Translational Examination of Changes in Baroreflex Function After Renal Denervation in Hypertensive Rats and Humans

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Abstract—Renal denervation has shown promise in the treatment of resistant hypertension, although the mechanisms underlying the blood pressure (BP) reduction remain unclear. In a translational study of spontaneously hypertensive rats (n=7, surgical denervation) and resistant hypertensive human patients (n=8; 5 men, 33–71 years), we examined the relationship among changes in BP, sympathetic nerve activity, and cardiac and sympathetic baroreflex function after renal denervation. In humans, mean systolic BP (SBP; sphygmomanometry) and muscle sympathetic nerve activity (microneurography) were unchanged at 1 and 6 months after renal denervation (P<0.05). Interestingly, 4 of 8 patients showed a 10% decrease in SBP at 6 months, but sympathetic activity did not necessarily change in parallel with SBP. In contrast, all rats showed significant and immediate decreases in telemetric SBP and lumbar sympathetic activity (P<0.05), 7 days after denervation. Despite no change in SBP, human cardiac and sympathetic baroreflex function (sequence and threshold techniques) showed improvements at 1 and 6 months after denervation, particularly through increased sympathetic baroreflex sensitivity to falling BP. This was mirrored in spontaneously hypertensive rats; cardiac and sympathetic baroreflex sensitivity (spontaneous sequence and the Oxford technique) improved 7 days after denervation. The more consistent results in rats may be because of a more complete (>90% reduction in renal norepinephrine content) denervation. We conclude that (1) renal denervation improves BP in some patients, but sympathetic activity does not always change in parallel, and (2) baroreflex sensitivity is consistently improved in animals and humans, even when SBP has not decreased. Determining procedural success will be crucial in advancing this treatment modality. (Hypertension. 2013;62:533-541.) • Online Data Supplement

Key Words: denervation • hypertension • translational medicine

In Europe and the United States, ≈15% to 30% of adults diagnosed with hypertension are resistant to pharmacological intervention.1,2 That is, their blood pressure (BP) fails to meet target levels despite treatment with >3 antihypertensive medications. This is termed drug-resistant hypertension and has been highlighted as a growing problem worldwide.3,4 Drug-resistant hypertension particularly emphasizes the inadequacies of current pharmacological-based treatment regimes in a significant proportion of patients.5

It is not surprising, therefore, that device-based therapy developed from classic hypotheses regarding sympathetic activity and hypertension6,7 has received renewed focus as a treatment for difficult-to-control hypertension.8–12 Among these, catheter-based renal denervation (RDN) has been shown to safely and effectively decrease BP in patients with resistant hypertension.9,11 According to the Symplicity trial investigators, RDN decreased systolic BP (SBP; n=46, −20 mm Hg) in resistant hypertensives as early as 1 month postprocedure13 and continued to decrease and maintain a lower BP for ≤24 months (n=18).14 The Symplicity-2 investigators purport a 83.7% success rate (a reduction in SBP >10 mm Hg) at 6 months post-RDN.9

Despite the recent accumulation in publications relating to RDN in humans,9,11,13,15 as well as many previous animal studies,5,7,15 little is known of the mechanisms by which RDN reduces BP. It has been hypothesized that decreased renalafferent input into the brain stem reduces reflex-evoked increases in both sympathetic nerve activity (SNA) and BP.20 In this context, selective renal afferent denervation (dorsal rhizotomy) in rats with chronic renal failure abolishes hypertension in these animals.21 Furthermore, both single-unit and multiunit SNA decreased in patients who responded to RDN.22 Conversely,
Brinkmann et al.\textsuperscript{18} reported that neither average group BP nor muscle SNA (MSNA) was altered in 11 resistant hypertensive patients studied 3 to 6 months after RDN. This raises the important issue of preprocedural screening to determine responders. Interestingly, cardiac baroreflex function has been demonstrated to improve in some humans\textsuperscript{20} and animals who respond to RDN with a decrease in SBP.\textsuperscript{23} Changes in baroreflex function after RDN may reflect a decrease in renal nerve afferent input into the brain stem. Consistent with this hypothesis, selective renal afferent denervation resulted in improved cardiac baroreflex sensitivity in spontaneously hypertensive (SH) rats.\textsuperscript{24} Despite studies reporting either a change in baroreflex function in humans after RDN\textsuperscript{20} or a lack of change,\textsuperscript{18} interindividual differences in the response of the cardiac and sympathetic baroreflex have been overlooked. It is possible that improvements in baroreflex function are linked to reduced SNA and BP outcome after RDN, especially because chronic carotid sinus stimulation is antihypertensive.\textsuperscript{12}

