

Research Article

Neuroanatomical Basis of BP-Elevation Response Evoked by High Frequency Electronic Stimulation in Renal Artery

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Abstract

Background: High frequency electric stimulation has emerged to detect renal nerve by evoking BP response. However, its relationship with the renal nerve distribution is not clear. This study was designed to evaluate the renal nerve distribution by annular electrode catheter via High Frequency Electric Stimulation (HFS).

Methods: A total of 8 Chinese Kunming dogs were included. HFS was performed from proximal to distal renal artery. HFS positive sites (Systolic BP increased at least 10mmHg when HFS delivery) were identified as to HFS positive (HFSP) group and other negative sites were identified as to HFS negative (HFSN) group. Renal nerve distribution at HFSP and HFSN sites was analyzed.

Results: Finally, 12 renal arteries were included, 144 bipolar HFS were performed at distal, middle and proximal bilateral renal artery. 25/144(17.4%) of HFS might evoke positive autonomic response. 48% (12/25) were located at proximal renal artery, 28% (7/25) at middle renal artery, and 24% (6/25) at distal renal artery. In HFSP group, stimulation caused a significant BP elevation of $13.4 \pm 4.1/9.9 \pm 4.9$ mmHg ($P < 0.001$ for both). In HFSN group, stimulation only increased BP by $1.4 \pm 3.7/1.3 \pm 2.5$ mmHg ($P = 0.36$). The mean area of nerves in HFSP sites was 0.41 ± 0.19 μm^2 and the average number of nerves was 7.16 ± 3.94 , whereas in HFSN sites were 0.18 ± 0.06 μm^2 and 3.48 ± 2.23 , respectively ($p < 0.001$).

Conclusion: Renal afferent sympathetic nerves can be detected effectively by HFS. The BP-elevation response to HFS may be one useful marker to trace the sympathetic-enriched sites and be potentially applied to determine the targeted ablation site.

Keywords: Blood pressure; High-frequency stimulation; Annular electrode catheter; Renal nerves

Introduction

In recent years, a novel minimally invasive endovascular intervention, Renal Sympathetic Denervation (RSD), has been extensively studied as a new approach to treating resistant hypertension [1-3]. Ablation from distal to proximal renal artery was used in Simplicity-HTN serial trials. However, it is difficult to judge whether the ablative point selected by the intervention list is the correct nerves-enriched site, due to heterogeneity of renal nerves distribution. Therefore, how to identify and destroy the high nerve density site of renal sympathetic nerves is a key to successful RSD procedure. In previous animal studies, high frequency stimulation (HFS) was used to identify renal sympathetic nerves with systolic BP elevation of at least 10 mmHg, which disappeared or diminished

subsequently after successful ablation with significant BP reduction at 3 months [4,5]. However, the exact relationship between BP response and renal nervous distribution is not clear. Consequently, the present animal study was designed to reveal the relationship between BP response and renal nerve distribution, and provide the neuroanatomical evidence for RSD guided by high frequency stimulation.

Methods

Animals

The Chinese Kunming dog breed was originally developed from the local hybrid dogs by crossing local native dogs with working dogs [6]. They have natural high BP [7] and therefore are ideal experimental animals used for this study. Eligible dogs were older than 3 years and had a Systolic Blood Pressure (SBP) of 140mmHg or more (under anesthesia and via invasive BP monitoring). After selection, a total of 8 healthy Chinese Kunming dogs, weighing between 30 and 35kg, were enrolled in the present study. The rationale related to using Chinese Kunming dogs as experimental animals, the source, breeding, feeding conditions, and the experimental protocol of this study were reviewed and approved by the animal experimentation ethics committee of the Chongqing Medical University, following the guidelines of the National Institutes of Health and of the Declaration of Helsinki for the care and uses of laboratory animals.

Animal Preparation and Renal Angiogram

The dogs were anesthetized with 3% sodium pentobarbital (30 mg/kg) by intraperitoneal injection, followed by a maintenance dose of 5 mg/kg per hour. Penicillin was given intramuscularly after the experimental procedure for the prevention of infection. The

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surface ECG was continuously recorded throughout the procedure by a multichannel recorder (Sichuan Jinjiang Electronic Science and Technology Company, China). The bilateral femoral artery was punctured under sterile conditions, and 2000 IU unfractionated heparin was administered. Continuous invasive BP monitoring was recorded via the left femoral artery catheterization. Bilateral renal angiography via right femoral catheterization was performed using a 6F JR4 Judkins catheter (Cordis Corporation Miami, FL). If renal artery abnormalities were found, or arterial diameters were below the minimum acceptable size (<4 mm, using inner diameter of 6F JR4 Judkins catheter as reference), or main renal artery length was shorter than 20 mm, the renal artery or dog would be excluded from the study.

