Selective Renal Denervation Guided by Renal Nerve Stimulation in Canine

A Method for Identification of Optimal Ablation Target

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Abstract—Renal nerve stimulation (RNS) can result in substantial blood pressure (BP) elevation, and the change was significantly blunted when repeated stimulation after ablation. However, whether RNS could provide a meaningful renal nerve mapping for identification of optimal ablation targets in renal denervation (RDN) is not fully clear. Here, we compared the antihypertensive effects of selective RDN guided by two different BP responses to RNS and explored the nerve innervations at these sites in Kunming dogs. Our data indicated that ablation at strong-response sites showed a more systolic BP-lowering effect than at weak-response sites (P=0.002), as well as lower levels of tyrosine hydroxylase and norepinephrine in kidney and a greater reduction in plasma norepinephrine (P=0.004 for tyrosine hydroxylase, P=0.002 for both renal and plasma norepinephrine). Strong-response sites showed a greater total area and mean number of renal nerves than weak-response sites (P=0.012 for total area and P<0.001 for mean number). Systolic BP-elevation response to RNS before RDN and blunted systolic BP-elevation to RNS after RDN were correlated with systolic BP changes at 4 weeks follow-up (R=0.649; P=0.012 and R=0.643; P=0.013). Changes of plasma norepinephrine and renal norepinephrine levels at 4 weeks were also correlated with systolic BP changes at 4 weeks (R=0.837, P<0.001 and R=0.927, P<0.001). These data suggest that selective RDN at sites with strong BP-elevation response to RNS could lead to a more efficient RDN. RNS is an effective method to identify the nerve-enriched area during RDN procedure and improve the efficacy of RDN. **(*Hypertension*. 2019;74:536-545. DOI: 10.1161/HYPERTENSIONAHA.119.12680.)**

● **Online Data Supplement**

Key Words: blood pressure ■ denervation ■ dogs ■ hypertension ■ kidney

Catheter-based renal denervation (RDN) becomes a hot topic for hypertension treatment and is pursued in interventional cardiology since Krum et al[1] reported their very promising results.[2] However, disputation of this technique raised because of some negative results given by several sham-controlled studies.[3–5] These conflicting results inspired researchers to reappraise the limitations of previous studies and to improve the efficacy of RDN itself. Recent studies have confirmed that antihypertensive drugs need to be well controlled during RDN trials, and this confounding factor had huge impacts on the results.[6–8] More importantly, how to verify optimal ablation sites, assess successful renal nerve denervations during the procedure, and identify responsive hypertensive populations are urgently needed.[9,10] Both SPYRAL OFF-MED and ON-MED studies[6,8] have confirmed the moderate blood pressure (BP)–lowering effects (a reduction in office systolic BP [SBP] by 10 mm Hg and 24-hour ambulatory BP by 5.5 mm Hg from SPYRAL OFF-Med study) by numerous ablations (43.8/per patient). Even with the large number of ablations, >20% of patients were observed as either nonresponders or had elevated BP. Latest studies have provided solid anatomic and histological basis for renal nerve stimulation (RNS).[11–13] They demonstrated that renal nerves consisted of efferent sympathetic, parasympathetic, and afferent sensory fibers and distributed unequally both along the length and around the 4 circumferential quadrants of renal arteries. The physiological response of these nerve fibers to RNS are different: stimulations could lead to increase, decrease, and no changes in BP, respectively.[14–21] The BP response to RNS depends on the overall responses of these nerve fibers at this site. Although we still do not fully understand the mechanism of

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how the different types of nerves fibers around the renal arteries work together for the regulation of BP response to RNS, it should not prevent us from using integrated physiological approaches to distinguish the innervation sites with dominant BP-regulation function for selective denervation. RNS may provide a meaningful method for renal nerve mapping and selective RDN.[9,10] This method may improve the efficacy and safety with less ablations for more reduction in BP.[22] However, arguments on whether RNS is efficient enough to distinguish different innervations and why the whole bundle stimulation is able to identify optimal target sites for selective ablations exist. Consequently, the hypothesis which BP changes in response to RNS can be quantitatively measured and guide RDN therapy is brought up and to be tested. It helps not only to identify the nerve-enriched area but also to guide the interventionalists with recommended site for ablation.

Methods

The authors declare that all supporting data and techniques are available within the article and in the online-only Data Supplement; detailed data are available from the corresponding author on reasonable request.

Study Design

A total of 24 healthy adult Chinese Kunming dogs, weighed 25 to 35 kg, were enrolled in the present study and underwent bilateral renal arteriography. Anatomically eligible dogs were randomly assigned into 3 groups: strong-response sites (SRS) ablation (SRA) group, weak-response sites (WRS) ablation (WRA) group, and RNS-control group (RSC). The dogs in SRA group underwent RNS and ablation at SRS (defined as site with maximum SBP-elevation >10 mm Hg during RNS). The dogs in WRA group underwent RNS and ablation at WRS (defined as site with SBP-elevation ≤10 mm Hg during RNS). In RSC group, dogs underwent RNS but no ablation (Figure 1). Invasive femoral artery pressures were monitored, and blood samples were collected at baseline. At 4 weeks follow-up, all dogs were anesthetized and underwent a surface ECG and the second intervention procedure followed by invasive femoral artery pressure monitoring, renal artery angiography, and arterial blood sampling. Finally, dogs were euthanized with an overdose of intravenous sodium pentobarbital (200 mg/kg). Renal arteries with surrounding tissues and renal cortex were harvested for subsequent analysis.

Experimental Animal and Preparation

Chinese Kunming dog was chosen for the study because of its natural hypertension and high sympathetic tone. A series of preclinical studies involving catheter-based RDN technique have been successfully conducted on Chinese Kunming dogs by our team.[22–24] The experiment was approved by the Animal Experimentation Ethics Committee of the Chongqing Medical University, following the guidelines of the National Institutes of Health for the care and use of laboratory animals. Standard food and water feedings (online-only Data Supplement) were given by appointed person in Laboratory Animal Center of Chongqing Medical University throughout the experimental period.

The dogs were anesthetized with 3% sodium pentobarbital (dosage:30 mg/kg per dog) intraperitoneally, followed by a maintenance dose of intravenous 5 mg/(kg·h) via trace syringe pump. Penicillin was given intraperitoneally after the interventional procedures for the prevention of infection. Bilateral femoral arteries were punctured under sterile conditions, one for continuous BP monitoring and another for catheter accessing, then an amount of 2000 IU unfractionated heparin was administered. Surface ECG and invasive left femoral artery pressure were continuously monitored throughout the whole procedure by a Multichannel Electrophysiology Management System (Sichuan Jinjiang Electronic Science and Technology Corporation, Chengdu, China).

Bilateral renal arteriography via right femoral artery was performed in all dogs using a 6F JR4 Judkins catheter (Cordis Corporation, Miami, FL). The dog would be excluded from the study if renal arteriography reported any renal artery abnormalities, such as a severe distortion or stenosis in renal artery, diameter of renal artery under 4 mm (using inner diameter of 6F catheter as reference).

RNS to Map Renal Nerves and Guide Selective Denervation

RNS was applied from the distal (the bifurcation of renal artery) to the proximal (the ostium of renal artery) segments of the bilateral renal arteries via a renal artery dedicated open-irrigated ablation catheter (AquaSense, Synaptic Medical Limited, Beijing, China). Electrical stimulations[17,22] were delivered at the frequency of 20 Hz, energy output at 15 mA, and pulse duration of 2 ms for 60 s by using a Nerve and Muscle Stimulator (Sichuan Jinjiang Electronic Science and Technology Corporation, Chengdu, China). A total of 2 to 4 target sites were selected in each renal artery. RDN was performed immediately at target site when BP returned to baseline after RNS by setting the temperature at 45°C, radiofrequency energy of 10 W and duration of 90 s. The impedance, power, and temperature were monitored during the ablation procedure. Saline was irrigated at 3 to 5 mL/min to decrease the temperature of tissue-electrode interface during radiofrequency energy delivery in using the Vation-CoolPump (Sichuan Jinjiang Electronic Science and Technology Corporation, Chengdu, China). Repeated RNS were performed immediately when the BP returned to baseline after RDN. RNS and RDN were performed via the same renal artery dedicated open-irrigated ablation catheter (AquaSense, Synaptic Medical Limited, Beijing, China). The catheter was positioned stationary in a cycle of RNS-RDN-repeated RNS to ensure that stimulation and ablation were performed at the same site. After ending a cycle of RNS-RDN-Repeated RNS, we proceeded to next target site (Figure 1).

