated with decreased complexity of atrial fibrillation. 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.

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 have an increased incidence of VAs, and augmented sympathetic nerve sprouting by NGF infusion leads to a greater incidence of sudden cardiac death and VAs in dogs. This sympathetic nerve sprouting hypothesis is also consistent with the evidence for β-blockade in preventing sudden cardiac death after MI. Linz et al demonstrated that the reduction of atrial sympathetic nerve sprouting after RDN in goats is associated with decreased complexity of atrial fibrillation. 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.

Injured nerves after interference of blood supply undergo Wallerian degeneration, which would be followed by axonal regeneration. Necrosis takes place early in the infarction, which may be followed by nerve regeneration 1 week later. Our experimental protocol was not designed to study early changes, such as nerve necrosis; however, we studied myocardial nerves density 3 weeks after MI. The presence of fewer sympathetic nerves in the infarcted myocardium in our data supports the novel idea that RDN can prevent or suppress sympathetic hyperinnervation after infarction. This is an important finding as such adverse cardiac nerve development is known to be arrhythmogenic and is associated with sudden cardiac death. This normalization of sympathetic hyperactivity is consistent with previous findings that RDN normalizes sympathetic nerve activity in hypertension. Our study corroborates this paradigm in that a catheter-based single intervention can reduce the magnitude of cardiac sympathetic nerve activity in a postinfarct model. For VA management, β-blockers are the first-line drugs. Though we did not study the effect of β-blocker in our experimental model, Linz et al showed that RDN is as effective as β-blockade in reducing PVCs and VF. The potential advantage of RDN as an alternative to β-blocker would be the lack of compliance concerns, generalized effects, and long-term costs.

Increased tissue NPY abundance was previously associated with increased sympathetic activity. 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. 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.
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 predominantly 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 RDN in the postinfarct myocardium, pointing to a novel antiarrhythmic effects in the post-MI setting relate predominantly to trigger reduction as opposed to substrate alteration. The present study assessed the role of RDN on VAs and reduction of cardiac sympathetic nerves that took place during the first 3 weeks post-MI; further studies are needed to determine whether these effects persist beyond this time period.

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. Another limitation of the study was the absence of comparison with β-blockade. In practice, RDN would be a single intervention after MI, as opposed to medical β-blockade with systemic effects and variable compliance. The present study assessed the role of RDN on VAs and reduction of cardiac sympathetic nerves that took place during the first 3 weeks post-MI; further studies are needed to determine whether these effects persist beyond this time period.

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Disclosures

Dr Nanthakumar is a consultant for Biosense Webster and St Jude Medical. S. Massé is a consultant for St Jude Medical. The other authors report no conflicts.

References

11. Jackson et al. RDN for Treating Ventricular Arrhythmias


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Effects of Renal Artery Denervation on Ventricular Arrhythmias in a Post-Infarct Model

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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 (≥200bpm, 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).
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. 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

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).
肾交感神经消融对心肌梗死后室性心律失常的作用

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背景——肾交感神经消融（renal denervation, RDN）在心律失常领域的应用目前仍处在初始阶段，其具体作用机制有待进一步阐明。本研究以梗死动物模型为研究对象，旨在明确 RDN 是否具有抗心梗后心律失常作用，以及是否可通过降低交感活性增加心肌获益。

方法和结果——以猪作为研究对象，植入单极除颤器以记录室性心律失常（ventricular arrhythmias, VAs）的发生，后通过植入方法栓塞其冠脉，制作猪心肌梗死模型。2 周后，将研究动物分为假手术组和 RDN 治疗组，使用 St. Jude EmglightHTN 篮状消融导管对 RDN 治疗组进行双侧肾动脉消融。自梗死后起，对心率和交感活性进行为期 3 周的观察记录。对心肌梗死动物进行组织学分析，并用神经纤维积分评价交感神经活性，分析结果显示 RDN 治疗组的神经纤维积分明显低于假手术组，且仅在 RDN 治疗组中存在神经结构破坏。自发性 VAs 发生率在 RDN 治疗组中下降 100%，而在假手术组中升高 75%（P = 0.03）。梗死区域心肌组织中，交感神经活性以及代表交感神经活性的神经肽-Y 含量明显增加，但在 RDN 治疗组中上述物质含量均明显降低。此外，在 RDN 治疗组中，心率最大值及平均值均明显降低。

