Substantial Reduction in Single Sympathetic Nerve Firing After Renal Denervation in Patients With Resistant Hypertension


Abstract—Renal denervation (RDN) has been shown to reduce blood pressure (BP) and muscle sympathetic nerve activity (MSNA) in patients with resistant hypertension. The mechanisms underlying sympathetic neural inhibition are unknown. We examined whether RDN differentially influences the sympathetic discharge pattern of vasoconstrictor neurons in patients with resistant hypertension. Standardized office BP, single-unit MSNA, and multi-unit MSNA were obtained at baseline and at 3-month follow-up in 35 patients with resistant hypertension. Twenty-five patients underwent RDN, and 10 patients underwent repeated measurements without RDN (non-RDN). Baseline BP averaged 164/93 mm Hg (RDN) and 164/87 mm Hg (non-RDN) despite use of an average of 4.8±0.4 and 4.4±0.5 antihypertensive drugs, respectively. Mean office BP decreased significantly by −13/−6 mm Hg for systolic BP (P<0.01) and diastolic BP (P<0.05) with RDN but not in non-RDN at 3-month follow-up. RDN moderately decreased multi-unit MSNA (79±3 versus 73±4 bursts/100 heartbeats; P<0.05), whereas all properties of single-unit MSNA including firing rates of individual vasoconstrictor fibers (43±5 versus 27±3 spikes/100 heartbeats; P<0.01), firing probability (30±2 versus 22±2% per heartbeat; P<0.02), and multiple firing incidence of single units within a cardiac cycle (8±1 versus 4±1% per heartbeat; P<0.05) were substantially reduced at follow-up. BP, single-unit MSNA, and multi-unit MSNA remained unaltered in the non-RDN cohort at follow-up. RDN results in the substantial and rapid reduction in firing properties of single sympathetic vasoconstrictor fibers, this being more pronounced than multi-unit MSNA inhibition. Whether the earlier changes in single-unit firing patterns may predict long-term BP response to RDN warrants further exploration. (Hypertension. 2013;61:457-464.) ● Online Data Supplement

Key Words: resistant hypertension • sympathetic nervous system • microneurography • single-unit • renal denervation

Arterial hypertension is a major contributor to global cardiovascular morbidity and mortality.1 Although the pathophysiology of hypertension is complex and multifactorial, sympathetic activation is a pivotal mechanism contributing to the development and perpetuation of high BP and its adverse cardiovascular consequences.2-7 Many patients with essential hypertension are characterized by increased sympathetic nerve firing rates in postganglionic fibers directed to the skeletal muscle vascular bed,8 accompanied by enhanced cardiac and renal sympathetic nerve activity.2,3,9 The magnitude of sympathetic excitation is directly linked to the disease progression and contributes considerably to hypertension-related end organ damage including left ventricular hypertrophy10 and left ventricular diastolic dysfunction.11 Although evidence for increased sympathetic outflow to the periphery has been determined primarily by using multi-unit muscle sympathetic nerve activity (MSNA) recordings, more recent findings applying technically challenging single nerve recording have demonstrated that firing patterns of single muscle sympathetic fibers provide more specific and quantitative information on central sympathetic drive. Indeed, in patients with mild essential hypertension, sympathetic discharge from single muscle vasoconstrictor neurons is more pronounced than in moderate and severe hypertension, whereas multi-unit MSNA is comparable across all grades of the disease.6 Assessment of the pattern of single-unit vasoconstrictor firing has also provided evidence for a distinct difference in central sympathetic outflow between lean and obese hypertensive patients.12 Furthermore, a direct relationship between single nerve firing rates and left ventricular mass, as assessed by magnetic resonance imaging, has recently been demonstrated in essential hypertension.13

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Given the importance of sympathetic excitation in hypertension and cardiovascular disease, strategies to specifically target chronic sympathetic overactivity are likely to be of substantial clinical benefit. Indeed, in very recent developments, the application of catheter-based renal denervation (RDN) has been demonstrated to result in safe and effective BP lowering and reduced noradrenaline (NA) spillover and postganglionic efferent multi-unit MSNA in patients with resistant hypertension (RH).14–18 However, the exact mechanism underlying sympathetic inhibition associated with RDN is unknown. We, therefore, investigated whether and to what extent ablation of renal sympathetic nerves may restrain abnormal patterns of sympathetic nerve firing in high-risk patients with RH.