To gain insight into the depressor mechanisms of RDN and because translational physiology is essential for our understanding of hypertension, we completed the following aims in both SH rats and human resistant hypertensive patients. We examined whether changes in SNA and baroreflex control of falling and rising BP were temporally related to changes in BP after RDN in SH rats and whether these data were mirrored in resistant hypertensive humans receiving catheter-based RDN.

**Methods**

**Human Study**

**Patients**

After the study received local NHS (National Health Service) and Trust ethical approval, we recruited 13 resistant hypertensive patients for treatment using the Symplicity catheter-based RDN method. Patients were recruited from our specialist hypertension clinic and were defined as drug-resistant hypertensives only after their treatments were optimized/thoroughly screened for white coat hypertension as per the National Institute of Clinical Excellence guidelines in the United Kingdom for the management of BP. All patients were either taking (5/8) or deemed intolerant to spironolactone (a potassium-sparing diuretic that is also an aldosterone receptor antagonist), suggesting that the hypertension was not secondary to subclinical hyperaldosteronism. Drug-resistant hypertension was defined as a clinic SBP >160 mm Hg or 24-hour ambulatory SBP >135 mm Hg, despite treatment with ≥3 antihypertensive medications including a diuretic.\textsuperscript{1} Full pre- and post-RDN data were achieved for 8 of 13 patients; 2 patients had a weight loss of >5 kg, making interpretation of changes in MSNA invalid; 2 patients failed to report for follow-up; and finally, 1 patient was excluded because of stroke during the follow-up period. In 1 patient, the microneurography recordings could not be completed; therefore, data for MSNA and baroreflex data are presented for 7 patients. Table 1 outlines patient demographics and antihypertensive medication (see Table S1 in the online-only Data Supplement for details and comorbidities) for the 8 completed subjects. Body mass remained stable throughout the study (Table 2). Medications did not change in 6 of 8 patients. In 1 patient, spironolactone was stopped, and the dose of doxazosin was decreased within the first 6 months postprocedure because the patient showed a significant decrease in SBP with symptoms of postural hypotension (participant 6). The other patient had doses of antihypertensive medications increased 1 month postprocedure because their BP persistently increased after RDN (participant 5). Drug adherence was assessed throughout the study via a patient self-reporting process.

**Catheter-Based RDN**

RDN was completed using the Symplicity Catheter System (Medtronic). Pain during the procedure was controlled using titrated doses of intravenous fentanyl and midazolam. A 6F femoral arterial sheath was sited under local anesthesia. The procedure was performed under anticoagulation with a bolus of intravenous heparin (100 IU/kg); the activated clotting time was checked 30 minutes into the procedure, and a further intravenous heparin bolus was administered, if required, to maintain an acute clotting time of >250 ms. Four to 8 radiofrequency ablations along both main renal arteries were applied (maximum 8 W, maximum 75°C for 2 minutes). The distance between ablations was >0.5 mm. The catheter was rotated =60° between ablations, which were applied from distal to proximal in a spiral pattern. Patients were loaded on aspirin (300 mg) before the procedure and discharged with a 4-week course of aspirin (75 mg) once daily, unless already on long-term antplatelet therapy. Patients were advised to continue all of their current antihypertensive medications on discharge.