结论——在猪心梗模型中，RDN 可显著降低心梗后 VAs 发生率及交感神经对心脏重构的影响。这可能是 RDN 治疗心梗后 VAs 的理论基础。

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关键词：心律失常 ■ 冠状动脉闭塞 ■ 微循环 ■ 心肌梗死 ■ 神经肽-Y ■ 肾交感神经消融术

来自Koruth和Dukkipati的社论

已有研究通过猪冠脉栓塞模型证实 RDN 可有效降低心梗后急性期左室舒张末压的升高、抑制期前收缩的发生、降低室颤（ventricular fibrillation, VF）的发生率[6]。另一项对猪急性心梗模型的研究发现 RDN 通过延长心室有效不应期（ventricular effective refractory period, ERP）、延长心室动作电位时程、减小动作电位时程离散度，可有效降低心梗后室性心律失常（ventricular
目前已知肾交感神经消融具有多重作用,对肾交感神经传出纤维活性(即去甲肾上腺素溢出率)、左室舒张末压的升高、急性心梗后室颤的发生均有抑制作用。未设置假手术对照组的大量研究均显示肾交感神经消融具有降压作用,然而 SYMPLICITY HTN-3 研究结果呈阴性,目前肾交感神经消融的临床应用范围尚未明确。

研究现状

- 目前已知肾交感神经消融具有多重作用,对肾交感神经传出纤维活性(即去甲肾上腺素溢出率)、左室舒张末压的升高、急性心梗后室颤的发生均有抑制作用。
- 未设置假手术对照组的大量研究均显示肾交感神经消融具有降压作用,然而 SYMPLICITY HTN-3 研究结果呈阴性,目前肾交感神经消融的临床应用范围尚未明确。

本研究新发现

- 本研究发现通过介入方式进行肾交感神经射频消融只能对行走在动脉外膜或紧贴动脉外膜走行的神经束和轴突产生破坏。
- 在猪心梗后模型中,肾交感神经消融可降低窦性心律并降低心梗后室性心律失常发生率。
- 肾交感神经消融后,梗死部位电生理改变外,还存在交感神经密度及神经肽-Y 含量的减少。

方法

实验分组

本研究分为 2 组,共计 14 只猪:
1. MI 后假手术组 (n = 8)
2. MI 后 RDN 治疗组 (n = 8)

实验方案

- 每只动物需完成以下 3 部分研究。
- 实验动物前期准备
  - 实验动物前期准备
  - 基线研究
  - 中点研究(中点研究完成后 2 周)
  - 终点研究(中点研究完成后 1 周/终点研究完成后 3 周)

实验动物同期健康

10-12 周龄健康的克郡猪,雌雄不限,体重 26-51 kg。饲养于圣迈克尔医院李嘉诚研究院动物中心。所有实验动物基线数据测量前于该动物中心至少饲养 5 天。术前禁食过夜,采用肌肉注射叔丁酚、氯胺酮、甲苯噻嗪及阿托品进行麻醉。采用异氟烷进行麻醉时(≤5%),行气管插管机械通气,通过持续通入异氟烷及氧气维持麻醉。由于心电图胸导联应用限制,研究中采用肢体导联进行心电图记录。左右侧胸壁分别粘贴与体外双向除颤仪相连的电极片,以备术中紧急除颤时使用。

心脏超声

心脏超声测量方法详见增补材料中方法学部分。

ICD植入

无菌条件下,在颈部侧面左侧颈静脉旁行一切口,置入类固醇洗脱 IS-1 起搏 / 除颤双极电极(Durata; St Jude Medical, Minneapolis, MN), 在 X 线透视下并将其嵌入右室心尖部,该电极与单腔 ICD (Current or Fortify; St Jude Medical) 相连。电极植入后通过 X 线透视确认位置正确,并同时确认电极感知功能良好 (>5mV)、阻抗正常 (500-1200 Ω)。后将 ICD 置于胸腔囊袋中并进行缝合。ICD 内置程序由 VF 相关设置及 VT 监测相关设置组成,当心率≥270 bpm (监测 20 个心动周期) 时,ICD 在 VF 模块控制下完成充电后放电 35J 进行除颤,VT
动脉血压、ECG记录及心脏电生理研究