The HFS Protocol and Autonomic Responses

An annular catheter with 4 electrodes (from 1 to 4) and diameter of 5 mm (designed by Prof. Yin, made by Synaptic Medical Limited, Beijing, China) was applied. When the annular catheter was placed properly in renal artery, it may divide renal artery into 4 parts on cross section. HFS was delivered at 2 adjacent electrodes, the electrode combination was 1-2, 2-3, 3-4 and 4-1 respectively, and these combinations might cover the circumference of renal artery (Figure 1). HFS was applied from the distal to the proximal segments of bilateral renal artery. The number of attempts of HFS at proximal, middle, and distal segments of renal arteries depended on the ease of access and length of renal artery. Rectangular electrical stimuli were delivered at a frequency of 20 Hz, amplitude to 8 V, and pulse duration of 2 ms for 60s, using a Nerve and Muscle Stimulator (XinNuo B100; Sichuan Jinjiang Electronic Science and Technology Company, Sichuan, China). Meanwhile, the 4 electrode position of annular catheter was identified under fluoroscopic guidance, and the positive and negative BP response sites on HFS were recorded for subsequent analysis. The positive BP response was defined as elevation of SBP by ≥ 10 mm Hg during HFS according to studies by Chinushi et al. [4] and Lu et al. [5]. The dogs were sacrificed after the HFS experiment.

Specimen Collection and Histopathology

Paraffin rods were specifically prepared. It was designed and made according to diameter of renal arteries as determined by renal angiography. Bilateral renal arteries with attached abdominal aorta and kidneys were collected from the 8 Chinese Kunming dogs. The paraffin rod was inserted into renal artery so as to keep its anatomical structure and morphology. The superior and inferior portions of proximal, middle and distal renal artery were marked by red and black stitches, respectively. Each artery with surrounding soft tissue was cut at 4- to 5-mm intervals and equally divided into proximal, middle, and distal segments. The artery samples were fixed in 4% neutral-buffered paraformaldehyde for 24 hours, dehydrated with an alcohol concentration gradient, soaked in different concentrations of xylene, embedded in paraffin wax, and then sectioned. Each renal artery was sectioned at approximately 1-mm intervals from distal (kidney) to proximal (aorta) and cut into 4- μ m slices; 10 serial samples of each section were obtained, and stained with Hematoxylin and Eosin (H&E) and Tyrosine Hydroxylase (TH). Digital images from H&E-stained and TH-stained histological sections were performed with the aid of an Olympus Scan scope BX51 (Olympus, JP) at 40x magnification with microscope scale (500 μ m, 1 μ m=0.9 pixel). TIF compression with dpi 432 was used. The images were divided into 4 quadrants on the basis of stitches labeling and analyzed with image analysis software (Image-Pro Plus 6.0, Media Cybernetics, USA). Each quadrant's nerve bundles were manually traced along the myelin by "irregular" tool, then through "count-view statistic" option to obtain the quadrant's

nerve area. Measurements of the distance from the luminal surface to each nerve and nerve area were performed in each quadrant around the renal artery (Figure 2).

BP data analysis

The mean BP 20-s before each bipolar HFS was used as baseline value. Each HFS of 60-s phases was subdivided into 20-s phases. In our previous preclinical study, we found that BP would reach peak during second 20-s phase of HFS. Therefore, the mean BP at second 20-s phase was used as the evoked BP value for analysis (Figure 3).

Statistical analysis

All continuous data were presented as mean \pm SD, and categorical variables were expressed as proportions. Differences of BP between positive and negative sites, distance from the luminal surface of the renal arteries to each nerve, number of nerves and nervous areas at different response to HFS were compared using student t test or paired t test, if appropriate. A 2-tailed value of $P < 0.05$ was regarded as statistically significant. All the statistical analyses were performed with SPSS statistical software (version 17.0; Chicago, IL).