Assessments of Plasma Norepinephrine, Kidney Tissue Norepinephrine, and Tyrosine Hydroxylase

Four milliliters of baseline blood samples from the right femoral vein were collected in EDTA tubes to measure the plasma norepinephrine levels. After high-speed centrifugation, the plasma samples were stored at −80°C until assay. Four weeks after the interventional procedures, all dogs underwent invasive BP monitoring and bilateral renal arteriography via the femoral artery. Blood samples were collected and stored in the same way as above-mentioned. The plasma norepinephrine was assayed by ELISA kits (MB-5231A; Mei Biao Biological Technology, Co, Ltd, Jiangsu, China) according to the manufacturer's instructions.

Tissue specimens of renal cortex were harvested from all dogs and immediately frozen in liquid nitrogen until assay. The local norepinephrine in kidney was assayed by high-performance liquid chromatography. The expression of tyrosine hydroxylase (TH) in kidney was measured by Western blot (the detailed methods are presented in the online-only Data Supplement).

Determination the Nerve Innervations of Mapping Sites by Histology

Dogs were euthanized after completing all follow-up items, and bilateral renal arteries with surrounding tissues were harvested immediately and fixed in 10% neutral buffered formalin for at least 36 hours, then subjected to alcoholic dehydration and embedded in paraffin. Renal arteries were sectioned at ≈5-mm intervals from distal (bifurcation) to proximal (aorta) and cut into 4-μm slices with a total of 5 serial slices obtained from each section. Slices from each section were stained with Masson trichrome to locate the ablation area. Slices from RSC group were stained with appropriate markers to label renal efferent sympathetic (TH), afferent sensory (CGRP [calcitonin gene–related peptide]), and parasympathetic nerve fibers (nNOS [neuronal NO synthase]). The distance from the luminal surface of the renal arteries to each nerve, the area, and number of the nerves were measured with Image-ProPlus 6.0 (the detailed methods are presented in the online-only Data Supplement).

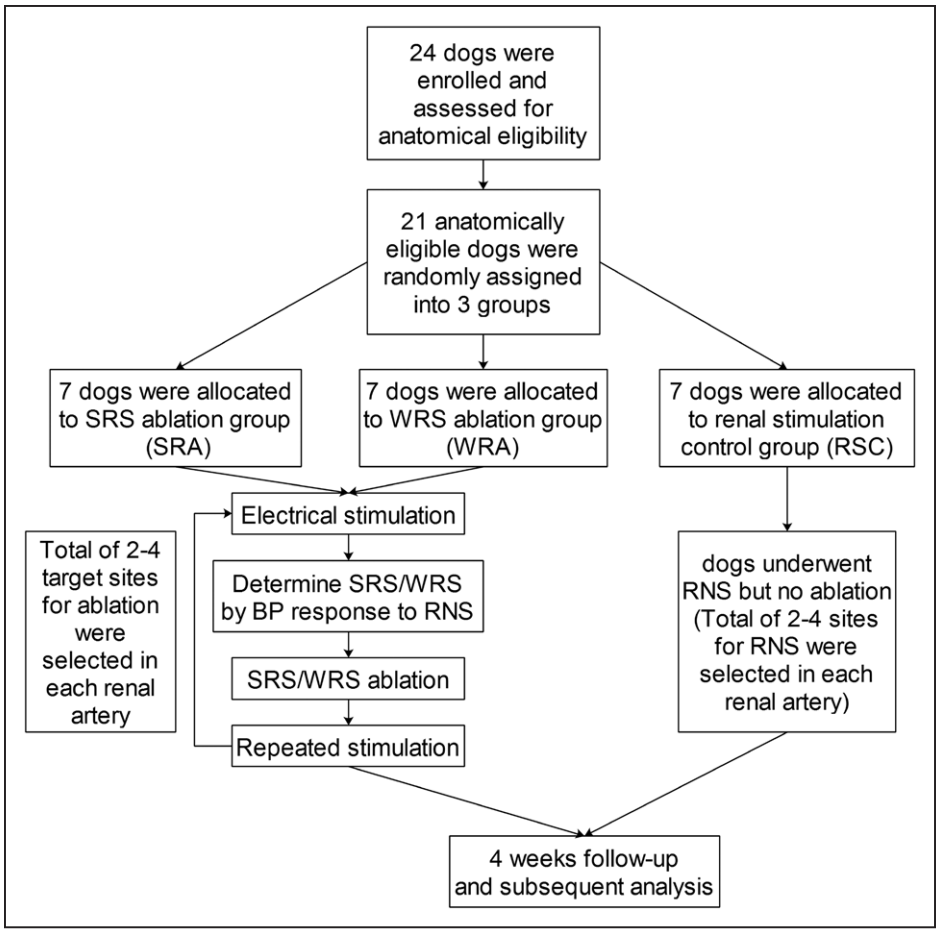

Figure 1. The flow chart of the procedure. RNS indicates renal nerve stimulation; RSC, RNS-control group; SRA, SRS ablation; SRS, strong-response site; WRA, WRS ablation; and WRS, weak-response site.

Statistics

Continuous variables were expressed as mean±SD or median with range when appropriate. Categorical variables were reported by frequencies and percentages. BP response in 1 dog was derived from the average BP response at all sites. The differences of variables among 3 groups were analyzed with the use of 1-way ANOVA, followed by post hoc analysis with least significant difference *t* test to evaluate the differences between individual variables. If homogeneity of variances was violated, the differences of variables among 3 groups were analyzed using Welch ANOVA, followed by post hoc analysis with Games-Howell test. ANCOVA was applied to adjust for baseline BP measurements. Comparison of variables before and after intervention was performed with paired *t* test, whereas unpaired *t* test was applied to the comparison of variables between 2 groups. Pearson correlation was used to assess the correlation between 2 continuous variables. Differences of mean SBP changes at SRS and WRS were analyzed by 2-way repeated measures ANOVA. Post hoc pairwise comparisons were applied when a significant interaction effect was observed. Two-sided $P<0.05$ was defined as statistical significances. All statistical analyses were performed with SPSS statistical software (version 23.0, Chicago, IL).

Results

A total of 21 out of 24 Chinese Kunming dogs (7 in SRA group, 7 in WRA group and 7 in RSC group) completed all the research items and were included in data analysis. No renal artery dissection, stenosis, or other complications associated with RNS and RDN procedure was observed, whereas 3 out of 24 were excluded because of their anatomic ineligible revealed by renal arteriography.

Distribution of Sites With Different BP Responses to RNS

A total of 156 available sites of RNS before RDN were attempted in SRA and WRA group. SRS was seen at 73 sites, and the distribution in the proximal, middle, and distal renal artery segments were 30 (41%), 24 (33%), and 19 (26%), respectively. In contrast, the WRS was seen at 83 sites, 17 (20%) were in the proximal renal artery segments, 27 (33%) in the middle, and 39 (47%) in the distal renal artery segments. Further description for procedural data of RNS was summarized in Table S1 in the online-only Data Supplement.