Methods

Subjects

The study was approved by the Institutional Ethics Committee, and written informed consent was obtained from all patients. Thirty-five non-smoking patients (27 male; 8 female) with established RH were enrolled in our therapeutic RDN program as extensions to the Symplicity protocols (NCT00884433), of whom 25 (RDN) were prospectively assigned to treatment group and 10 patients (non-RDN) were assigned to continued medical care. Twelve patients were included in the Symplicity HTN-2 trial. Patients underwent a complete medical history and physical examination, assessment of vital signs, and review of medication. Patients were interviewed whether they had taken their complete medication at defined doses. Treating physicians and patients were instructed not to change medications except when medically required. Hypertension was diagnosed based on the current European Society of Hypertension and European Society of Cardiology guidelines for the management of arterial hypertension.19 Only patients without evidence of secondary forms of hypertension such as primary aldosteronism, renovascular hypertension, pheochromocytoma, Cushing’s disease, and others as assessed by physical examination, biochemical and imaging studies, were included. Four patients diagnosed with obstructive sleep apnea but adequately treated with continuous positive airway pressure therapy were also included. RH was defined according to the current state of the art and the American Heart Association.20

All patients were studied at baseline and at 3-month follow-up. The non-RDN group also served to assess reproducibility of measurements of BP, multi-unit MSNA, and single-unit MSNA.

Study Protocol

The RDN procedure is approved in Australia by the Therapeutic Goods and Drug Administration. Subjects were comprehensively examined in a quiet room and in a comfortable position. Measurements in the RDN group were obtained at baseline (before RDN) and at 3 months after the procedure. Non-RDN patients underwent the comparable measurements at baseline and at 3-month follow-up without having the intervention. On the first visit, BP was measured as described below followed by fasting biochemistry assessments. On the second visit, patients were studied in the supine position after a standardized light breakfast. Participants were asked to empty their bladder to minimize the possible effects of bladder distension on sympathetic activity assessment. Subjects were asked to refrain from alcoholic beverages for at least 48 hours before a study protocol.

Serum and Urine Biochemistry

Routine blood tests (hemoglobin, glucose level, HbA1c, lipid profile, kidney and liver function, electrolytes), urinary albumin-to-creatinine ratio (morning spot urine), and estimated glomerular filtration rate calculated using the Modified Diet in Renal Disease21 formula were performed in all patients before study enrollment. To assess safety of the procedure, kidney function tests were repeated at 3-month follow-up.

Office-Seated and Ambulatory BP

Average sitting office BP was measured after at least 5 minutes of rest on both arms and was calculated as the average of 3 consecutive measurements within a 2-minute interval at baseline and during each visit at follow-up with a validated device (Omron HEM-907, Omron Healthcare Singapore PTE Ltd). The arm with higher BP readings was used for subsequent measures.

To exclude pseudo-RH, all participants underwent 24-hour BP and heart rate monitoring (ABPM) using a validated device (Spacelabs 90207 or 90217 recorder; Spacelabs Healthcare, WA) at baseline as described previously.22 As recommended in the current guidelines, only ABPM data fulfilling the described standards with regard to the proportions of valid values for the day and night periods recordings were used for analysis.23 At 3-month follow-up, ABPM recordings were only available from 13 patients as the remaining 12 patients were participants of the Symplicity HTN-2 trial, the protocol of which required ABPM to be performed at 6-, but not at 3-month follow-up.

MSNA Recording

After 15 minutes of rest, MSNA was recorded continuously over a 20-minute period by obtaining concurrent measurements of multi-unit and single-unit recordings of postganglionic sympathetic nerve activity using microneurography (662C-3 Nerve Traffic Analysis System, Bioengineering of Iowa University, IA) from the right peroneal nerve from all participants. A tungsten active high-impedance microelectrode (UNA40FOT; FHC, Bowdoinham, ME) was inserted directly into the peroneal nerve posterior to the fibular head. The electrode was manipulated to obtain a high-quality image of single vasocostrictors that appeared out of the multi-unit MSNA fibers. The single-unit fibers were obtained from 2 different sites where achievable. A reference uninsulated microneurography needle (UNA40F2S; FHC, Bowdoinham, ME) was positioned at a distance of 2 to 3 cm from the recording active electrode. The neural signals were amplified, filtered, rectified, and integrated to obtain a voltage display of sympathetic nerve activity. MSNA was identified through careful inspection of the voltage neurogram as described previously.9,10

Catheter-Based RDN

Bilateral RDN was performed in 1 session using a radiofrequency catheter (Simplicity; Medtronic Ardian Inc, Palo Alto, CA) introduced into each renal artery via femoral access as described previously.14,15,17 To minimize local visceral pain during the energy delivery, anxiolytics and analgesics were administered intravenously.