详解增补材料方法学部分。

心肌梗死模型制作

通过经皮球囊扩张术造成冠脉LAD中段完全闭塞，具体过程详见增补材料。【增补材料，内容见http://circinterventions.ahajournals.org/content/suppl/2017/03/03/CIRCINTERVENTIONS.116.004172.DC1】

肾交感神经消融

通过股动脉入路，后经X线透视分别进行右、左肾动脉造影，并置入指引导管，后通过射频消融方法完成RDN。具体过程详见增补材料。

神经微丝积分

标本置于10%福尔马林中固定后，制作成4 μm后切片进行免疫组化染色。神经微丝蛋白抗体（clone alpha F11; Dako）经MACH4试剂盒稀释1200倍后使用。染色切片使用Image Scope（Leica）放大20倍进行图像观察及分析。为进行图像标注及积分，首先描绘出动脉外膜的外侧缘，并沿上述描迹线向外测量500 μm, 连接测量点呈线。同时描绘出动脉外膜的内侧缘（即外膜与中膜的分界线）。对神经微丝蛋白阳性结构进行计数。神经微丝蛋白阳性结构呈现一致性（两个截面，但均与同一条血管相关联）则积1分。如神经束存在明显的轴突膨胀或出现巨噬细胞，则计为该神经束受损。上述分析由一名神经学家（Kiehl博士）完成，研究分组对该分析人员进行了设盲。

自发性心律失常的判定

所有ICD记录数据均由一名电生理专家单独判定，研究分组对该专家进行了设盲。如一段心律表现为突然发作及终止，同时远场心电记录存在相关性形态变化，该段心律即可判定为VA。由于ICD编码及存储容量的限制，本研究仅对持续超过20个心动周期的VAs进行了记录分析。

窦性心率过速的记录

研究对窦性心率过速时的平均及最高心率通过ICD进行了记录，方法详见增补材料中研究方法部分。

心脏交感神经活性定量分析

方法详见增补材料中研究方法部分。

NGF及NPY的Western Blot染色

左室组织NGF及NPY的Western Blot染色结果分析方法详见增补材料中研究方法部分。该部分结果分析由一位技术人员完成，研究分组对该分析人员进行了设盲。

统计方法

两组间发生自发性VAs的动物比例采用McNemar检验法进行比较（发生任何心律失常者：未发生心律失常者）。自发性VAs发作次数及ICD放电次数（包括适当的和不适当的）的比较，采用负二项混合效应回归模型进行定量分析，将时间及RDN治疗/假手术均作为因变量进行分析。电生理研究中诱发性VAs的发作次数及RDN对NGF和NPY表达的影响，通过Mann-Whitney检验进行均数比较；窦性心动过速最快心率通过重复测量数据的方差分析进行比较。组织切片中交感神经密度通过Mann-Whitney检验进行比较，心脏超声参数、ERP, 以及RDN对血压的影响采用双侧重复测量数据的方差分析进行比较。统计分析通过Stata 13/14版本及GraphPad Prism 5完成。P<0.05认为有统计学意义。两组间交感神经有效性评分采用Mann-Whitney检验进行比较。两组间有效神经定量及受损神经定量通过双侧方差分析进行比较。