In addition, 41 of 156 sites (26 in SRA and 15 in WRA group) showed a BP drop up to −10.7±6.6 mm Hg during the first several seconds and BP elevation during the remaining RNS procedure (Figure S1A). Our classification principle for these sites was based on the maximum SBP response during RNS. Nine sites in 3 dogs showed a continuous drop in BP response to RNS, which were not included in the analysis. And ablation was not conducted because of the limited quantities. (Figure S1B).

BP Response to RNS Before and After RDN

Before RDN, RNS increased the mean SBP of SRS by −0.4±6.8, 7.2±1.7, 8.4±3.6, 11.9±4.3, 16.4±5.1, and 17.9±8.1 mm Hg ($P=0.891$, $P=0.003$, $P=0.001$, $P<0.001$, $P<0.001$,

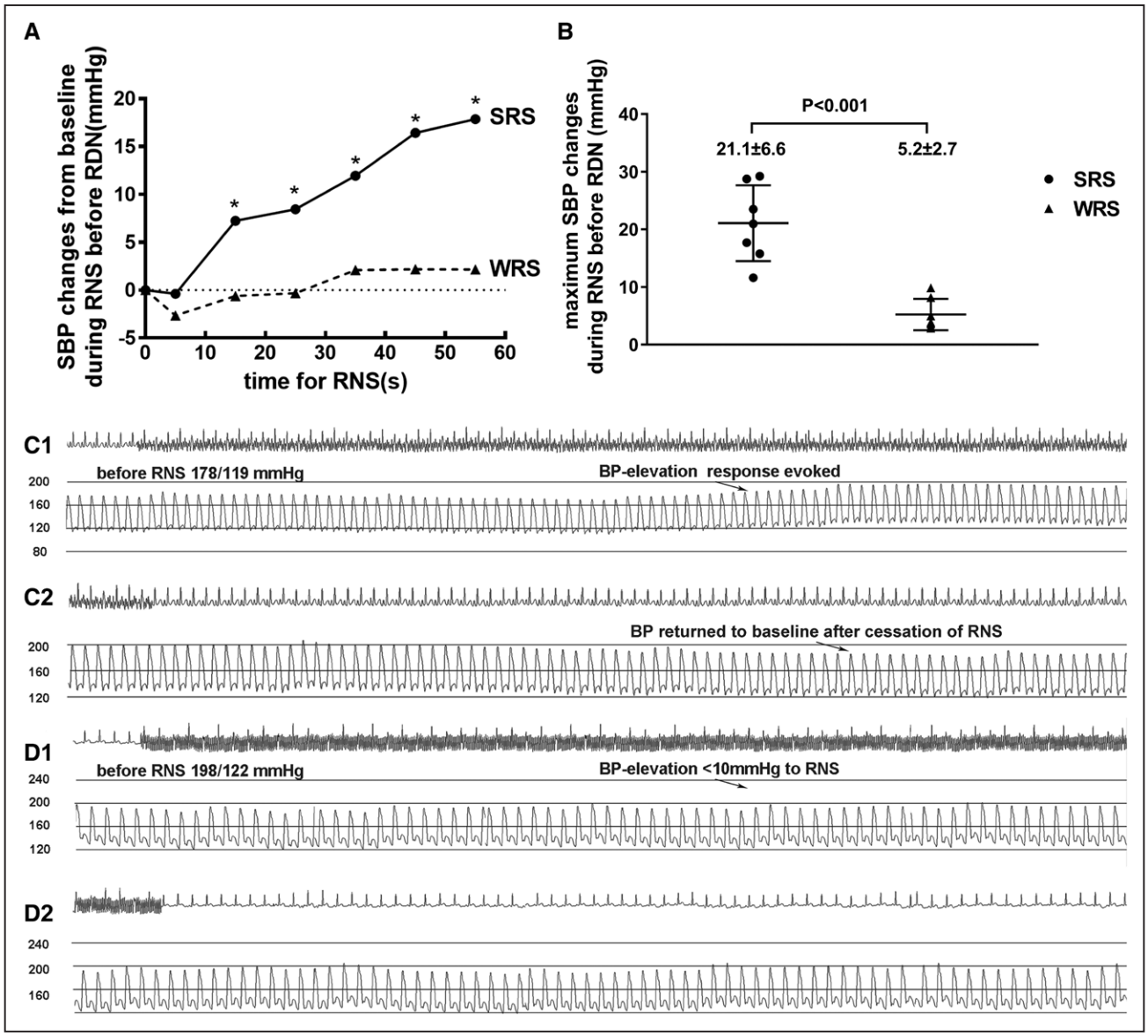

Figure 2. Blood pressure (BP) response to renal nerve stimulation (RNS) at strong-response site (SRS) and weak-response site (WRS). **A**, BP response to RNS at SRS and WRS, **P*<0.05 for SRS vs WRS at this period of time. **B**, Maximum BP changes between SRS and WRS. **C** and **D**, Representative image for the BP changes during RNS; **C** for SRS and **D** for WRS. Differences of systolic BP (SBP) changes were analyzed by 2-way repeated measures ANOVA. Post hoc pairwise comparisons were applied when a significant interaction effect was observed. Comparison of maximum BP changes and mean SBP changes between SRS and WRS were performed using the unpaired *t* test. RDN indicates renal denervation.

P<0.001, respectively; Figure 2A, 2C1 and 2C2) at the first, second, third, fourth, fifth, and sixth 10 s during RNS. Similarly, the mean SBP of WRS were increased by −4.3±5.5, −0.6±5.0, −0.3±3.7, 1.9±4.0, 2.2±5.2, and 2.0±4.0 mm Hg (*P*>0.05 for all; Figure 2A, 2D1, and 2D2). Statistically significant differences were found between SRS and WRS at the second, third, fourth, fifth, and sixth 10 s during RNS (*P*=0.009, *P*=0.003, *P*=0.003, *P*=0.001, and *P*=0.002).

For the maximum BP changes, in SRA group, before RDN, RNS increased the SBP/ diastolic BP (DBP) from 181.1±16.9/112.8±12.0 to 201.7±15.7/121.7±13.8 mm Hg (*P*<0.001/*P*=0.001). After RDN, SBP and DBP in response to RNS increased from 183.7±15.3/116.3±11.4 to 190.9±16.1/119.4±11.6 mm Hg (*P*=0.017/*P*=0.116). In WRA group, before RDN, RNS increased the SBP/DBP from 194.2±18.6/122.6±26.5 to 199.4±17.8/125.8±27.7 mm Hg (*P*=0.002/*P*=0.030). After RDN, SBP/ DBP in response to RNS increased from 193.7±16.8/121.8±28.6 to 198.1±16.4/125.8±29.4 mm Hg (*P*=0.004/*P*=0.016).

Before RDN, greater SBP-elevation to RNS was observed in SRA group than in WRA group (21.1±6.6 versus 5.2±2.7 mm Hg, *P*<0.001, Figure 2B). After RDN, the SBP-elevation in response to RNS was significantly blunted (8.2±4.5 versus 21.1±6.6 mm Hg, *P*=0.001) in SRA group. Comparison of pre- and post-RDN, DBP in SRA group and SBP in WRA group showed a similar trend (8.9±4.0 versus 3.1±4.5 mm Hg, *P*=0.009 and 5.2±2.7 versus 4.4±2.6 mm Hg, *P*=0.003; Figure 3A, 3B, and 3C).