Peri- and Postprocedural Medications

To assess the effects of RDN on BP, multi-unit MSNA, and single-unit MSNA, baseline medication was kept unchanged for at least 6 weeks before RDN and this treatment was maintained until 3 months follow-up. Similarly, baseline medication was not altered throughout the study period in the 10 patients who did not undergo RDN. Medication records of each patient were reviewed and documented at each visit. Female subjects were postmenopausal and were not receiving hormone replacement therapy. Patients initially treated with antidepressants, thyroids hormones, and phosphodiesterase inhibitors that influence sympathetic activity were not included in this study.

Data Analysis

Multi-Unit and Single-Unit MSNA

Over a period of 15 minutes, MSNA bursts were identified and sympathetic activity was calculated as burst frequency (bursts/min) and as burst incidence (bursts/100 heartbeats). Resting assessment of single-unit MSNA were thoroughly analyzed for 3- to 5-minute period as described in detail previously.23 For the analysis of single-unit recordings, we applied the methods of Macfield,24 which use stringent criteria for the acceptance of nerve firing. Detailed description of single-unit and multi-unit MSNA are provided in the online-only
Data Supplement. The analysis of single-unit and multi-unit MSNA was performed by an experienced investigator without knowledge of the patient identity or treatment status.

Statistical Analysis
Data are presented as the mean±SEM. Statistical analysis was performed using SigmaStat Version 3.5 (Systat Software, Point Richmond, CA). The variability of multi-unit and single-unit in the short term (<1 hour) and in the long term (3 months) was assessed by the 95% CI of the individual differences relative to the mean of the repeated measurements. The inter individual variability was assessed as the coefficient of variation, defined as the SE of the mean of the repeated measure divided by the mean of the 2 measurements. The short-term CIs were estimated in 21 patients in whom we were able to obtain 2 recording sites at each investigational visit. Long-term variability was analyzed in the 10 patients who were studied at baseline and 3-month follow-up. For patients who underwent RDN, changes in BP, single-unit MSNA, and multi-unit MSNA at 3-month follow-up were compared with baseline measurements using a paired t test. When the data did not pass the normality test, a square root transformation was used before performing t tests. A value of P<0.05 was considered significant.

Results

Baseline Characteristics
Baseline clinical characteristics of the 25 treated patients (RDN) and 10 patients who did not undergo RDN (non-RDN) are summarized in Table 1. The RDN cohort had a mean age of 57±2 years and non-RDN, 59±4 years. Body mass index was 32±1 kg/m² (RDN) and 30±2 kg/m² (non-RDN), waist circumference was 104±5 cm (RDN) and 108±6 cm (non-RDN), and waist-to-hip ratio was 1.0±0.1 (RDN) and 1.0±0.0 (non-RDN). On average, RDN patients were taking 4.8±0.4 antihypertensive drugs, including angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers or dual blockade (12 patients), β-blockers, calcium-channel blockers, diuretics, aldosterone antagonists, α-blockers, vasodilators, and centrally acting sympatholytic agents. Coronary artery disease was diagnosed in 24% and type 2 diabetes mellitus in 16% of the RDN patient cohort. Obstructive sleep apnea was previously diagnosed in 4 of 35 investigated patients, each of whom was on continuous positive airway pressure therapy, which was unaltered during the study. Average creatinine-based estimated glomerular filtration rate of the RDN patients was 74±3 mL/min per 1.73m².

Non-RDN patients were on an average of 4.4±0.5 antihypertensive drugs. Detailed BP lowering drugs and medical history of the non-RDN patient cohort are presented in Table 1. Average creatinine-based estimated glomerular filtration rate of the non-RDN patients was 83±3 mL/min per 1.73m².

Baseline average office-seated systolic blood pressure (SBP) and diastolic blood pressure (DBP), and heart rate (HR) in RDN and in non-RDN patients are shown in Table 1.