结果

本研究中共计14只猪进行了随机分组。假手术组中2只动物于MI及假手术后死亡，未完成为期3周随访。其中1只在随访结束前2天死于笼中，另一只死于终点数据采集前的诱导麻醉。上述2只死亡动物均未纳入最终分析。两组基线体重、性别、年龄均无显著差异。
图 2. 肾交感神经消融（renal denervation, RDN）对肾交感神经出芽的影响
A. 左侧 A1、A2 所示为假手术组（RDN-）及 RDN 治疗组（RDN+）中有效神经束（黑色箭头所示）计数。B. 图 A1 中有效神经束的放大图像。
C. 采用有效神经微丝积分定量分析显示，RDN (+) 组（n = 5）有效神经微丝积分明显低于 RDN (-) 组（n = 6），** 表示与 RDN (-) 组相比 P = 0.0043（Mann-Whitney 检验）。
D. 左侧 D1、D2 所示为假手术组（RDN-）及 RDN 治疗组（RDN+）中有效神经束（黑色箭头所示）及受损神经束（红色箭头所示）计数。E. 图 D2 中受损神经束的放大图像。F. 有效及受损神经积分定量分析显示，RDN (+) 组（n = 5）受损神经积分明显高于 RDN (-) 组（n = 6），** 表示与 RDN (-) 组相比 P = 0.0005（双侧方差分析[ANOVA]）。
RDN 效果及肾交感神经损伤情况

为确认消融的有效性，研究中对每条肾动脉均进行了仔细探查，RDN 治疗组的所有动脉均可见消融损伤，假手术组中未见损伤部位。我们对神经微丝进行了特殊染色，并对动脉外膜至距离外膜 500 μm 之内的区域进行分析，得出神经微丝积分，并以此对有效神经束及受损神经束进行定量分析。在 RDN 治疗组中，动脉中膜以外至距离外膜 500 μm 范围内的有效神经微丝积分明显低于假手术组（28.7±2.17 vs. 97.05±17.80；P = 0.004；图 2A 至 2C）。RDN 治疗组中所有动脉均可见消融损伤，假手术组中未见损伤部位。我们对神经微丝进行了特殊染色，并对动脉外膜及距离外膜 500 μm 之内的区域进行分析，得出神经微丝积分，并以此对有效神经束及受损神经束进行定量分析。在 RDN 治疗组中，动脉中膜以外至距离外膜 500 μm 范围内的有效神经微丝积分明显低于假手术组（28.7±2.17 vs. 97.05±17.80；P = 0.004；图 2A 至 2C）。

在血管外膜外，距离外膜 500 μm 范围内的神经轴突几乎完全消失，有效神经微丝积分分别为 16.90±3.05 和 67.94±11.31（P < 0.001）。受损或断裂神经积分，在 RDN 治疗组中显著高于假手术组，分别为 19.0±3.4 和 0（图 2D 至 2F）。综合分析上述数据可见，RDN 可有效作用于肾交感神经，除可在成神经断裂外还会造成健全神经减少。在动脉外膜以外，有效神经微丝也存在减少，但未达统计学差异。

自发性及诱导性 VAs 发作情况

图 3 为在 3 周观察时间内 ICD 所记录的自发性 VAs 的发生率。在 RDN 治疗组中，83%（5/6）动物在 MI 后出现自发性 VAs，但在 RDN 治疗后无动物（0/6）发作自发性 VAs（P = 0.07，McNemar 检验；图 3A）。在假手术组中，50%（3/6）动物在 MI 后假手术前出现自发性 VAs，在假手术后 1 周自发性 VAs 发生率仍为 50%（3/6）（P = 0.61，McNemar 检验；图 3A）。在 RDN 治疗组中，MI 后 2 周内共有 14 次自发性 VAs 发生（1.17 次/只•周）。以此计算，RDN 治疗组自发性 VAs 个体周发生率下降了 100%，而假手术组中自发性 VAs 个体周发生率增加了 57%（P = 0.61，负二项混合效应回归模型，图 3A 至 3C）。

本研究中两组间适当的 ICD 放电率（P = 0.31）及不适当的 ICD 放电率（P = 0.96，图 4A 至 4C）均无显著差异。在诱导麻醉时，动物常会发生严重的窦性心动过速，若发生在 ICD 感知期内，常导致不适当放电的发生。研究中有 6 只动物发生了不适当放电，均发生于麻醉期，发现上述情况后，我们停止了麻醉中阿托品的使用，此后麻醉期间不适当放电的发生率大大减低。

在诱导 VAs 发作的电生理研究中，假手术组在假手术前诱发性 VAs 发生率均为 5/6。RDN 治疗组中，RDN 治疗后诱发性 VAs 发生率均为 3/6（交互作用 P = 1.0）。

