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ORIGINAL ARTICLE

Mapping Renal Innervations by Renal Nerve Stimulation and Characterizations of Blood Pressure Response Patterns

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Abstract

Increased sympathetic nervous activity is one of main contributors to pathogenesis and progression of hypertension. Renal denervation (RDN) has been demonstrated as a potential therapy for treatment of hypertension; however, lack of indicators of intra-/post-procedure results in inconsistent clinical outcomes. Renal nerve stimulation (RNS), a simple and promising method, could evoke elevated blood pressure as an intraoperative indicator for RDN. But related researches on patterns of blood pressure responses to RNS are still incomplete. To investigate and categorize the phenotypes of blood pressure response to RNS and heart rate alteration before and after RNS, 24 Chinese Kunming dogs were used to perform RNS from bifurcation to ostium of renal arteries after angiography, and a total of 483 stimulated sites were complete. We identified five different patterns of blood pressure response to RNS in 483 stimulated sites, (1) continuous ascending and finally keeping steady above baseline (26.9%), (2) declining and then rising over baseline (11.8%), (3) declining and then rising but below baseline (14.5%), (4) fluctuating in the vicinity of baseline (39.5%), and (5) continuous declining and finally keeping steady below baseline (7.2%), and found no difference in RR intervals among five blood pressure responses before and after renal nerve stimulation. Renal nerve stimulation could elicit different patterns of blood pressure response, which could potentially assist in distinguishing sympathetic-excitatory sites and sympathetic-inhibitory sites from mixed nerve components, which might help to improve the efficacy of RDN.

Keywords Hypertension · Renal denervation · Renal nerve stimulation · Blood pressure response

Introduction

Overactivated sympathetic nervous system has been generally recognized as one of major pathogenesis factors responsible for essential hypertension, which is a pivotal risk factor for cardiovascular events. In 2009, Krum et al. performed a proof-of-principle trial of renal sympathetic denervation in patients with resistant hypertension by a dedicated catheter and demonstrated a significant reduction in blood pressure (BP) with satisfying safety profile [1]. Both efficacy and safety have also been proved by subsequent clinical trials [2–5]; however, in Symplicity HTN-3 [6], the first double-blinded randomized controlled trial, the BP difference was not observed between the denervated and sham groups at 6 months. Although the detailed mechanisms of renal denervation (RDN) to attenuate sympathetic nerve activity and reduce blood pressure have not been fully elucidated yet, effective ablation of sympathetic-excitatory fibers has been proposed and contributed to its lowering BP effect. It becomes apparent that RDN, as an innovative and interventional therapeutic strategy, is indeed effective for hypertensive patients.

However, reduction in BP was not observed among approximately 25~30% patients undergoing RDN, and this phenomenon was consistently observed across all energy-based RDN studies such as radiofrequency, ultrasound, and alcohol

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[4, 7]. Townsend and Sobotka [8] expressed that those non-responders may reflect either technical failures or suboptimal patient selection given the lack of predictors of BP-lowering success. Furthermore, results from clinical trials also showed increased blood pressure in some patients after RDN at 6-month follow-up [2–5], which runs counter to the purpose of procedure. Thus, indicators before, during, and after RDN procedure to predict and confirm a successful sympathetic denervation are urgent unmet clinical needs for this therapy.

Recent studies [9–11] have shown that renal nerve stimulation has emerged as a simple and feasible method to guide RDN and improve its efficacy. Ablation on BP-elevated sites evoked by RNS could attenuate BP and sympathetic activity in animals and patients with far less non-responders [12–15]. We also found that the BP-lowering effect was proportional to the increase in BP by RNS [13]. Other studies [9–11, 14] have illustrated the different nerve distributions from the perspectives of histology and function. Besides, van Amsterdam [16] revealed sensory afferent nerves, sympathetic efferent nerves, and parasympathetic efferent nerves from the aspect of anatomy structures around renal artery from human specimens, while Kiuchi [17] et al. held different views and categorized these nerves as "pressor nerves" and "depressor nerves" based on the BP responses to stimulation from the aspect of nervous functions, providing convincible evidence for the rationale of renal denervation on sympathetic-excitatory sites guided by RNS, which might have more potential to result in predictable efficacy and optimize RDN. We believe that, in any case, it is counterbalance between sympathetic-excitatory and sympathetic-inhibitory fibers that accounts for the net effect of BP responses.