Procedural Aspects
Renal angiograms were performed before the introduction of the RF treatment catheter via femoral access and anatomic eligibility, and absence of significant vascular pathology was confirmed in all patients. An average of 9.4±0.4 ablation treatments using a predetermined treatment protocol and algorithm were delivered in each patient without any peri- or postprocedural complications. Angiographic evaluation before and directly after RDN revealed no compromise of treated arteries. There were no intra- or periprocedural complications. No short-term (at 3-month follow-up) adverse events related to the procedure were noted in any of the treated patients. There were no significant alterations in kidney function assessed by estimation of glomerular filtration rate based on serum creatinine (74±3 versus 74±3 mL/min per 1.73m²; P=0.95), plasma potassium (3.9±0.07 versus 4.0±0.09 mmol/L; P=0.3), and sodium (140±0.4 versus 139±0.5 mmol/L; P=0.096) levels after RDN.

Effects of RDN
Average office SBP and DBP at baseline and at 3-month follow-up for the RDN cohort is depicted in Table 2. RDN significantly reduced SBP (P<0.001) and DBP (P<0.05) at 3-month follow-up (Table 2). Mean decrease in sitting office BP was −13/−6 mm Hg (SEM 3/2) for SBP and DBP after RDN. There were no changes in resting office HR (68±3 versus 69±3 bpm; P=0.51) after RDN (Table 2). Average office SBP and DBP remained unchanged in the non-RDN group (SBP: 164±8 versus 163±6 mm Hg; DBP: 87±4 versus 88±5 mm Hg; Table 4).

Table 1. Baseline Characteristics of the Treated Patient Cohort (Renal Denervation) and Untreated Hypertensive Patients (Non-Renal Denervation)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Renal Denervation (n=25)</th>
<th>Non-Renal Denervation (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>57±2</td>
<td>59±4</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>18/7</td>
<td>9/1</td>
</tr>
<tr>
<td>CAD</td>
<td>6 (24%)</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>T2DM</td>
<td>4 (16%)</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>OSA</td>
<td>3 (12%)</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>Number of antihypertensive drugs</td>
<td>4.8±0.4</td>
<td>4.4±0.5</td>
</tr>
<tr>
<td>ACEI</td>
<td>14 (56%)</td>
<td>5 (50%)</td>
</tr>
<tr>
<td>ARB</td>
<td>21 (84%)</td>
<td>9 (90%)</td>
</tr>
<tr>
<td>ACEI+ARB</td>
<td>12 (48%)</td>
<td>4 (40%)</td>
</tr>
<tr>
<td>β-Blocker</td>
<td>10 (40%)</td>
<td>4 (40%)</td>
</tr>
<tr>
<td>Calcium-channel blocker</td>
<td>23 (92%)</td>
<td>9 (90%)</td>
</tr>
<tr>
<td>α-Blockers</td>
<td>5 (20%)</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>Diuretics (thiazide type or loop)</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Aldosterone antagonists</td>
<td>11 (44%)</td>
<td>3 (30%)</td>
</tr>
<tr>
<td>Centrally acting sympatholytics</td>
<td>15 (60%)</td>
<td>3 (30%)</td>
</tr>
<tr>
<td>Office SBP, mm Hg</td>
<td>165±4</td>
<td>164±8</td>
</tr>
<tr>
<td>Office DBP, mm Hg</td>
<td>93±3</td>
<td>87±4</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>68±3</td>
<td>64±5</td>
</tr>
</tbody>
</table>

Data are presented as mean±SEM and percentage (%). CAD indicates coronary heart disease; T2DM, type 2 diabetes mellitus; OSA: obstructive sleep apnea; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; bpm, beat per minute.
Analysis of ambulatory BP monitoring revealed that RDN resulted in a significant reduction in daytime SBP (151±4 versus 140±4 mm Hg; P<0.05) and DBP (89±4 versus 82±3 mm Hg; P<0.05), whereas the changes in night-time SBP (135±5 versus 129±5 mm Hg; P=0.76) and DBP (76±3 versus 71±4 mm Hg; P=0.97) after the procedure were not statistically significant.

Daytime SBP (152±9 versus 156±10 mm Hg; P=0.49) and daytime DBP (90±7 versus 91±6 mm Hg; P=0.67) as well as night-time SBP (138±7 versus 138±8 mm Hg; P=0.96) and night-time DBP (76±4 versus 78±4 mm Hg; P=0.52) remained unchanged at 3-month follow-up in non-RDN.