心肌交感神经密度

图 5 所示为假手术组及 RDN 治疗组心肌梗死区交感神经分布情况。经像素数目分析，假手术组交感神经密度明显高于 RDN 治疗组（3.55±0.71 mm² vs. 1.39±0.37 mm²；P = 0.009）。按所占面积百分比分析，RDN 治疗组交感神经所占梗死区面积亦明显小于假手术组。
图 4. 肾交感神经消融（renal denervation，RDN）对 ICD 放电次数的影响
A. ICD 放电 1 次后成功转复的室性心动过速（ventricular tachycardia，VT）发作记录。VT 平均周期为 185 ms（324 bpm），远场信号显示为规则、宽大波形符合室性心动过速表现。
B. 两组手术前后适当放电总数比较。RDN 治疗前后适当放电数无显著差异（P = 0.31，负二项混合效应回归模型）。
C. 两组手术前后不适当放电总数比较。RDN 治疗前后不适当放电数无显著差异（P = 0.96，负二项混合效应回归模型），两组均为 n = 6。

心肌 NPY 及 NGF 表达

通过 Western blot 分析可见在 RDN 治疗组中梗死区（P = 0.002）及后壁非梗死区（P = 0.04）NPY 表达均明显低于假手术组（图 6A 至 6B）。与此 NPY 表达相反，NGF 在 RDN 治疗组中梗死区（P = 0.03）及后壁非梗死区（P = 0.03）表达均明显高于假手术组（图 6C 至 6D）。
窦性心动过速时的平均及最高心率

在假手术组中，手术前后窦性心动过速时平均心率分别为 224±2 及 234±4 bpm，在 RDN 治疗组分别为 225±7 及 219±5 bpm（交互作用 P = 0.04）。在假手术中，手术前后窦性心动过速时最高心率分别为 261±7 及 276±13 bpm。而在 RDN 治疗组中，RDN 术后窦性过速时最高心率显著降低，分别为 271±10 及 254±13 bpm（交互作用 P = 0.03；图 7）。

讨论

本研究通过随机方法设置假手术对照，并采用盲法进行终点分析，猪模型中对 RDN 通过降低交感神经活性发挥抗心梗后心律失常作用这一假说进行了验证。研究证实 RDN 可降低 MI 后 3 周内自发性 VAs 的发生率。经 RDN 腹动脉，其外膜及外膜交感神经末梢发生损伤。在相当一部分研究中有效的交感神经末梢末梢发生未通过组织学方法得到证实，证实 RDN 后相关交感末梢损伤是本研究的重要原因之一。此外，本研究还证实 RDN 后心脏除交感末梢末梢密度减低外，还同时存在心肌 NPY 表达减少，窦性心动过速是平均心率及最高心率均减低等心脏交感神经活性降低的多种表现。这些发现为 MI 后 3 周内心肌基质改变及触发、维持 VAs 高发生率的机制阐明提供了基础。

RDN 对自发性及诱导性 VAs 发生的影响

主要是由于存活心肌传导纤维的传导速度减慢所致\[9\]，而诱发性心律失常大多是由于梗死后瘢痕形成所致，因此本研究中会出现RDN对自发性VAs和诱导性VAs具有不同作用的现象。综上，RDN通过降低自律性减少触发活动而不是通过改变基质来发挥其减少MI后VAs发生的作用。RDN通过降低交感神经效应，减少异位起搏所诱发的折返激动或对折返环的电生理特性发挥调节作用。