However, renal nerve fibers vary significantly regarding the number and size, as well as their distance from the lumen in the proximal and distal segments of the main stem and branches [18], making it unpractical to perform RDN procedure with a fixed ablation pattern. Therefore, instant blood pressure response to RNS becomes an ideal and reliable indicator for both mapping renal innervation and assessment of complete denervation. Unfortunately, there are limited researches on the blood pressure responses to RNS. Herein, we aimed to investigate and categorize the phenotype of blood pressure responses to RNS and heart rate alteration before and after RNS for improvement of efficacy of RDN.

Materials and Methods

Experimental Protocol and Animal Preparation

Twenty-four Chinese Kunming dogs with either gender (weight, 25–35kg, age,>3 years) were used for the experiment, and this canine model is characterized by naturally high blood pressure and sympathetic tone according to our previous studies [12, 13]. General anesthesia was induced by 3% sodium pentobarbital (Xiya Reagent, Shandong, China) at 30 mg/kg intraperitoneally, followed by maintenance of anesthesia at a dose of 5mg/kg/h through trace syringe pump. Artificial airway was established if limb muscle relaxation and shallow and slow breath were observed. SIMV-PC mode of ventilator (WATO EX-55, Mindray, Shenzhen, China) was applied, and pulse oxygen saturation was recorded by clamping the sensor on the tongue (BeneVision N12, Mindray, Shenzhen, China). Pure oxygen was inhaled at a flow rate of 2L/min if necessary. After skin preparation, both surface electrocardiogram (ECG) and invasive blood pressure were monitored and continuously recorded by a Multichannel Electrophysiology Management System (Sichuan Jinjiang Electronic Science and Technology Corporation, Chengdu, China). The bi-spectral index was used for estimation of proper anesthesia and maintenance of a stable physiologic status. Renal angiography was performed to determine whether renal arteries were eligible for experiments. If renovascular abnormalities such as severe renal artery stenosis, less than 4mm in diameter, were observed, the animal was excluded from the study. Each animal received stimuli from bifurcation to ostium in both renal arteries.

Renal Nerve Stimulation

A dedicated saline-irrigated catheter (AquaSense, Synaptic Medical Limited, Beijing, China) for bipolar electrical stimulation was introduced into the left and right renal arteries, respectively, via the sheath in right femoral artery under the guidance of fluoroscope. RNS was performed from the bifurcation to ostium at 15mA output, 10 Hz, and pulse duration of 2ms for 60 s by a nerve and muscle stimulator (SynNuo-C4, Sichuan Jinjiang Electronic Science and Technology Company, Chengdu, China). A new target site would not receive renal nerve stimulation until blood pressure remains steady for at least 60 s. This procedure was repeatedly executed until the entire main renal artery was covered and the distance between stimulated sites is at least 1–2 length of the electrode tips in the same quadrant.

2000IU of unfractionated heparin was administered during the procedure. Penicillin was given intramuscularly after the procedure to prevent postoperative infection.

Assessment of Heart Rate

To assess whether alterations of heart rate can be utilized as an index in response to RNS for mapping renal nerves, we obtained RR intervals before and after RNS. QRS waves were counted at baseline and right after RNS to assess heart rate variability for 9–10 s, because it is unpractical to predefine an exact period to count the integer of RR intervals. We measured the precise length of time axis using the built-in tool of

the Multichannel Electrophysiology Management System after counting the number of RR intervals.

Data Collection and Processing

Successful performance of electrical stimulation was determined by the interferences on ECG. BP response to RNS was observed and categorized paralleling to interferences of ECG. The baseline of blood pressure at each site was obtained from relatively steady BP (defined as SBP_{max}−SBP_{min} in response to RNS was observed and categorized closest to the onset of RNS. The 60-s stimulating phase was divided into six 10-s phases for statistical analysis.