Consistent with the BP response to RNS, an immediate and substantial SBP-elevation response was also seen during

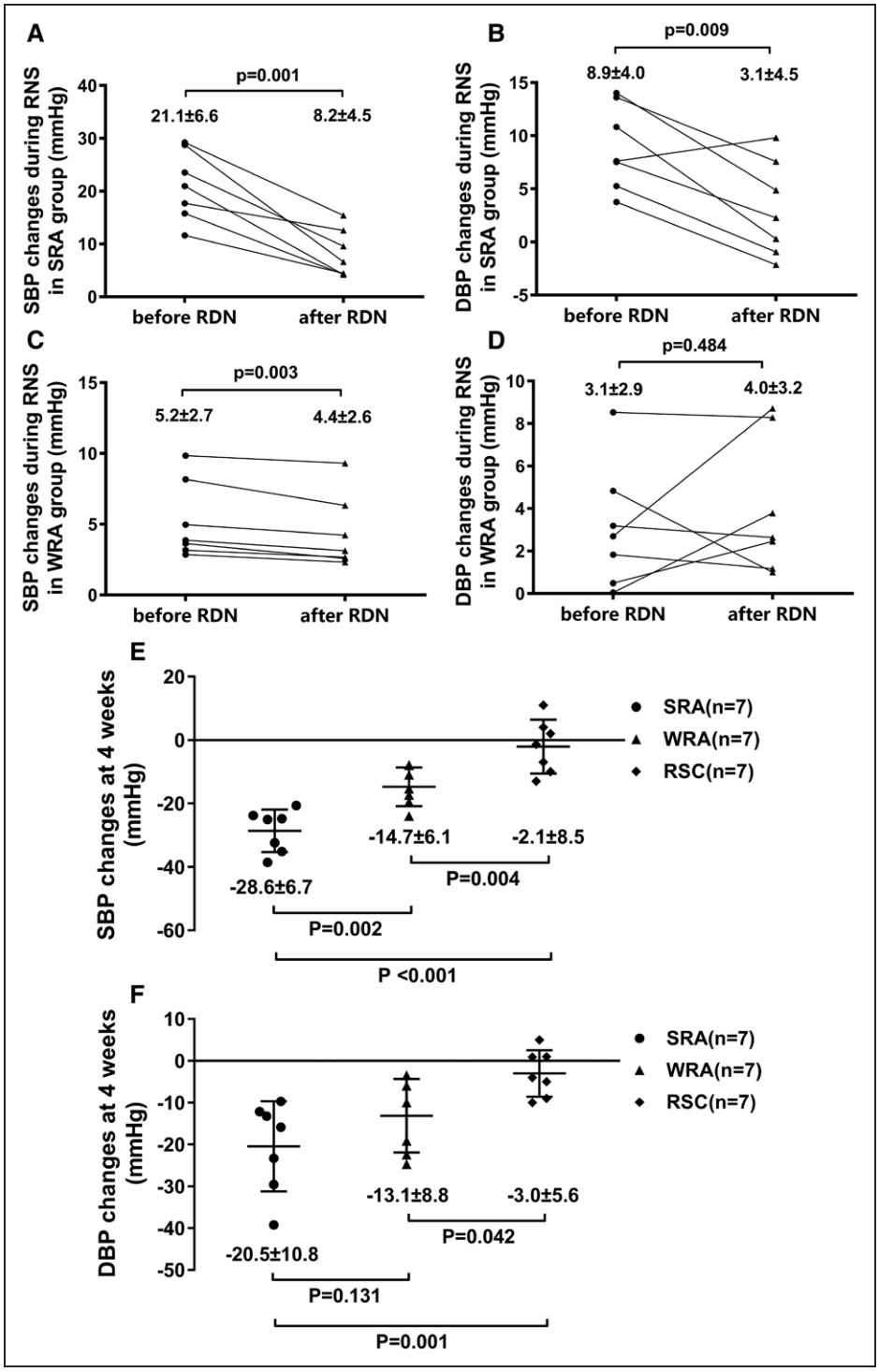

Figure 3. Blood pressure (BP) changes to renal nerve stimulation (RNS) before and after renal denervation (RDN); BP reduction at 4 wk among groups. **A** and **B**, BP response to RNS before and after RDN in strong-response site ablation (SRA) group. **C** and **D**, BP response to RNS before and after RDN in weak-response site ablation (WRA) group. **E** and **F**, Changes of BP at 4 wk after RDN. The differences of BP to RNS before and after RDN were analyzed with the use of the paired *t* test. The differences of BP among 3 groups were analyzed with the use of 1-way ANOVA, followed by post hoc analysis with least significant difference *t* test to evaluate the differences between individual variables. DBP indicates diastolic BP; RSC, RNS-control group; and SBP, systolic BP.

radiofrequency energy delivery. SBP-elevation response in RDN period was higher than that in RNS period (in SRA group, 28.6±11.7 versus 21.1±6.6 mm Hg, $P<0.005$; in WRA group, 17.0±9.3 versus 5.2±2.7 mm Hg, $P=0.008$). SBP changes during RDN were correlated with SBP-elevation response to RNS ($R=0.745$, $P=0.002$; Figure 5D).

BP-Lowering Effect of RDN Between SRA Group and WRA Group

RDN was successfully conducted in 14 dogs guided by the BP response to RNS. No significant differences were observed ($P=0.535$) between the SRA and WRA group in the number of ablation sites (Table S1). Impedance decreased from 211±18

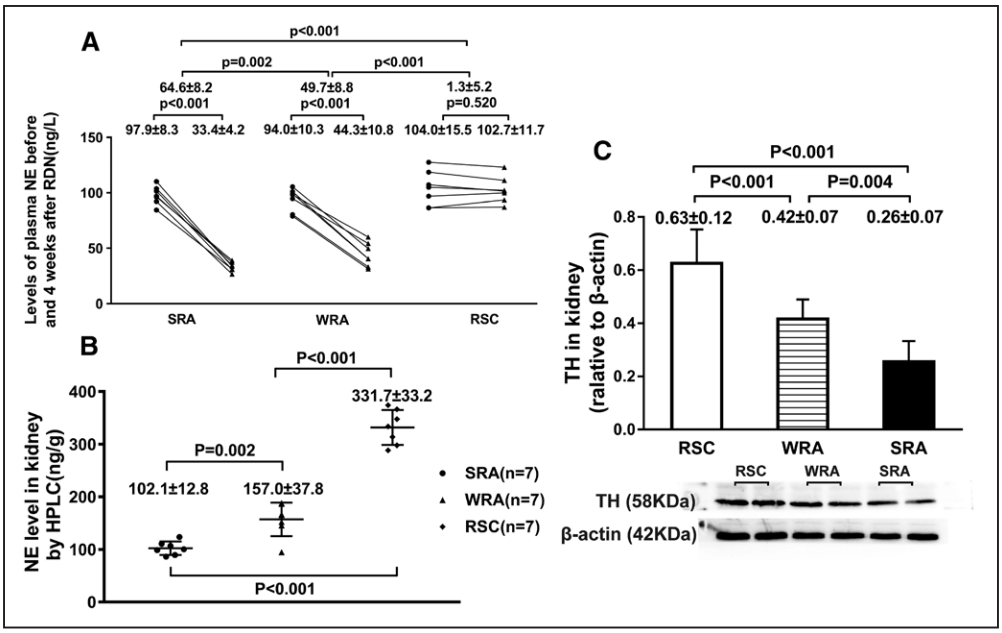

Figure 4. Levels of plasma norepinephrine (NE), renal NE, and renal tyrosine hydroxylase (TH). **A**, Levels of plasma NE before and 4 wk after renal denervation (RDN) among groups, bold dot for baseline and triangle for 4 wk follow-up. **B**, Levels of NE in kidney at 4 wk among groups. **C**, Protein expression levels of TH in kidney determined by Western blot analysis; comparison of plasma NE before and 4 wk after RDN with use of the paired *t* test. The differences of changes of value among 3 groups were analyzed with the use of 1-way ANOVA, followed by post hoc analysis with least significant difference *t* test to evaluate the differences between individual variables. HPLC indicates high-performance liquid chromatography; RSC, RNS-control group; SRA, strong-response site ablation; and WRA, weak-response site ablation.

before to 190±19 ohm after RDN (P<0.001). There was no significant difference in the changes of impedance before and after RDN between the SRA and WRA group (−21±8 versus −23±6 ohm, P=0.471).