**RDN对心室ERP的影响**

在Huang等\[7\]对非疾病模型动物进行RDN的研究以及Gu等\[12\]进行的星状神经节消融相关研究中均显示，交感神经节消融会心室ERP延长。本研究中，RDN治疗组的心室ERP在RDN后由基线时的196±23 ms延长至247±23 ms，该延长时间长于Huang等\[7\]所报道的ERP延长时间（均值大约为15 ms）。但本研究中假手术组和RDN治疗组的ERP延长幅度无明显差异，这表明在本研究中RDN可能导致了ERP的延长，但该作用可能被ICD电极与心肌连接处发生的进行性纤维化所造成的ERP延长所掩盖。本研究中所使用的ICD电极进行再次消毒后可能会造成ICD电极头端的类固醇涂层的流失而失去防止心肌纤维化的作用。
研究证实，在房颤情况下，RDN 可将平均心室率降低 24%；在窦性心律时，可使心率平均降低 20 bpm，这与本研究中发现的 RDN 对心率的影响是一致的。在对难治性高血压进行 RDN 治疗的 2 项研究中，所报道的 RDN 对窦性心率的影响要微弱得多，一项研究中心率降低了 2-3 bpm[14]，另一项研究中降低了 4 bpm[15]。RDN 后平均及最高心率的降低，连同自发性 VAs 发生率的降低，可能均与肾交感神经传入纤维活性降低介导的全身交感活性减低有关[16]。心率加快可能会导致心率相关性心肌缺血以及折返性 VAs 的发生[17]。

心脏交感神经及 NPY 分布情况

本研究中，RDN 组梗死区交感神经较假手术组减少。之前有研究显示急性 MI 会导致损伤，而后交感神经通过出芽方式在局部呈不均衡生长并导致心肌组织中交感神经的过度支配[19]。急性 MI 后存在交感神经密度增加的患者 VAs 发生率较高[18]。对犬的研究中发现 NGF 注射可促进交感神经再生，并增加心源性猝死及 VAs 的发生率[18]。β 受体阻滞剂可防止 MI 后心源性猝死的发生[19]。本研究中未设计对梗死早期心脏神经改变的研究，但对 MI3 周后心肌组织分布进行了分析，并发现梗死后心源性猝死发生率显著降低，1 周后心源性猝死发生[20]。本研究中未设计对梗死早期心脏神经改变的研究，但对 MI3 周后心肌组织分布进行了分析，并发现梗死后心源性猝死发生率显著降低，1 周后心源性猝死发生[20]。本研究中未设计对梗死早期心脏神经改变的研究，但对 MI3 周后心肌组织分布进行了分析，并发现梗死后心源性猝死发生率显著降低，1 周后心源性猝死发生[20]。本研究中未设计对梗死早期心脏神经改变的研究，但对 MI3 周后心肌组织分布进行了分析，并发现梗死后心源性猝死发生率显著降低，1 周后心源性猝死发生[20]。本研究中未设计对梗死早期心脏神经改变的研究，但对 MI3 周后心肌组织分布进行了分析，并发现梗死后心源性猝死发生率显著降低，1 周后心源性猝死发生[20]。
研究发现，RDN后NPY含量较前降低[28]。且在本研究中，在梗死区，RDN后NPY含量变化与RDN后交感神经密度及活性的变化是一致的，这表明RDN后非梗死区交感神经活性也同时出现了减低。

RDN对交感神经的影响

本研究中，RDN治疗组神经外膜的有效神经微丝积分显著减低，而动脉外膜以外区域的有效神经微丝积分并无显著改变（这可能是由于本研究统计效力不足，不足以显示动脉外膜以外区域有效神经微丝积分的统计学差异所致）。与预期相同，仅在RDN治疗组存在神经束受损，但在手术组无神经束损伤，这表明神经束损伤并非由于肾动脉器械退化损伤所致，而是由于RF所致。RDN后7天，再生轴突仍处于再生过程中。再生轴突及离断轴突的固有释放神经物质的能力[29,30]。不同物种间神经轴突再生所需时间不同[31-33]。在本研究中，仅对RDN后7天的交感神经进行了组织学分析，故研究结果无法延伸至其他时间范围。这一时间因素可解释研究中动脉外膜外侧大肾交感神经分布区域有效神经微丝积分存在差异的问题。免疫组化结果也证实了交感神经消融与自发性心律失常发生率减低之间的联系。