Changes in multi-unit and single-unit MSNA in the RDN cohort are described in Table 2. RDN moderately decreased multi-unit MSNA 3 months after RDN, expressed as bursts per minute (50±2 versus 45±3; P<0.05; Table 2) and bursts per 100 heartbeats (79±3 versus 72±4; P<0.05; Table 2). A more profound reduction in all properties of single-unit MSNA was observed, including firing rates of individual vasoconstrictor fibers (43±5 versus 27±3 spikes/100 heartbeats; P<0.01), firing probability (30±2 versus 22±2% per heartbeat; P<0.02), and the multiple firing incidence of single units (8±1 versus 4±1% per heartbeat; P<0.05). The reduction in office SBP was unrelated to the decrease in multi-unit MSNA (r=0.282; P=0.181) and to single-unit MSNA, including firing rates of individual vasoconstrictor fibers (r=0.003; P=0.98), firing probability (r=0.07; P=0.75), and the multiple firing incidence of single units (r=−0.16; P=0.46). The changes in multi-unit MSNA with RDN were unrelated to the single-unit firing rates (r=0.048; P=0.82), single-unit firing probability (r=0.08; P=0.71), or the multiple firing incidence of single units (r=−0.053; P=0.81).

Representative traces of continuous ECG, single-unit MSNA, and multi-unit MSNA obtained from 2 different units within 60 minutes in 21 patients is depicted in Table 3 and in the online-only Data Supplement. The mean differences and the variability in multi-unit and single-unit measurements between site 1 and site 2 recorded within 1 hour were small.

The reproducibility and coefficient of variation of BP, HR, multi-unit MSNA, and single-unit MSNA between the 2 visits in the non-RDN group are shown Table 4. All measurements remained unaltered at 3-month follow-up in non-RDN.

### Discussion

The present findings provide the first human evidence that catheter-based sympathetic RDN substantially reduces single-unit MSNA in high-risk patients with RH. The main findings of this study are that bilateral sympathetic RDN (1) results in a rapid and significant reduction in all properties of single-unit muscle sympathetic vasoconstrictor neurons; (2) reduces multi-unit MSNA; and (3) decreases BP in patients with RH. The inhibition of single-unit firing seems to be more pronounced than the reduction in multi-unit MSNA. The changes in properties of single unit nerve firing rates bore no direct association with concomitant changes in BP and multi-unit MSNA in RH.

There is convincing evidence of enhanced sympathetic activation in the development and progression of hypertension and related disease. The renal sympathetic nerves are pivotal in the pathology of human hypertension and experimental hypertension as evidenced by increased activity of single fibers of the renal nerves. More recent data indicate that increased single-unit firing is more closely related to certain grades of essential hypertension than multi-unit firing. Additionally, an augmented firing rate in individual neurons has been shown

### Table 2. Office-Seated Blood Pressure and Heart Rate, Single-Unit and Multi-Unit MSNA in Patients Who Underwent Renal Denervation at Baseline and at 3-Month Follow-Up

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>3-Month Follow-Up</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP, mm Hg</td>
<td>164±3</td>
<td>151±4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>93±3</td>
<td>87±3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>68±3</td>
<td>69±3</td>
<td>0.51</td>
</tr>
<tr>
<td>Multi-unit MSNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bursts per minute</td>
<td>50±2</td>
<td>45±3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Bursts per 100 heartbeats</td>
<td>79±3</td>
<td>72±4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Single-unit MSNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Firing rate, spikes per 100 heartbeats</td>
<td>43±5</td>
<td>27±3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Firing rate, spikes per minute</td>
<td>27±3</td>
<td>17±2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Firing probability, % per heartbeat</td>
<td>30±2</td>
<td>22±2</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Firing probability, % per burst</td>
<td>41±3</td>
<td>29±2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Incidence of multiple spikes, per heartbeat</td>
<td>8±1</td>
<td>4±1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Incidence of multiple spikes, per burst</td>
<td>12±2</td>
<td>6±1</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Incidence of multiple spikes, per firing burst</td>
<td>25±3</td>
<td>17±2</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Data are presented as mean±SEM. SBP indicates systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; bpm, beat per minute; MSNA, muscle sympathetic nerve activity.
Recent findings from our laboratory demonstrate that cardiac NA spillover is enhanced in individuals with increased incidence of multiple firing during a sympathetic burst, perhaps providing the mechanistic link between increased single-unit firing and left ventricular hypertrophy. Against this background, RDN may prove to be a rational approach to specifically target mechanisms underlying disease development and progression associated with chronic sympathetic overactivity.