Exclusion criteria of stimulated sites for data collection and analysis are the following: (1) high-low BP wave alternans (Figure 1A); (2) magnitude of respiratory variation in arterial BP over 10mmHg (SBP_{max}−SBP_{min}>10mmHg in a respiratory circle, Figure 1B); and (3) indistinct electrical interferences on ECG after contrast agent administration to confirm the adherence of catheter to the intima via the saline-irrigated catheter, while BP response observed (Figure 1C). Of note, all the stimulated sites would be categorized regardless of these listed exclusion criteria. These criteria were only applied to BP data collection and analysis due to the large fluctuations of blood pressure.

Statistics

Continuous variables were presented as mean ± standard deviation, and categorical variables were expressed as proportion, outputting from SPSS software (version 25.0, IBM Corps., Armonk, NY). The differences of variables among different BP responses and variation trends from 0 to 60s during RNS were analyzed with repeated measures analysis of variance (RM ANOVA). First, Mauchly's test of sphericity was used for parametric distribution to determine which tests should be applied for significance levels. Second, if P<0.05, then Roy's largest root of multivariate testing was applied; otherwise, within-subject effect testing would be applied. For each BP response pattern, ΔSBP-time curve was exhibited by GraphPad Prism 5 (GraphPad Prism Software Inc., San Diego, California). Heart rate alterations were compared by paired *t* test before and after stimulation. Two-sided P<0.05 was considered statistically significant.

Results

Blood Pressure Responses to Renal Nerve Stimulation

Renal nerve stimulations were performed on 483 sites in 24 dogs. Five different blood pressure responses have been observed as followed, and the proportion of each pattern was shown in Figure 2:

1) Continuous ascending and finally keeping steady above baseline (26.9%, Figure 3A)
2) Declining and then rising over baseline (11.8%, Figure 3B)
3) Declining and then rising but below baseline (14.5%, Figure 3C)
4) Fluctuating in the vicinity of baseline (39.5%, Figure 3D)
5) Continuous declining and finally keeping steady below baseline (7.2%, Figure 3E)

Of note, the elevation/reduction began mildly in the first 10 s, and the most significant changes were observed within 11–20 s, while the turning point may occur at 20~30 s if reduced blood pressure turned to increased blood pressure response (pattern 2 and pattern 3), followed by a relatively steady blood pressure curves until the suspension of RNS.

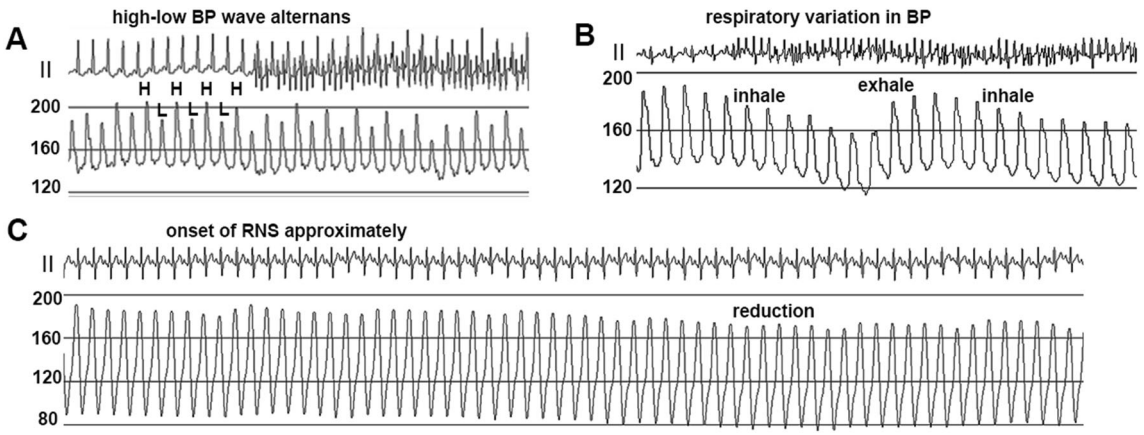

Figure 1 Typical excluded examples. **A** For high-low blood pressure wave alternans, **B** for respiratory variation in blood pressure, **C** for unclear interferences of ECG after confirmation of adherence to the intima with occurrence of the blood pressure response