Results

Effects of HFS on BP

Four left renal arteries were excluded. Two were due to dual renal artery and diameter less than 4 mm. Another 2 were due to the annular electrode catheter could not be introduced to middle and distal segment of renal artery. Finally, 12 renal arteries were included, and 144 bipolar HFS were performed at distal, middle and proximal segments of bilateral renal arteries. 25/144 (17.4%) of HFS might evoke positive

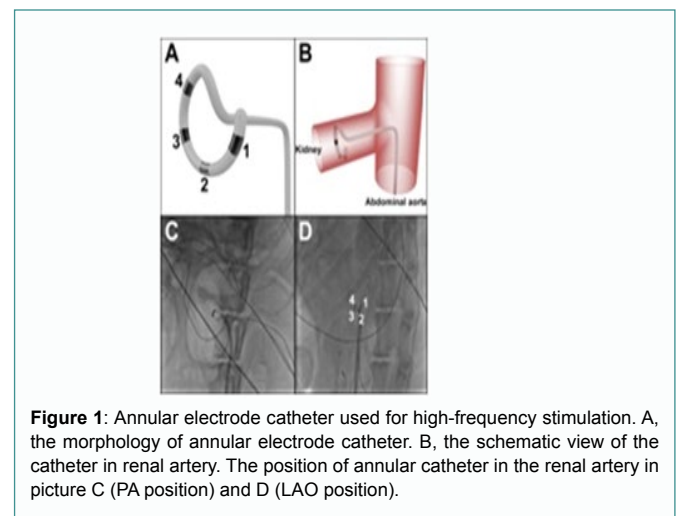


Figure 1: Annular electrode catheter used for high-frequency stimulation. A, the morphology of annular electrode catheter. B, the schematic view of the catheter in renal artery. The position of annular catheter in picture C (PA position) and D (LAO position).

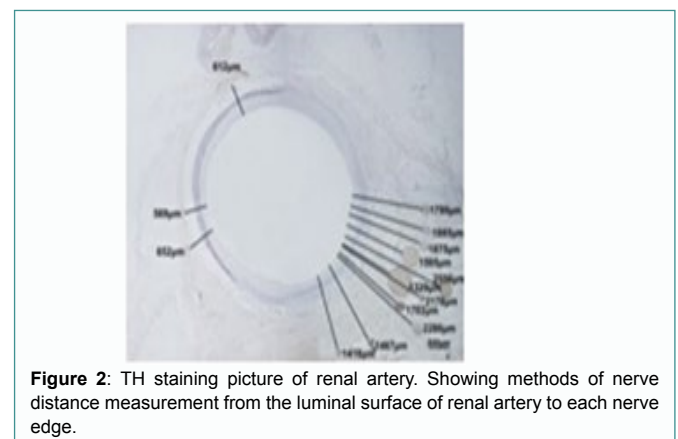


Figure 2: TH staining picture of renal artery. Showing methods of nerve distance measurement from the luminal surface of renal artery to each nerve edge.

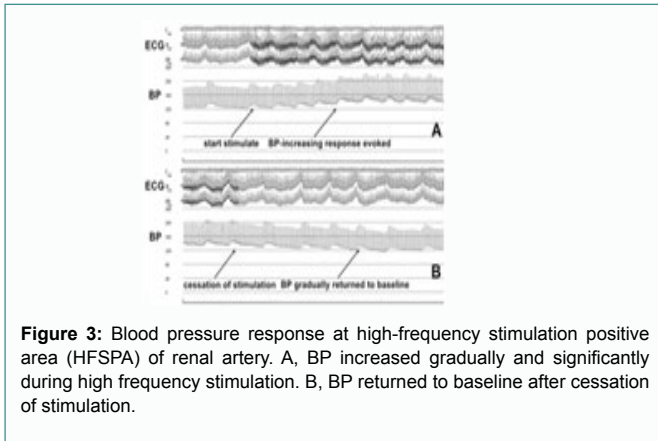


Figure 3: Blood pressure response at high-frequency stimulation positive area (HFSPA) of renal artery. A, BP increased gradually and significantly during high frequency stimulation. B, BP returned to baseline after cessation of stimulation.