No significant differences showed at baseline among groups in SBP (189.6±23.5 versus 194.9±21.6 versus 210.6±18.8 for SRA versus WRA versus RSC, P=0.188) and DBP (114.7±12.2 versus 111.9±18.3 versus 125.4±18.7 for SRA versus WRA versus RSC, P=0.304). However, the SBP/DBP in SRA group and WRA group were significantly decreased at 4 weeks after interventions, comparing with that in RSC group (SBP/DBP: 28.6±6.7/20.5±10.8 versus 2.1±8.5/3.0±5.6 mm Hg, P<0.001/P=0.001 for SRA versus RSC group and 14.7±6.1/13.1±8.8 versus 2.1±8.5/3.0±5.6 mm Hg, P=0.004/P=0.042 for WRA versus RSC group). In addition, the reduction of SBP in SRA group was greater than that in WRA group (28.6±6.7 versus 14.7±6.1 mm Hg, P=0.002; Figure 3E and 3F). Comparison of the 4 weeks change between the groups, adjusted for baseline measures by use of ANCOVA, showed similar differences for SBP and DBP (SBP/DBP: 29.9±6.9/21.2±7.4 versus 15.2±6.8/14.7±7.5 versus 0.4±7.1/0.7±7.7 for SRA versus WRA versus RSC; P=0.001/P=0.118 for SRA versus WRA; P=0.001/P=0.004 for WRA versus RSC and P<0.001/P<0.001 for SRA versus RSC).

Levels of Plasma Norepinephrine Before and 4 Weeks After RDN

The plasma norepinephrine concentrations at baseline showed no significant differences among groups (P=0.300). Both SRA and WRA group showed marked reductions in plasma norepinephrine at 4 weeks after RDN (97.9±8.3 at baseline to 33.4±4.2 ng/L at 4 weeks, P<0.001 in SRA group and 94.0±10.3 at baseline to 44.3±10.8 ng/L at 4 weeks, P<0.001 in WRA group; Figure 4A).

In addition, the reduction of plasma norepinephrine in SRA group was greater than that in WRA group (64.6±8.2 versus 49.7±8.8 ng/L, P=0.002; Figure 4A). The reduction of plasma norepinephrine was significantly correlated with SBP reduction after 4 weeks (R=0.837, P<0.001; Figure 5F).

Levels of Norepinephrine and TH in Kidney at 4 Weeks Follow-Up

As shown in Figure 4B, the cortical norepinephrine concentrations were significantly lower in SRA group (102.1±12.8 ng/g, P<0.001) and WRA group (157.0±37.8 ng/g, P<0.001) compared with RSC group (331.7±33.2 ng/g) at 4 weeks follow-up. The level of norepinephrine concentrations in SRA group was lower than that in WRA group (102.1±12.8 versus 157.0±37.8 ng/g, P=0.002). Furthermore, renal cortical norepinephrine concentrations were significantly correlated with SBP reduction after 4 weeks (R=0.927, P<0.001; Figure 5E).

The significantly lowering protein expressions of TH in kidney in SRA group and WRA group were observed in comparison with RSC group (P<0.001 for both). In addition, the expression of TH in SRA group was much lower than in WRA group (P=0.004; Figure 4C).

Correlation Between the BP-Elevation Response to RNS and BP-Lowering Effect of RDN

As shown in Figure 5, the SBP changes at 4 weeks were correlated with SBP-elevation response to RNS before RDN (R=0.649; P=0.012; BP-elevation response in 1 dog were derived from the average maximal BP response at all sites).

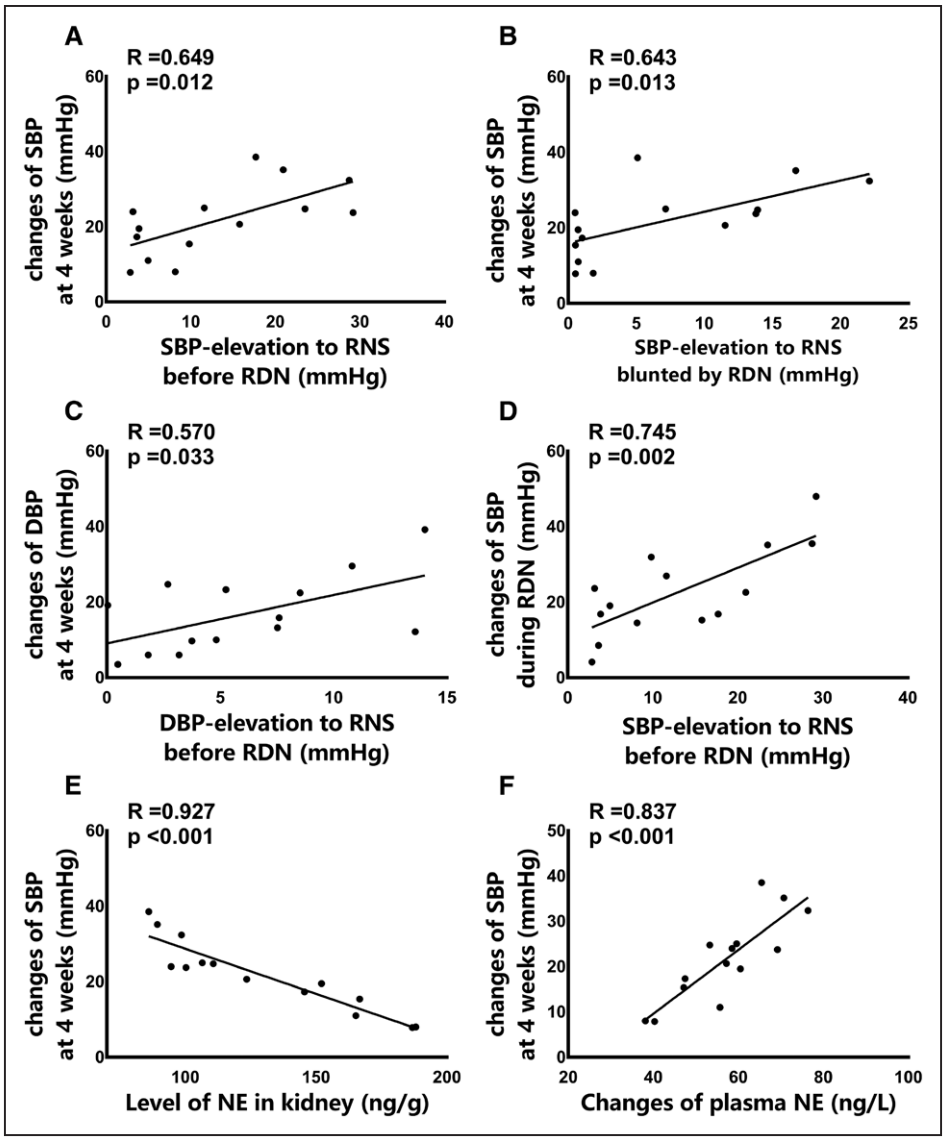

Figure 5. Correlation analysis on blood pressure lowering effect and blood pressure response to renal nerve stimulation, renal norepinephrine, plasma norepinephrine. **A**, Correlation between systolic blood pressure (SBP) changes at 4 wk follow-up and SBP-elevation response to renal nerve stimulation before renal denervation (RDN). **B**, Correlation between SBP changes at 4 wk and SBP-elevation to RNS blunted by RDN. **C**, Correlation between diastolic BP (DBP) changes at 4 wk and DBP-elevation response to RNS before RDN. **D**, Correlation between SBP changes during RDN and SBP-elevation response to RNS before RDN. **E**, Relationship between levels of norepinephrine in kidney and SBP changes at 4 wk. **F**, Relationship between changes of plasma NE and SBP changes at 4 wk. Pearson correlation was used to assess the correlation. NE indicates norepinephrine, and RNS, renal nerve stimulation.

These findings were seen in DBP as well (R=0.570; P=0.033). What is more, SBP changes at 4 weeks were also correlated with blunted SBP-elevation to RNS after RDN (the difference between RNS-induced BP elevation before and after RDN at the same site; R=0.643; P=0.013).