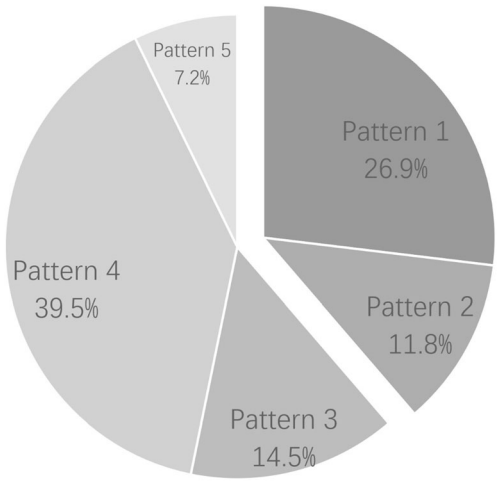

Figure 2 Proportions of patterns 1 to 5

Of 483 RNS sites, 191 were eligible for statistical analysis by dividing RNS into 6 equal parts for data processing and data hierarchy, as well as baseline was documented (Table 1).

Herein, Roy's largest root of multivariate testing was applied for RM ANOVA because of $P<0.05$ by Mauchly's test of sphericity. Systolic BP changed significantly from 1st 10 s to 60 s in patterns 1–5 by repeated measures analysis of variance ($P=0.016$). There is significant difference in each pattern ($P<0.05$) by repeated measures analysis of variance. Additionally, variation trends between pattern 1 and pattern 2, pattern 2 and pattern 3, and pattern 3 and pattern 5 changed significantly by 2-way repeated measures analysis of variance ($P=0.0003$, 0.0002, 0.003, respectively).

Examples of typical BP responses of all five patterns are presented in Figure 3.

Heart Rate

The alterations of RR intervals before and after RNS for each pattern were presented in Figure 4. Our results showed that RR intervals were not changed significantly by RNS in any pattern.

Discussion

Inconsistent conclusions of clinical trials, elevated BP in some patients after RDN, and variance of autonomic nerve contribution make it necessary to apply auxiliaries for location of proper sites to ablate and assessment of the procedural endpoint. Indicators such as renal artery vasodilation after RDN [19], elevated blood pressure response during radiofrequency delivery [20], cardiac baroreflex sensitivity [21], and ambulatory arterial stiffness [22] have been validated to predict the efficacy of RDN. However, to a more time-efficient extent, RNS could lead to an immediate BP response in real time by stimulating renal nerves, producing the integrated effects, while the elevation response to RNS would be blunted after sufficient ablation [10–13], indicating that nerve bundles have been ablated partially or totally. Classification of BP response might provide instant information of potential targeted ablation sites for interventionalists to perform a more efficient renal denervation.

Different phenotypes of BP responses were attributed to variance in proportion of sympathetic-excitatory fibers and sympathetic-inhibitory fibers. While non-response type (pattern 4) held the highest proportion (39.5%), it may indicate very few renal nerves traverse in the range of stimulated breadth and depth around the renal artery that hardly elicits macroscopic BP response, implying that a number of RDN sites separating from RNS may fall flat. Likewise, ablation on sympathetic-inhibitory fibers may neutralize the BP-lowering effects. It might be one of the possible reasons why blood pressure remained unchanged in some patients, even increased [2–5]. On the other hand, a net rise in blood pressure (patterns 1 and 2) held the proportion of 38.7% as ideal targeted sites to ablate. Fudim et al. called the elevated BP response sites (or pressor spot) as "hot spot" and the reduced BP response sites (or inhibitory) as "cold spot," which could be used to identify sympathetic-excitatory fibers, avoid sympathetic-inhibitory fibers, and thus guide selective RDN [15].

Recent studies demonstrated that selective afferent renal denervation by capsaicin could lead to a significant decrease in the sympathoexcitation and BP in 5/6 nephrectomy rats [23] and 2-kidney 1-clip hypertensive rats [24], emphasizing the role of afferent fibers contributing to the maintenance of hypertension.