Importantly, our findings indicate that the potential beneficial effects of RDN are not restricted to the reduction in BP and multi-unit MSNA, but extend further to all properties of single-unit muscle vasoconstrictor neurons. RDN considerably reduced the activity of single vasoconstrictor neural fibers, including firing frequency, firing probability, and the incidences of multiple spikes firing (firing salvos) within 1 cardiac interval.

The magnitude of the sympathetic inhibition of all features of single-unit muscle vasoconstrictor neurons seemed to be more pronounced than that of multi-unit activity in our patients with RH. Not unexpectedly and in line with previous findings, the pattern of firing in single muscle sympathetic discharge was unrelated to multi-unit MSNA. The properties of single-unit vasoconstrictor neurons in patients with RH in our study are not dissimilar to those described in obese individuals, perhaps indicating that excess body weight is an important driver of altered central sympathetic outflow in patients with RH. Indeed, average BMI was in the obese range in our study cohort. Nevertheless, hemodynamic, metabolic, reflex, and psychological changes that may influence the properties of sympathetic neural firing discharge after RDN cannot be ruled out. Along these lines, it has been shown that a more disturbed firing pattern is associated with anxiety proneness.

The observation of a more pronounced effect of RDN on single-unit firing rather than multi-unit MSNA may provide insights into the neural mechanisms of BP elevation in RH, perhaps indicating that elevated sympathetic drive in these patients is not simply a function of recruitment of additional

Figure 1. Representative recordings of ECG, single-unit, and multi-unit muscle sympathetic nerve activity (MSNA) in a patient with resistant hypertension at baseline and 3 months after renal denervation (RDN).

Figure 2. Representative recordings of ECG, single-unit, and multi-unit muscle sympathetic nerve activity (MSNA) in a patient with resistant hypertension at baseline and at 3-month follow-up without having undergone renal denervation (non-RDN).
firing units (as reflected by changes in MSNA) but also, and possibly more importantly, a function of an increase in the firing rate, probability of firing, and incidence of multiple firing of any particular single unit. Whether RDN induced abrogation of renal signaling via afferent nerves projecting to certain brain stem nuclei may preferentially alter the firing pattern of single units thereby contributing to the effects observed in our study remains to be elucidated. Clearly, increased NA release from subcortical brain regions has been demonstrated in human hypertension.\textsuperscript{30-32} The observed changes in the firing pattern of single-unit vasoconstrictor fibers may also have a profound effect on renal NA spillover, which is reduced by \textasciitilde47\% after RDN.\textsuperscript{14} Although a correlation between multi-unit MSNA and renal NA spillover has previously been demonstrated,\textsuperscript{33} more recent studies have revealed a close link between the incidence of multiple firing during a sympathetic burst and regional NA spillover, in this instance from the heart.\textsuperscript{35} In analogy, a similar relation between single-unit firing pattern and NA release from the kidneys would be expected to result in an even more pronounced and clinically meaningful reduction of renal NA spillover, given that neuronal reuptake of released NA is substantially less in the kidneys (\textasciitilde50\%) as compared with the heart (\textasciitilde90\%).\textsuperscript{34} The commonly observed pattern of a gradual further increase in the magnitude of the BP reduction over time being most pronounced at \textasciitilde6 to 12 months post-RDN may also be reflective of central mechanisms integrating afferent signaling from the kidneys and efferent sympathetic outflow. Overall, our findings of beneficial effects of RDN on individual firing rates may be of clinical significance as single-unit