由此得出结论，RDN降低MI后自发性VAs的发生，可能与降低MI后心脏交感神经活性有关。该抗MI后心律失常作用主要是通过减少触发活动，而非通过改变电生理特性来实现的。本研究中相关结果的测量均是通过设备记录客观量完成的，不存在主观偏差问题。本研究为心梗后通过RDN降低交感神经活性提供了新的视角，最主要的是提出了降低VAs发生率的药物-心轴。但其临床应用仍需临床随机对照研究来确认。

局限性

为确保动物的存活率，本研究采用了使左室射血分数（ejection fraction，EF）轻度下降，梗死面积相对较小的MI模型。尽管如此，由于目前临床中将相当一部分MI患者于早期接受PCI治疗，而并未发生或仅有轻度EF降低，使我们的研究结果仍具有一定临床参考价值。本研究的另一不足在于未与β受体阻滞剂疗效进行对照。在实际临床中，应用β受体阻滞剂会产生全身效应，此外药物疗效有赖于患者的依从性。而RDN治疗仅需MI后单次介入治疗即可完成。本研究对MI发生后前3周内，RDN对VAs的预防作用及降低心脏交感神经活性的作用进行了评估，接下来的研究将对更长时间内RDN作用的持续性进行评价。

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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 SYMPLICITY 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.

**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-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

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 antiarrhythmic 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-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
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 SYMPLECTITY 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 assessed 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 neuregion abundance of neuropeptide-Y in the infarct zone.

Studies 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-chamber 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-mm-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 ≥200 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 cannulated under fluoroscopic guidance. RDN was performed by delivering radiofrequency energy as described in Methods in the Data Supplement.
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 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.
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).
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.7 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 on 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 sympa-
thetic 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 hypoth-
thesis is also consistent with the evidence for 
β-blockade in 
preventing sudden cardiac death after MI.19 Linz et al dem-
onstrated 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 car-
diac sympathetic nerve sprouting.21

To explore the mechanism of the actions of RDN on car-
diac 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 despite a high level of NGF 3 weeks 
after MI induction suggests that RDN counteracts NGF-
mediated nerve growth in the infarcted heart. In infarcted 
heart failure models, NGF has been shown to be down-
regulated in response to sympathetic hyperactivity,22 and 
this can lead to decreased sympathetic nerve density in the 
failing heart. Similarly, RDN may have mitigated the sympa-
thetic hyperactivity seen after MI in our animal model 
and, therefore, attenuated the downregulation of NGF.

Injured nerves after interference of blood supply undergo 
Wallerian degeneration, which would be followed by axo-
nal regeneration.18,23 Necrosis takes place early in the infarc-
tion, which may be followed by nerve regeneration 1 week 
later.24 Our experimental protocol was not designed to study 
early changes, such as nerve necrosis; however, we studied 
myocardial nerves density 3 weeks after MI. The presence 
of fewer sympathetic nerves in the infarcted myocardium in 
our data supports the novel idea that RDN can prevent 
or suppress sympathetic hyperinnervation after infarction. 
This is an important finding as such adverse cardiac nerve 
development is known to be arrhythmogenic and is associ-
ated with sudden cardiac deaths.18 This normalization of 
sympathetic hyperactivity is consistent with previous find-
ings that RDN normalizes sympathetic nerve activity in 
hypertension.18 Our study corroborates this paradigm in that 
a catheter-based single intervention can reduce the magni-
tude of cardiac sympathetic nerve activity in a postinfarct 
model. For VA management, β-blockers are the first-line 
drugs.25 Though we did not study the effect of β-blocker in 
our experimental model, Linz et al26 has shown that RDN is 
as effective as β-blockade in reducing PVCs and VF. The 
potential advantage of RDN as an alternative to β-blocker 
would be the lack of compliance concerns, generalized 
effects, and long-term costs.

Increased tissue NPY abundance was previously associ-
ated 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.28 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 nonin-
farcted sites as well.
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 unhealthy 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 predominantly 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. Another limitation of the study was the absence of comparison with β-blockade. In practice, RDN would be a single intervention after MI, as opposed to medical β-blockade with systemic effects and variable compliance. The present study assessed the role of RDN on VAs and reduction of cardiac sympathetic nerves that took place during the first 3 weeks post-MI; further studies are needed to determine whether these effects persist beyond this time period.

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References