Pattern 1: Continuous ascending and finally keeping steady above baseline

Our previous study [13] illustrated the greater elevation of BP, the more nerve bundles around the renal arteries, and the more superior BP-lowering effects after RDN. Although we successfully located the ablated regions by Masson staining, immunohistochemistry failed to stain these three nerve fibers mentioned above to reveal the exact associations between nerves and BP responses due to sufficient destruction.

Pattern 2: Declining and then rising over baseline

Unlike pattern 1, blood pressure decreased at the beginning of RNS, and it took 20 s around to have blood pressure increased based on our data and statistical results. Ablation on either pattern 1 or pattern 2 could also decrease blood pressure in canine at 4-week follow-up [13].

Pattern 3: Declining and then rising but below baseline

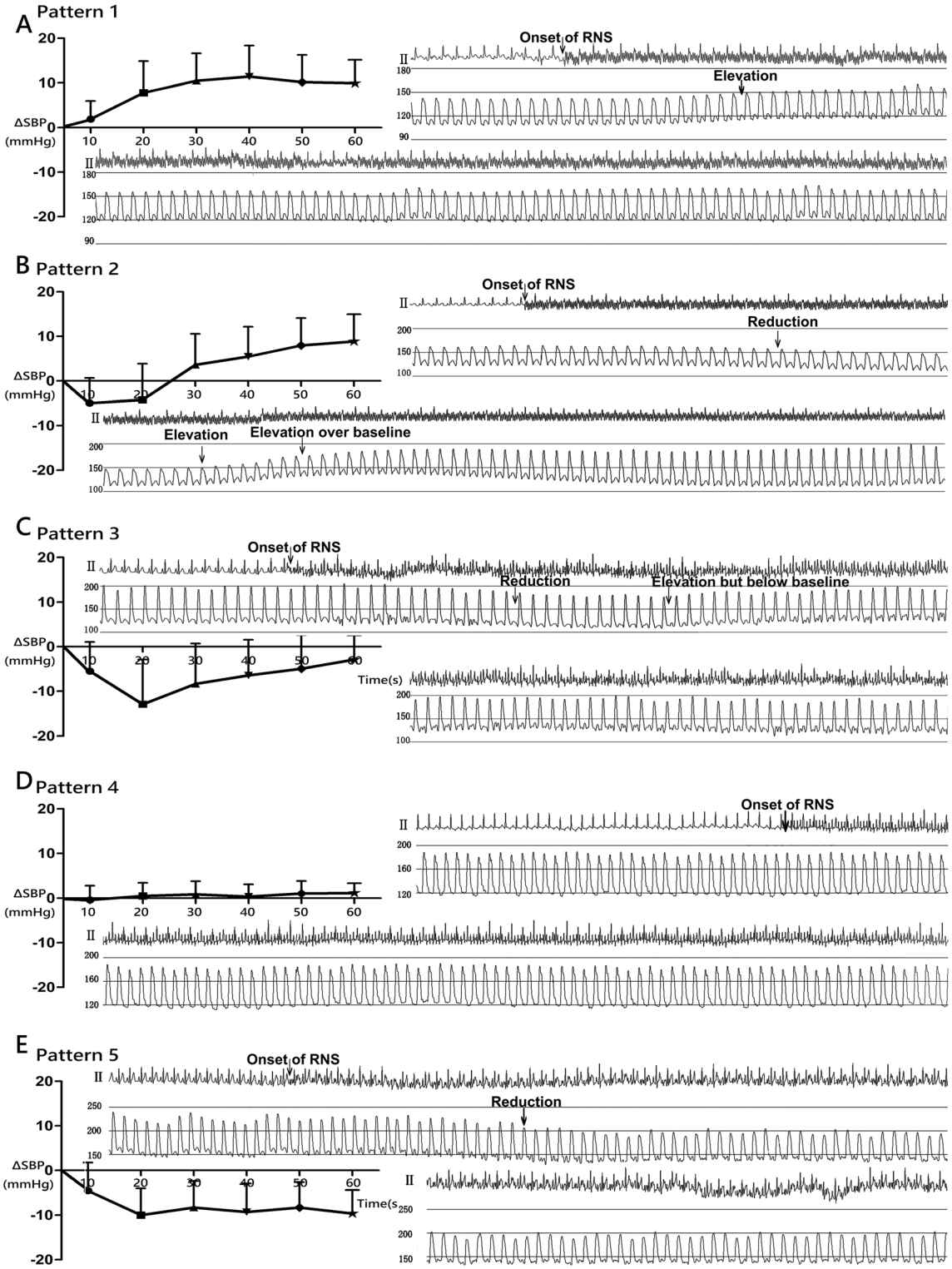

Figure 3 Five patterns of mean change in systolic blood pressure (ΔSBP) response and a typical case for each pattern of BP responses to RNS. Mean change of ΔSBP and a classical example of BP responses to RNS of **A** pattern 1, **B** pattern 2, **C** pattern 3, **D** pattern 4, and **E** pattern 5

Similar to pattern 2, RNS evoked a significant decrease in blood pressure for 20 s around, but followed by a minor increase without exceeding the baseline. Ablation on these sites might elicit a sustained increase in BP by destructing parasympathetic nerves (or sympathetic-inhibitory nerves), leading to tilting the balance in favor of overactivated sympathetic nervous system. Therefore, this sort of sites should be avoided.