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Site 1</th>
<th>Site 2</th>
<th>Mean Difference</th>
<th>95% CI</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Multi-unit MSNA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bursts per minute</td>
<td>51±3</td>
<td>51±3</td>
<td>−0.4±1.9</td>
<td>[−4.4; 3.5]</td>
<td>6.7%</td>
</tr>
<tr>
<td>Bursts per 100 heartbeats</td>
<td>76±3</td>
<td>78±3</td>
<td>−0.7±2.4</td>
<td>[−5.6; 4.3]</td>
<td>6.4%</td>
</tr>
<tr>
<td><strong>Single-unit MSNA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Firing rate, spikes per 100 heartbeats</td>
<td>43±5</td>
<td>45±6</td>
<td>−2.3±2.7</td>
<td>[−7.8; 3.2]</td>
<td>16.2%</td>
</tr>
<tr>
<td>Firing rate, spikes per minute</td>
<td>29±4</td>
<td>30±5</td>
<td>−1.9±1.2</td>
<td>[−6.2; 2.4]</td>
<td>16.6%</td>
</tr>
<tr>
<td>Firing probability, % per heartbeat</td>
<td>30±3</td>
<td>30±3</td>
<td>−0.7±1.7</td>
<td>[−4.3; 2.9]</td>
<td>15.4%</td>
</tr>
<tr>
<td>Firing probability, % per burst</td>
<td>39±3</td>
<td>40±4</td>
<td>0.2±1.9</td>
<td>[−3.7; 4.0]</td>
<td>13.7%</td>
</tr>
<tr>
<td>Incidence of multiple spikes, per heartbeat</td>
<td>9±2</td>
<td>10±2</td>
<td>1.0±0.6</td>
<td>[−2.3; 0.3]</td>
<td>21.1%</td>
</tr>
<tr>
<td>Incidence of multiple spikes, per burst</td>
<td>12±2</td>
<td>13±2</td>
<td>−0.7±0.7</td>
<td>[−2.3; 0.7]</td>
<td>19.5%</td>
</tr>
<tr>
<td>Incidence of multiple spikes, per firing burst</td>
<td>27±3</td>
<td>28±3</td>
<td>1.3±1.4</td>
<td>[−4.2; 1.6]</td>
<td>16.3%</td>
</tr>
</tbody>
</table>

CV indicates coefficient of variability; MSNA, muscle sympathetic nerve activity.
assessments of sympathetic nervous system (SNS) function are of great importance in identifying the relative role of sympathetic contributions to cardiovascular disease and in establishing the extent of changes induced by therapeutic interventions. Moreover, studies evaluating improvements in BP response to SNS interventions are needed to identify the degree of sympathetic overactivity that can be modulated by RDN or to determine whether the decrease in the firing rate, firing probability, and a reduction in the incidence of multiple spikes of individual vasoconstrictor fiber within 1 cardiac cycle associated with RDN may retard disease progression and improve cardiovascular outcomes.

Study Limitations
The small number of patients included in this study is a limitation. However, we applied the sophisticated and technically challenging single-unit MSNA recordings simultaneously with multi-unit MSNA. As single-unit MSNA has not yet been elucidated in RH, our study provides the first evidence of baseline characteristics of single sympathetic discharge pattern in this patient cohort and its response to intervention therapy. Second, given the observation from previous studies that the magnitude of the BP reduction tends to increase over time, the short-term follow-up of 3 months as reported here may not be sufficient to explore the full extent of the effects of RDN on single-unit and multi-unit MSNA and the related changes in BP. This needs to be explored in further longer term follow-up studies.

Strengths of our study include the precise evaluation of the effects of therapeutic sympathetic RDN on the characteristics of firing in single-unit muscle sympathetic fibers, multi-MSNA, and BP responses in high-risk patients with RH in whom we observed increased resting sympathetic outflow despite use of multi-drug regimens. Second, antihypertensive medication was unchanged in all patients irrespective of the procedure and maintained at follow-up thereby eliminating potential confounding effects of changes in antihypertensive treatment on BP and MSNA. Furthermore, we carefully evaluated hypertensive status and performed repeated measurements demonstrating that single-unit and multi-unit sympathetic neural firing pattern and BP remains unaltered over a 3-month period in patients who did not undergo the RDN procedure. Although this was a proof of concept study to demonstrate the effects of RDN on multi-unit and single-unit firing characteristics compared with a matched non-treated cohort, the non-blinded nature of our study is a limitation.

Perspectives
Given the mounting evidence of the importance of sympathetic activation in hypertension, ablation of the renal sympathetic nerves seems to be a promising approach for the treatment of RH and several conditions associated with chronic sympathetic excitation. Our findings indicate that renal nerve ablation may serve as a tool to beneficially influence the firing pattern of single-unit sympathetic vasoconstrictor fibers in patients with RH with important clinical implications relating to sympathetic inhibition and improved BP control. These findings merit further studies to delineate whether changes in the firing pattern of single-units may predict continued BP response to RDN and whether these beneficial changes are sustained over time.