Nerve Innervations at SRS and WRS

As shown in Figure 6, hyperplasia of collagen fibers (colored blue) was found in ablated areas. Changes of renal artery in Masson trichrome stain could be used to locate the mapping and ablating site. The total area of renal nerves in SRS was larger than that in WRS (0.65±0.34 versus 0.27±0.17 mm^2, P=0.012). At the same time, SRS had greater mean number of nerves than WRS (9.2±1.9 versus 3.1±1.4, P<0.001). However, no statistical difference was found in mean distance from lumen to nerve between SRS and WRS (2.26±0.89 versus 1.72±0.52 mm, P=0.255).

Also, markers for renal efferent sympathetic nerves (labeled by TH), renal sensory afferent nerves (labeled by CGRP), and parasympathetic nerve fibers (labeled by nNOS) were positive in renal nerve bundles. Renal efferent sympathetic nerves were predominant among these 3 nerve types (Figures S2 and S3).

Discussion

The current study compared the BP-lowering effects of selective RDN guided by two different BP responses to RNS and explored the nerve innervations at sites with different BP changes. The main findings were as follows: (1) Ablation at SRS showed a more superior BP-lowering effect than at

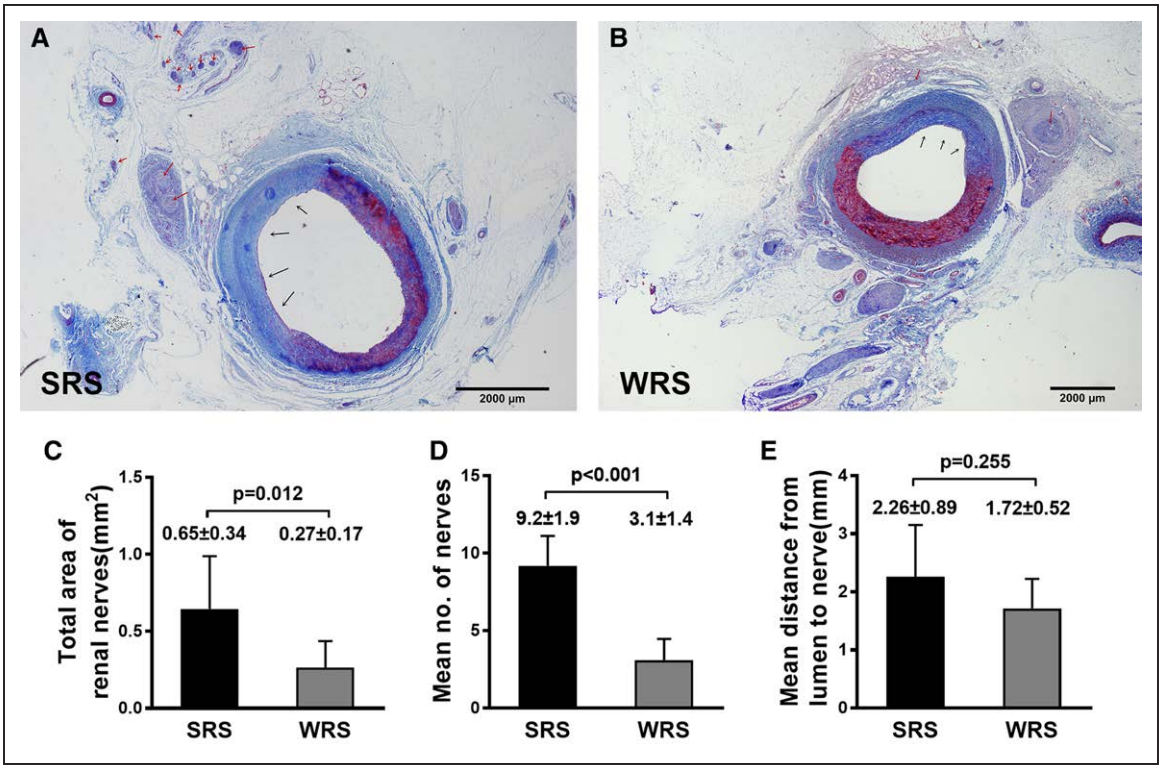

Figure 6. Difference in nerve distribution between strong-response site (SRS) and weak-response site (WRS). **A** and **B**, Representative Masson staining image for SRS and WRS. The red arrows indicated renal nerve bundle, and the black arrows indicated ablation area. Comparison of variables between 2 groups with use of the unpaired *t* test.

WRS, as well as a lower level of TH and norepinephrine in kidney and a greater reduction in plasma norepinephrine. (2) SRS showed a greater total area and mean number of renal nerves than WRS, and (3) SBP-elevation response to RNS before RDN and blunted SBP-elevation to RNS after RDN were correlated with the SBP changes at 4 weeks follow-up.

Our study extended the previous results from the following 3 aspects: first, the hypothesis that RNS may provide a useful method for renal nerve mapping was proved histologically. Second, RDN was performed under the guidance of BP responses to RNS rather than the unselected RDN in previous studies. Third, comparison of the BP-lowering effect of ablation at sites with two different BP responses to RNS was made.

The more superior BP-lowering effects and greater plasma norepinephrine reduction in SRA group were most likely due to destructions of more renal nerves. In this regard, our study revealed greater total area and mean number of renal nerves at SRS than those at WRS. In other word, we agreed RNS can outline the anatomic basis for mapping the nerve-enriched sites. Referring to the finding reported by Sakaoka et al,[25] radiofrequency energy of 10 W for 90 s in our study was sufficient to destroy most of the renal nerves. To perform ablation at nerve-enriched site indicated the opportunity to destroy more nerves. Additionally, renal norepinephrine levels have been correlated with ablation-induced neural injuries.[26] TH was the rate-limiting enzyme in catecholamine synthesis and was regarded as indicative of efficient ablation. We documented a lower norepinephrine concentration and TH expression in renal tissue in SRA than in WRA group. Thus, to perform ablation at sites with strong BP-elevation response to RNS could lead to a more efficient RDN.

Previous studies revealed significant variation of BP-lowering effects after unselected RDN.[1–3] Especially, a few studies showed no BP reduction or even had BP elevation after RDN.[6,8] However, our study showed a significant BP-lowering effect both in SRA group and WRA group. We speculated that the variation of BP-lowering effect may be related to ablation of confounding sites which should not be ablated.

Abundant evidence, including our data, have demonstrated that renal nerves consist of renal efferent sympathetic, afferent sensory and parasympathetic nerve fibers.[11–13,27] Destruction of efferent sympathetic nerves can result in a significant reduction of water and sodium reabsorption and also inhibition of renal renin-angiotensin system overactivation.[28,29] Renal afferent nerves are associated with central sympathetic outflow to peripheral circulation system.[30] Injury of both renal efferent sympathetic and afferent nerves was beneficial for the treatment of hypertension. However, the physiological function of parasympathetic nerves remained unclear to date.

This study showed that RNS can result in substantial BP elevation, and the change was significantly blunted when repeated stimulation after ablation, which was consistent with the findings of published researches.[21] But still, the underlying pathophysiological mechanisms of BP response to RNS have not been fully delineated yet. The majority of researches[10,31,32] revealed that RNS-induced BP changes are due to an increase in the central sympathetic tone through sympathoexcitatory renal afferent pathways. First, this immediate and substantial BP-elevation response occurred at 10 to 20 s after RNS started and accompanied

by a widespread vasoconstriction in both visceral and musculocutaneous beds, which suggests the activation of renal afferent fibers could elicit a reflex increase in efferent sympathetic activity. Second, increased serum catecholamine and the heart rate variability during RNS and upregulated cardiac autonomic nervous activity after RNS also reflected an increase in systemic sympathetic nervous activity via stimulation of renal afferent pathway. Finally, stimulation of renal efferent nerves potentially increased arterial pressure secondary to the increased tubular sodium reabsorption, renin secretion, or renal vascular resistance. Theoretically, activation of renal efferent nerve would take more time to induce the BP-elevation response.