Table 1 Baseline and 1st to 6th 10 s of systolic blood pressure (SBP) and diastolic blood pressure (DBP) (mmHg)

		Baseline	1st 10s	2nd 10s	3rd 10s	4th 10s	5th 10s	6th 10s
SBP	Pattern 1	181.5±17.8	1.9±4.1	7.8±7.1	10.5±6.2	11.4±5.0	10.2±6.1	9.9±5.2
	Pattern 2	181.2±14.2	4.8±5.6	−4.4±8.02	4.1±7.5	5.9±7.33	8.8±7.9	10.3±10.4
	Pattern 3	192.5±18.5	−5.5±6.6	−12.9±10.1	−8.4±9.1	−6.4±8.0	−4.9±7.6	−2.9±5.5
	Pattern 4	180.5±16.38	−0.4±3.17	0.5±3.0	0.9±3.0	0.3±2.8	1.0±2.9	1.2±2.2
	Pattern 5	191.8±13.1	−4.5±6.3	−10.0±6.0	−8.3±5.9	−9.3±6.4	−8.3±5.7	−9.6±5.2
DBP	Pattern 1	112.5±15.3	1.8±1.1	5.9±5.3	7.0±5.2	6.7±5.4	5.5±4.8	5.5±5.2
	Pattern 2	110.5±13.9	−3.2±4.4	−3.0±7.0	2.5±5.2	1.6±5.3	2.2±4.9	3.6±5.9
	Pattern 3	122.3±13.5	−4.0±4.3	−8.3±7.7	−4.1±6.5	−2.8±4.9	−2.1±4.0	−1.6±3.8
	Pattern 4	111.3±17.1	−0.1±2.5	1.3±3.2	0.9±2.5	0.5±3.2	0.8±3.4	0.7±3.2
	Pattern 5	117.3±9.2	−3.0±3.5	−3.7±6.3	−2.6±5.7	−2.9±5.5	−3.0±5.2	−2.5±5.5

Pattern 4: Fluctuating in the vicinity of the baseline

As is mentioned above, this pattern held the highest proportion, emphasizing the necessity of RNS for guidance on RDN. The effort to denervate these sites solely may not influence the blood pressure when following up.

Pattern 5: Continuous declining and finally keeping steady below the baseline

These sites (35 out of 483) were distributed among 17 dogs. To prevent the occurrence of more severe hypertension caused by reduced parasympathetic nerve activity during the post-procedural follow-up, ablation on these sites should be avoided.