autonomic response. 48% (12/25) positive autonomic response was located at proximal renal artery, 28% (7/25) at middle renal artery, and 24%(6/25) at distal renal artery. Baseline BP value at site with positive autonomic response was 179.6±12.8/124.6±9.3mmHg, and BP increased gradually to 192.9±14.1/134.6±10.9 mmHg during bipolar HFS, systolic BP elevation was 13.4±4.1mmHg (95%CI:11.7-15.1, P<0.001) and diastolic BP was 9.9±4.9mmHg (95%CI:7.9-12.0, P<0.001). Baseline BP value at site with negative autonomic response was 179.4±11.6/124.5±10.7 mmHg, and 180.9±12.3/125.8±11.1mmHg during bipolar HFS, the change of BP was 1.4±3.7/1.3±2.5mmHg (p=0.361 for SBP, p=0.359 for DBP) (Figure 4). 36 of 119 HFS negative sites showed a decrease in BP during high frequency stimulation. 33% (12/36) cold spots were located at proximal renal artery, 42% (15/36) at middle renal artery, and 25% (9/36) at distal renal artery (Table 1 and Figure 5). The number of renal nerves in high frequency stimulation positive (HFSP) site was significant higher than that in high frequency stimulation negative (HFSN, p<0.001) site, and the area of renal nerves at HFSP site was also significant larger than that at HFSN site. However, there was no difference of the distance from lumen to renal nerve between HFSP and HFSN sites (p=0.659).

The evaluation of renal nerves distribution at different renal segment

The number of renal nerves at middle and distal segment was higher than that at proximal segment (P<0.009), and the distance from the luminal surface of the renal arteries to nerve at proximal segment was farther than that at middle and distal segment (P<0.02). However, the mean renal nerve area had no difference among 3 renal segments (Table 2).

Discussion

This study firstly explored the anatomic substrate of BP elevation response induced by high frequency stimulation. The main findings of this study were that the number and area of renal nerves at BP positive response to HFS were very richer than those at negative response. It means HFS may effectively found nerves-enriched sites. RDN is a minimally invasive percutaneous procedure which aims to disrupt sympathetic nerve afferent and efferent activity by the application of radiofrequency energy directly within the renal artery wall. During the past several years, catheter-based RDN has emerged as a promising therapeutic option for drug-resistant hypertension and other cardiovascular disease [8-11]. Despite achieving great success in previous cohort studies [1-3], the Simplicity HTN3 trial [12] failed to demonstrate a significant BP reduction compared with sham-controlled group. The reason of failure has been elucidated extensively. Besides crucial design differences between Simplicity

HTN-3 and previous, unblinded and mostly observational studies, the disappointing results could be partly due to insufficient renal nervous ablation [13], and the key of the RDN procedure is the achievement of effective ablation in bilateral renal arteries. Briasoulis et al. [14] analyzed the ablation data of Symplicity HTN-3, and found that when bilateral renal artery ablation sites>16 points, blood pressure decreased significantly when compare to the sham group, whereas when the ablation sites 8-16 points, there was no statistically significant decrease in blood pressure compared with the sham group. This phenomenon suggests that some of the ablation sites in Symplicity HTN-3 are ineffective or the investigator had not truly ablated the renal nerves. In current stage, the BP-lowering effect of RDN is highly variable and the rates of non-response to RDN vary between 8-37% [15]. Although several factors, including baseline SBP, vascular aging and stiffness, and the use of central sympatholytic agents, design of catheter will impact the efficacy of RDN, how to find and destroy renal nerves is the most important for RDN. Our

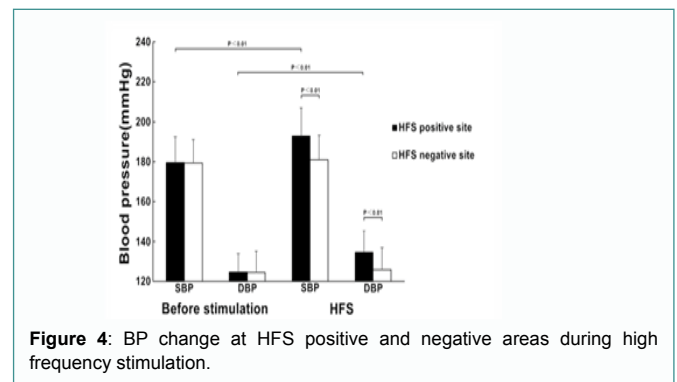


Figure 4: BP change at HFS positive and negative areas during high frequency stimulation.