Moreover, in accordance with the report from de Jong et al[19] and Murai et al,[20] our study also observed a continuous BP decrease during RNS at a few sites. This may relate to the activation of the sympatho-inhibitive renal afferent nerve or parasympathetic nerve fibers by RNS.[10,19] We speculated that ablation at these sites showing a BP drop response to RNS may not be beneficial to BP reduction. This may help to explain the variation of BP-lowering effect after unselected RDN. Thus, RNS may be able to identify the suggested ablation site, as well as the nonrecommended ablation site.

We think that the effects of electric stimulation on BP are integrated results from the dominant components of nerve fibers in the bundle; ablations of these sites should cause corresponding effects on BP depending on the dominated type of nerve fibers on the sites. Probably the sites with strong BP-elevation response to RNS indicated that the predominance of enriched efferent sympathetic or sensory afferent area should be the ideal target site for ablation.

Consistent with the clinical research conducted by de Jong et al,[17] the present study showed that the SBP changes at 4 weeks after RDN were correlated with SBP-elevation response to RNS before RDN. In other word, the BP-elevation response to RNS may be a promising marker for the prediction of BP-lowering effect after RDN. Moreover, the correlation between antihypertensive response and blunted SBP-elevation to RNS could provide a reasonable end point for RDN procedure, that is, ablation procedure would be terminated when there was no BP response to repeated RNS.

It should be noted that the average number of ablation sites in each dog is 6 (range 5–8). This number is much lower than previous research on unselected RDN.[1–3,6,8] Although RDN in the current trials appeared to be safe, the increased the number of ablation sites may be associated with potential risk of acute and chronic injury of renal arteries. Selective RDN guided by RNS could reduce excessive ablations and the risk of therapy-related side effect without weakening the BP-lowering effect.

Limitations

Despite the advantage of selective RDN, three limitations need to be considered. First, the working principle of different nerve fibers around renal arteries for the regulation of BP response to RNS needs more histological evidence. However, to quantitate the specific nerve types at the ablation sites was an elusive goal due to the low/no expression of nerve markers after ablation in this study (Figure S4). Second, the effect of ablating certain sites of continuous BP drop response to RNS was not analyzed due to the limited number of these sites. Third, the present study was conducted in animals with natural high BP. Considering the differences in neuroanatomy between humans and animals, the conclusion of present study should be proved by well-designed clinical trials in human.

In conclusion, selective RDN at sites with strong BP-elevation response to RNS could lead to more efficient RDN. RNS is an effective method to identify the nerve-enriched area during RDN procedure and to improve the efficacy of RDN.

Perspectives

Anatomic Basis for Nerve Mapping

This study demonstrates that strong BP-elevation response to RNS is indicative of local abundance of nerves. The hypothesis that RNS may provide a useful method for renal nerve mapping was proved histologically.

Identification of Optimal Ablation Target

Ablation at SRS showed a more superior BP-lowering effect than at WRS, as well as a lower level of TH and norepinephrine in kidney and a greater reduction in plasma norepinephrine. These results provide insights for interventionalists to more effectively identify the optimal ablation target. In our speculation that different BP responses to RNS indicated the difference of overall responses to different nerve types at these sites. RNS, one integrated physiological approach, would be a promising method for distinguishing the innervation sites with dominant BP-regulation function for selective denervation.

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Disclosures

None.

References

1. Krum H, Schlaich M, Whitbourn R, Sobotka PA, Sadowski J, Bartus K, Kapelak B, Walton A, Sievert H, Thambar S, Abraham WT, Esler M. Catheter-based renal sympathetic denervation for resistant hypertension: a multicentre safety and proof-of-principle cohort study. *Lancet*. 2009;373:1275–1281. doi: 10.1016/S0140-6736(09)60566-3
2. Esler MD, Krum H, Sobotka PA, Schlaich MP, Schmieder RE, Böhm M. Renal sympathetic denervation in patients with treatment-resistant hypertension (The Symplicity HTN-2 Trial): a randomised controlled trial. *Lancet*. 2010;376:1903–1909. doi: 10.1016/S0140-6736(10)62039-9
3. Bhatt DL, Kandzari DE, O'Neill WW, D'Agostino R, Flack JM, Katzen BT, Leon MB, Liu M, Mauri L, Negoita M, Cohen SA, Oparil S, Rocha-Singh K, Townsend RR, Bakris GL; SYMPLICITY HTN-3 Investigators. A controlled trial of renal denervation for resistant hypertension. *N Engl J Med*. 2014;370:1393–1401. doi: 10.1056/NEJMoa1402670
4. Desch S, Okon T, Heinemann D, Kulle K, Röhnert K, Sonnabend M, Petzold M, Müller U, Schuler G, Eitel I, Thiele H, Lurz P. Randomized sham-controlled trial of renal sympathetic denervation in mild resistant hypertension. *Hypertension*. 2015;65:1202–1208. doi: 10.1161/HYPERTENSIONAHA.115.05283
5. Mathiassen ON, Vase H, Bech JN, et al. Renal denervation in treatment-resistant essential hypertension. A randomized, SHAM-controlled, double-blinded 24-h blood pressure-based trial. *J Hypertens*. 2016;34:1639–1647. doi: 10.1097/HJH.0000000000000977
6. Townsend RR, Mahfoud F, Kandzari DE, et al; SPYRAL HTN-OFF MED trial Investigators*. Catheter-based renal denervation in patients with uncontrolled hypertension in the absence of antihypertensive medications (SPYRAL HTN-OFF MED): a randomised, sham-controlled,