Wehrwein et al. demonstrated that the axons of many sympathetic preganglionic neurons are relatively short while sympathetic postganglionic axons are much longer, contrary to axons of parasympathetic neurons. Of note, the axonal diameter of preganglionic neurons (1~5μm) is greater than that of postganglionic neurons (0.1~2μm), which is proportional to the transmission velocity. On the other hand, preganglionic fibers are lightly myelinated B fibers or unmyelinated C fibers, while postganglionic fibers consist of unmyelinated C fibers. Above all, the transmission speed of parasympathetic fibers is faster than that of sympathetic fibers [25]. In the case of pattern 2 and pattern 3, we believe that delayed elevation of BP response to RNS is evoked when there are sympathetic outflows from the central nervous system, but the parasympathetic manifestation presents earlier because of the higher speed of parasympathetic fibers. After that comes the increase in BP elicited by the superior effects of sympathetic nerves. The net rise or decline of blood pressure response to RNS depends on the proportion of different nerve fibers stimulated. On the whole, ablation on pattern 1 and/or pattern 2 could produce more predictive BP-lowering effects combined with confirmative RNS after RDN, while ablation on pattern 3 and/or pattern 5 might induce an increase in blood pressure at follow-up, leading to tilting the balance in favor of overactivated sympathetic nervous system.

RR intervals before and right after RNS did not reach the significant difference in five patterns of blood pressure responses. Consistent with Tsai et al. [26], they found that there were no significant changes of 24-h average RR intervals at 1 and 2 months after renal denervation in dogs. Moreover, Esler

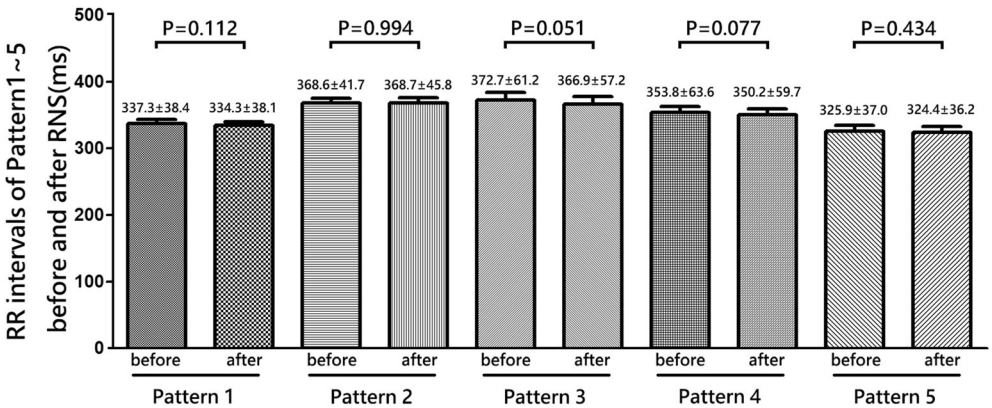

Figure 4 Comparison of heart rates (RR intervals, ms) before and after RNS

et al. [27] demonstrated that heart rate is more likely an indicator of cardiac rather than renal sympathetic activities.

According to previous clinical trials including the most recent SPYRAL HTN-OFF MED pivotal [2–5], approximately 25~30% patients exhibited increased blood pressure after RDN, regardless of radiofrequency, ultrasound, or other energies. Although we can't prove that pattern 3 and pattern 5 are elicited by renal nerve stimulation on sympathetic-inhibitory fibers histologically, such results may in part explain the phenomenon that BP of these patients increased after renal denervation at follow-up.

Not only can renal nerve stimulation hunt for the potentially proper denervation sites, but assess whether ablated sites have been completely denervated. Based on our statistics, sites recommended to ablate contributed for 38.7% (pattern 1 and pattern 2), so there's more chance (61.2%) to denervate non-response sites or sympathetic-inhibitory sites. It's essential to locate the sympathetic-excitatory sites to decrease the ablated number by RNS to alleviate the potential renovascular injury. Furthermore, RNS could assess and predict the success of RDN by another stimulation after ablation at the same site.