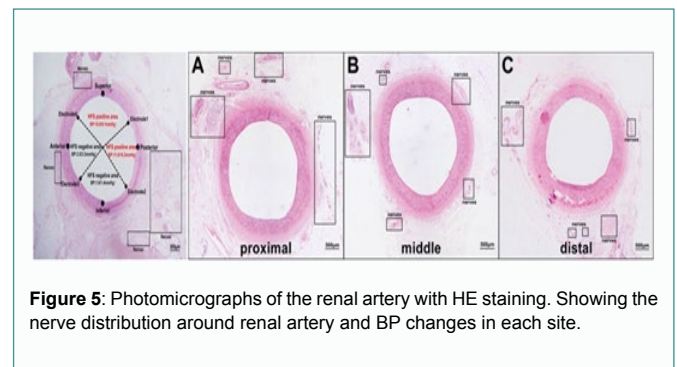


Figure 5: Photomicrographs of the renal artery with HE staining. Showing the nerve distribution around renal artery and BP changes in each site.

Table 1: Comparison of nerve anatomy between HFSP and HFSN sites (Mean±SD)

Group	Number of nerves	Area of nerves(mm ²)	Distance from nerves to lumen(mm)
HFSP	7.16±3.94	0.41±0.19	0.93±0.26
HFSN	3.48±2.23*	0.18±0.06 [#]	0.96±0.51

*P<0.001 VS HFSP; [#]P<0.001 VS HFSP

Table 2: Comparison of nerve density in proximal, middle and distal artery (Mean±SD)

Group	Number of nerves	Area of nerves (mm ²)	Distance from nerves to lumen (mm)
Proximal	13.25±4.65	0.54±0.27	1.05±0.45
Middle	18.75±5.57*	0.56±0.26	0.86±0.41 [▲]
Distal	17.87±4.82 [#]	0.74±0.33	0.88±0.36 [△]

*P=0.016 vs. Proximal; [#]P= 0.025 vs. Proximal; [▲]P=0.001 vs. Proximal;

[△]P=0.002 vs. Proximal.

pervious animal study [5] found that RDN guided by bipolar HFS via autonomic response would lead to significant reduction both BP and plasma norepinephrine after RDN of 3 months than sham group. In this animal study, we have verified that there was more renal nerve distribution at positive BP-elevation site than negative BP-elevation site. It means that BP-elevation response evoked by high frequency stimulation has solid neuroanatomical basis, and it also provides the theory basis for RDN guided by high frequency stimulation.

Limitations

Some limitations should be considered in interpreting the present results. Firstly, we didn't compare the BP-elevation response under different parameters of HFS, therefore, electrical stimulation parameters used in this animal study may not be optimal, it may influence inducing rate of positive BP-elevation response, and miss some effective ablation sites. Secondly, we have not explored the reason why inducing rate of positive BP-elevation response gradually decreased from proximal to distal renal artery found in this study. However, an anatomical study conducted by Sakakura et al. [16] may explain the phenomenon. They found that afferent nerve is richer at proximal than distal renal artery, and therefore, more easy to be detected by high frequency electrical stimulation, which is consistent to our results. Thirdly, the mechanism that high-frequency stimulation led to BP decrease was unclear yet. Okusa's [17] study of the neural circuits of renal found that vagus afferents were glutamatergic, which enabled them to be discriminated from cholinergic vagus efferents [18], and no evidence existed of vagus efferents in the kidney. However, Gattone's [19] study proved the presence of vagus afferents in the kidney, these vagus afferents targeted the nucleus of the solitary tract in the brain [20], an area that was important for regulation of autonomic function in the periphery. Wouter's [21] histological analysis showed that there were both sympathetic and parasympathetic nerves in the same nerve bundle. In another words, vagus afferents were also distributed in sympathetic-enriched sites. Therefore, the activation of vagus afferents may be the reason why high frequency stimulation led to a reduction of blood pressure. This requires further research to clarify its mechanism.

Conclusion

The BP-elevation response to HFS may be one useful marker to identify the renal afferent sympathetic-enriched sites and can be applied to determine the targeted ablation site.

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