proof-of-concept trial. *Lancet*. 2017;390:2160–2170. doi: 10.1016/S0140-6736(17)32281-X
7. Barber-Chamoux N, Esler MD. Predictive factors for successful renal denervation: should we use them in clinical trials? *Eur J Clin Invest*. 2017;47:860–867. doi: 10.1111/eci.12792
8. Kandzari DE, Böhm M, Mahfoud F, Townsend RR, Weber MA, Pocock S, Tsioufis K, Tousoulis D, Choi JW, East C, Brar S, Cohen SA, Fahy M, Pilcher G, Kario K; SPYRAL HTN-ON MED Trial Investigators. Effect of renal denervation on blood pressure in the presence of antihypertensive drugs: 6-month efficacy and safety results from the SPYRAL HTN-ON MED proof-of-concept randomised trial. *Lancet*. 2018;391:2346–2355. doi: 10.1016/S0140-6736(18)30951-6
9. Mahfoud F, Schlaich M, Böhm M, Esler M, Lüscher TF. Catheter-based renal denervation: the next chapter begins. *Eur Heart J*. 2018;39:4144–4149. doi: 10.1093/eurheartj/ehy584
10. Fudim M, Sobotka AA, Yin YH, Wang JW, Levin H, Esler M, Wang J, Sobotka PA. Selective vs. global renal denervation: a case for less is more. *Curr Hypertens Rep*. 2018;20:37. doi: 10.1007/s11906-018-0838-2
11. van Amsterdam WA, Blankestijn PJ, Goldschmeding R, Bleys RL. The morphological substrate for renal denervation: nerve distribution patterns and parasympathetic nerves. A post-mortem histological study. *Ann Anat*. 2016;204:71–79. doi: 10.1016/j.aanat.2015.11.004
12. Mompeo B, Maranillo E, Garcia-Touchard A, Larkin T, Sanudo J. The gross anatomy of the renal sympathetic nerves revisited. *Clin Anat*. 2016;29:660–664. doi: 10.1002/ca.22720
13. Sakakura K, Ladich E, Cheng Q, Otsuka F, Yahagi K, Fowler DR, Kolodgie FD, Virmani R, Joner M. Anatomic assessment of sympathetic peri-arterial renal nerves in man. *J Am Coll Cardiol*. 2014;64:635–643. doi: 10.1016/j.jacc.2014.03.059
14. Hilbert S, Kosiuk J, Hindricks G, Bollmann A. Blood pressure and autonomic responses to electrical stimulation of the renal arterial nerves before and after ablation of the renal artery. *Int J Cardiol*. 2014;177:669–671. doi: 10.1016/j.ijcard.2014.09.168
15. Gal P, de Jong MR, Smit JJ, Adiyaman A, Staessen JA, Elvan A. Blood pressure response to renal nerve stimulation in patients undergoing renal denervation: a feasibility study. *J Hum Hypertens*. 2015;29:292–295. doi: 10.1038/jhh.2014.91
16. Chinushi M, Suzuki K, Saitoh O, Furushima H, Iijima K, Izumi D, Sato A, Sugai M, Iwafuchi M. Electrical stimulation-based evaluation for functional modification of renal autonomic nerve activities induced by catheter ablation. *Heart Rhythm*. 2016;13:1707–1715. doi: 10.1016/j.hrthm.2016.04.021
17. de Jong MR, Adiyaman A, Gal P, Smit JJ, Delnoy PP, Heeg JE, van Hasselt BA, Lau EO, Persu A, Staessen JA, Ramdat Misier AR, Steinberg JS, Elvan A. Renal nerve stimulation-induced blood pressure changes predict ambulatory blood pressure response after renal denervation. *Hypertension*. 2016;68:707–714. doi: 10.1161/HYPERTENSIONAHA.116.07492
18. de Jong MR, Hoogerwaard AF, Gal P, Adiyaman A, Smit JJ, Delnoy PP, Ramdat Misier AR, van Hasselt BA, Heeg JE, le Polain de Waroux JB, Lau EO, Staessen JA, Persu A, Elvan A. Persistent increase in blood pressure after renal nerve stimulation in accessory renal arteries after sympathetic renal denervation. *Hypertension*. 2016;67:1211–1217. doi: 10.1161/HYPERTENSIONAHA.115.06604
19. de Jong MR, Hoogerwaard AF, Adiyaman A, Smit JJJ, Heeg JE, van Hasselt BAAM, Misier ARR, Elvan A. Renal nerve stimulation identifies aorticorenal innervation and prevents inadvertent ablation of vagal nerves during renal denervation. *Blood Press*. 2018;27:271–279. doi: 10.1080/08037051.2018.1463817
20. Murai H, Okuyama Y, Sakata Y, Kaneko S, Hamaoka T, Okabe Y, Usui S, Furusho H, Takamura M. Different responses of arterial blood pressure to electrical stimulation of the renal artery in patients with resistant hypertension. *Int J Cardiol*. 2015;190:296–298. doi: 10.1016/j.ijcard.2015.04.196
21. Chinushi M, Izumi D, Iijima K, Suzuki K, Furushima H, Saitoh O, Furuta Y, Aizawa Y, Iwafuchi M. Blood pressure and autonomic responses to electrical stimulation of the renal arterial nerves before and after ablation of the renal artery. *Hypertension*. 2013;61:450–456. doi: 10.1161/HYPERTENSIONAHA.111.00095
22. Lu J, Wang Z, Zhou T, Chen S, Chen W, Du H, Tan Z, Yang H, Hu X, Liu C, Ling Z, Liu Z, Zrenner B, Woo K, Yin Y. Selective proximal renal denervation guided by autonomic responses evoked via high-frequency stimulation in a preclinical canine model. *Circ Cardiovasc Interv*. 2015. 8(6).
23. Chen W, Du H, Lu J, Ling Z, Long Y, Xu Y, Xiao P, Gyawali L, Woo K, Yin Y, Zrenner B. Renal artery vasodilation may be an indicator of successful sympathetic nerve damage during renal denervation procedure. *Sci Rep*. 2016;6:37218. doi: 10.1038/srep37218
24. Wang Z, Chen S, Zhou T, Su L, Ling Z, Fan J, Chen W, Du H, Lu J, Xu Y, Tan Z, Yang H, Hu X, Li C, Yan X, Hu G, Liu C, Yin Y. Comparison of saline-irrigated catheter vs. temperature-controlled catheter for renal denervation in a canine model. *Am J Hypertens*. 2015;28:1434–1443. doi: 10.1093/ajh/hpv051
25. Sakaoka A, Terao H, Nakamura S, Hagiwara H, Furukawa T, Matsumura K, Sakakura K. Accurate depth of radiofrequency-induced lesions in renal sympathetic denervation based on a fine histological sectioning approach in a porcine model. *Circ Cardiovasc Interv*. 2018;11:e005779. doi: 10.1161/CIRCINTERVENTIONS.117.005779
26. Tzafriri AR, Mahfoud F, Keating JH, Markham PM, Spognardi A, Wong G, Fuimaono K, Böhm M, Edelman ER. Innervation patterns may limit response to endovascular renal denervation. *J Am Coll Cardiol*. 2014;64:1079–1087. doi: 10.1016/j.jacc.2014.07.937
27. Imnadze G, Balzer S, Meyer B, Neumann J, Krech RH, Thale J, Franz N, Warnecke H, Awad K, Hayek SS, Devireddy C. Anatomic patterns of renal arterial sympathetic innervation: new aspects for renal denervation. *J Interv Cardiol*. 2016;29:594–600. doi: 10.1111/joic.12343
28. Mahfoud F, Tunev S, Ewen S, Cremers B, Ruwart J, Schulz-Jander D, Linz D, Davies J, Kandzari DE, Whitbourn R, Böhm M, Melder RJ. Impact of lesion placement on efficacy and safety of catheter-based radiofrequency renal denervation. *J Am Coll Cardiol*. 2015;66:1766–1775. doi: 10.1016/j.jacc.2015.08.018
29. Yoshimoto M, Sakagami T, Nagura S, Miki K. Relationship between renal sympathetic nerve activity and renal blood flow during natural behavior in rats. *Am J Physiol Regul Integr Comp Physiol*. 2004;286:R881–R887. doi: 10.1152/ajpregu.00105.2002
30. Fujisawa Y, Nagai Y, Lei B, Nakano D, Fukui T, Hitomi H, Mori H, Masaki T, Nishiyama A. Roles of central renin-angiotensin system and afferent renal nerve in the control of systemic hemodynamics in rats. *Hypertens Res*. 2011;34:1228–1232. doi: 10.1038/hr.2011.115
31. Osborn JW, Foss JD. Renal nerves and long-term control of arterial pressure. *Compr Physiol*. 2017;7:263–320. doi: 10.1002/cphy.c150047
32. Kopp UC. Role of renal sensory nerves in physiological and pathophysiological conditions. *Am J Physiol Regul Integr Comp Physiol*. 2015;308:R79–R95. doi: 10.1152/ajpregu.00351.2014

Novelty and significance

What Is New?

- This study demonstrates that strong blood pressure (BP)–elevation response to renal nerve stimulation (RNS) is indicative of local abundance of nerves. Ablation at strong-response site showed a more superior BP-lowering effect than at weak-response site, as well as a lower level of tyrosine hydroxylase and norepinephrine in kidney and a greater reduction in plasma norepinephrine.

What Is Relevant?

- RNS helps not only to identify the nerve-enriched area but also to guide the interventionalists with recommended site for ablation. Potentially, selective renal denervation guided by RNS can achieve more effective BP reductions.

Summary

RNS provides insights for interventionalists to more effectively identify the optimal ablation target and would be a promising method for distinguishing the innervation sites with dominant BP-regulation function for selective denervation. Further research will study the mechanism of how the different nerves fibers around renal arteries work together for the regulation of BP response to RNS and the effect of ablating these sites with BP drop response to RNS. RNS may be able to identify the suggested ablation site as well as the nonrecommended ablation site.