Gal et al [28] reported the first study on BP response to RNS-guided renal denervation at 4–6 sites per artery in 8 patients to determine the feasibility. Mean ambulatory blood pressure monitoring (ABPM) was reduced from 153.3±12.9/89.0±3.5 to 135.0±9.4/73.6±13.5 mmHg with a mean of 3.5 antihypertensive drugs, comparing to a reduction of 9.0±11/6±7.4mmHg (baseline, 152.1±7.0/97.2±6.9mmHg) in the SPYRAL HTN-ON MED trial with 45.9±13.7 unguided ablation sites in patients with 2.2±0.9 antihypertensive drugs at 6-month follow-up [2]. Furthermore, as the post hoc analysis of data from the Symplicity HTN-3 reported, the number of ablation sites performed in each patient was highly correlated with post-procedure BP reductions [29]. However, the more sites we ablate, the more injury we cause. The main purpose is to alleviate overactivated sympathetic nervous system and rebuild the balance between sympathetic and parasympathetic nervous systems. Some researchers held the view that the elevation of BP is the result of pain by RNS, rather than response to RNS. As is mentioned above, we found that there's superior BP-lowering effect in groups that blood pressure response to RNS elevated more than that elevated minor after RDN [13]. Additionally, we observed such five different responses to RNS instead of the sole elevated BP response when stimulation was performed, even in one model. On the other hand, bi-spectral index during the RNS procedure remained stable (70~80), while the depth of anesthesia would alter if pain reaction was activated. We did notice that BP of some stimulated sites increased right after the onset of RNS for several seconds and returned around the baseline, followed by subsequent BP responses, which might be the consequence of pain.

We classified and explained the five patterns of BP response to RNS from the perspective of the physiology of different fibers. Each pattern depends on a variant proportion of neural fibers. RNS could identify the sympathetic-excitatory sites to be ablated and sympathetic-inhibitory sites to be avoided. Meanwhile, Murai [30] and de Jong [31] reported that both increase and decrease in BP responses during RNS had been observed in patients with resistant hypertension. On the other hand, we also noticed these five BP responses in patients with resistant hypertension. Catheter-based RDN, as a novel and minimal invasive treatment for resistant hypertension, arrhythmias, and heart failure, is strongly supported by clinical and experimental evidence. Efficacy of RDN could be improved by renal nerve stimulation. More preclinical studies and RCTs of RNS-guided RDN are needed to confirm its values and probe to the mechanism of both RNS and RDN more clearly.

Limitations

1) Data were collected from animal models, and more clinical data are needed to be analyzed and confirmed. More evidence, well-designed animal experiments, and clinical data are needed to validate.
2) RNS, as a rapid screening and confirmation of technical success of ablation methods, focuses mainly on renal afferents but fails to emphasize the effect of efferents which also play an indispensable role in long-term BP-lowering effects.
3) Although our previous studies have illustrated that diverse BP responses to RNS correspond to different distribution of nerve fibers, the five patterns above are based on the alteration of blood pressure during RNS. There's no histological evidence to support the exact relationship between five patterns and nervous fiber components due to the destruction of the bundles that traverse the adventitia of renal arteries, which is essential to assess the completion of ablation. Even though our previous study [13] has confirmed that the total area of renal nerves around the ablated sites in SRS was larger than that of WRS, we are trying to unveil the exact relationship between BP response and renal nerve distribution.

Conclusions

Renal nerve stimulation could elicit five patterns of blood pressure response, which could potentially assist in distinguishing sympathetic-excitatory sites and sympathetic-inhibitory sites from mixed nerve components, which might help to improve the efficacy of RDN.

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Author Contributions HZ, YL, YX, HL, WC, LS, ZL, and YY designed this experiment. YL, YX, KT, and XL prepared and recorded the intervention procedure. HZ, HL, YL, ZO, and WC performed the intervention procedure. HZ, YL, YX, HL, and ZO collected the data, and all authors participated in data analysis. HZ, YL, and YY composed this paper. All authors reviewed and approved for the final version of manuscript.

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Declarations

Ethics Approval The experimental protocol was approved by the Animal Experimentation Ethics Committee of Chongqing Medical University, in accordance with the guidelines of National Institutes of Health for the care and use of laboratory animals.

Conflict of Interest The authors declare no competing interests.

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