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Disclosures
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References
Renal denervation not only reduces blood pressure and multi-unit muscle sympathetic nerve activity, but also suppresses single-unit sympathetic nerve firing discharges, which may result in improved cardiovascular prognosis in this patient cohort.

**Summary**

Our findings are indicative of a favorable effect of renal denervation on single-unit and multi-unit sympathetic inhibition and blood pressure lowering. These changes are likely to attenuate the high cardiovascular risk in patients with resistant hypertension.
Substantial Reduction in Single Sympathetic Nerve Firing After Renal Denervation in Patients With Resistant Hypertension

Dagmara Hering, Elisabeth A. Lambert, Petra Marusic, Antony S. Walton, Henry Krum, Gavin W. Lambert, Murray D. Esler and Markus P. Schlaich

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SUBSTANTIAL REDUCTION IN SINGLE SYMPATHETIC NERVE FIRING AFTER RENAL DENERVATION IN PATIENTS WITH RESISTANT HYPERTENSION

Dagmara Hering, Elisabeth A Lambert, Petra Marusic, Antony S Walton, Henry Krum, Gavin W Lambert, Murray D Esler, Markus P Schlaich

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Short title: Renal denervation and sympathetic activity

Data analysis

Multi-unit and single-unit MSNA

The morphology of each spike was carefully determined. Only spikes with similar high amplitude and shape were calculated using our developed software that allowed the superimposition of all spikes to confirm that the raw signals was derived from the same single fibre. Only the units that fulfilled the criteria were included in the final assessment. For each unit the following properties of the single-units were evaluated:

1. The firing rate = number of spikes that occurred during the full length of the recording expressed as per 100 heartbeats and spikes per minute

2. The probability of firing = the percentage of heartbeats during which one or more spikes occurred (expressed as % of heartbeats and % of bursts)

3. Incidence of multiple firing = the percentage of heartbeats or bursts where at least two spikes occurred (expressed as % of heartbeat and burst). The incidence of multiple firing was also expressed as % of firing bursts after excluding all bursts where the vasoconstrictor neuron remained silent.

When two recording sites were obtained per subject, single unit data was taken as the average of the 2 sites.

The short-term reproducibility (< 60 minutes) of single-unit and multi-unit MSNA was obtained in 21 patients in whom we were able to obtain two different recording sites at each investigational visit (Figure S1, Figure S2 and Figure S3).
Figure S1. Reproducibility of multi-unit MSNA from 2 different units within 60 minutes in patients with resistant hypertension (n=21) expressed both as bursts/min. (Figure S1a) and bursts/100 heartbeats (Figure S1b).
**Figure S2a.**

*Firing rate (spikes/min.)*

![Graph showing firing rate (spikes/min.) for Site 1 and Site 2. The correlation coefficient R² = 0.7181.](image)

**Figure S2b.**

*Firing rate (spikes/100 heartbeats)*

![Graph showing firing rate (spikes/100 heartbeats) for Site 1 and Site 2. The correlation coefficient R² = 0.683.](image)

**Figure S2.** Reproducibility of firing rate of single-unit MSNA from 2 different units within 60 minutes in patients with resistant hypertension (n=21) expressed both as spikes/min. (Figure S2a) and spikes/100 heartbeats (Figure S2b).
Figure S3a. Firing probability (% bursts)

Figure S3b. Firing probability (% heartbeats)

**Figure S3.** Reproducibility of firing probability of single-unit MSNA from 2 different units within 60 minutes in patients with resistant hypertension (n=21) expressed both as % of bursts (Figure S3a) and % of heartbeats (Figure S3b).
Figure S4. Reproducibility of the incidence of multiple firing (salvos of firing) of single-unit MSNA from 2 different units within 60 minutes in patients with resistant hypertension (n=21) expressed as per total heartbeat (Figure S4a) and per total burst (Figure S4b).
Figure S4c. Reproducibility of the incidence of multiple firing (salvos of firing) of single-unit MSNA from 2 different units within 60 minutes in patients with resistant hypertension (n=21) expressed as per firing within a cardiac cycle.