**Study Day Measurements and Protocol**

All studies were performed in our Clinical Research Laboratory at the Bristol Heart Institute. Patients reported to the laboratory before and at 1 and 6 months after procedure. Three patients attended an additional study visit at 3 months, and these data are presented individually rather than averaged (Figure 2). Patients were asked to (1) take their antihypertensive medication as usual and (2) abstain from caffeine/alcohol/additional pain killers (eg, ibuprofen) and intense exercise 24 hours before the study day. All patients were studied at the same time of day on repeat visits.

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>Age, y</th>
<th>Sex</th>
<th>Height, m</th>
<th>Weight, kg</th>
<th>BMI, kg/m²</th>
<th>RDN responder (Y/N)</th>
<th>ACEI</th>
<th>ARB</th>
<th>β-ARB</th>
<th>CCI</th>
<th>Diuretic (Loop)</th>
<th>Diuretic (Thiazide)</th>
<th>Diuretic (K+ sparing)</th>
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Patient 1 tried various types of diuretic type antihypertensives but was determined intolerant to this class of drugs. RDN responders are classified as decrease in systolic blood pressure >10 mm Hg at 6 mo. ACEI indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; ARB, adrenergic receptor blocker; BMI, body mass index; CAS, centrally acting sympatholytic; CCI, calcium channel inhibitor; F, female; M, male; and RDN, renal denervation.
Table 2. Hemodynamic and Neural Response to Catheter-Based Renal Denervation 1 and 6 Months Postprocedure in Drug-Resistant Hypertensive humans

<table>
<thead>
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<th>Neural and Hemodynamic Variables</th>
<th>Pre-RDN</th>
<th>1 mo</th>
<th>6 mo</th>
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<tr>
<td>BMI, kg/m²</td>
<td>29.5±1.4</td>
<td>29.6±2.2</td>
<td>29.9±2.3</td>
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<tr>
<td>Clinic SBP, mm Hg</td>
<td>187±7</td>
<td>183±2</td>
<td>179±9</td>
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<td>Clinic DBP, mm Hg</td>
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<td>103±5</td>
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<td>Clinic MAP, mm Hg</td>
<td>136±6</td>
<td>130±7</td>
<td>128±6</td>
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<td>fSBP, mm Hg</td>
<td>172±7</td>
<td>171±11</td>
<td>166±12</td>
</tr>
<tr>
<td>fDBP, mm Hg</td>
<td>75±7</td>
<td>76±9</td>
<td>70±11</td>
</tr>
<tr>
<td>fMAP, mm Hg</td>
<td>106±6</td>
<td>105±11</td>
<td>100±11</td>
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<td>fMAP, mm Hg/DBP</td>
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<td>6±1</td>
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<tr>
<td>SV, ml</td>
<td>109±10</td>
<td>111±14</td>
<td>108±20</td>
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<td>CO, L/min</td>
<td>6.6±0.6</td>
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<td>TPR</td>
<td>21.0±1.9</td>
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<td>MSNA, bursts/100 heart beats</td>
<td>67±7</td>
<td>63±6</td>
<td>64±9</td>
</tr>
<tr>
<td>MSNA, bursts/min</td>
<td>38±2</td>
<td>39±3</td>
<td>38±4</td>
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</table>

Clinic blood pressures are taken on the same day as the study day with an automated cuff (mean of 3). BMI indicates body mass index; CO, cardiac output; DBP, diastolic blood pressure; DBPv, DBP variability; fDBP, finometer-based DBP; fMAP, finometer-based mean arterial pressure; fSBP, finometer-based systolic blood pressure; HR, heart rate; HRv, HR variability; MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity; RDN, renal denervation; SBP, systolic blood pressure; SBPv, SBP variability; SV, stroke volume; and TPR, total peripheral resistance.

After seated rest, clinic BP was assessed using standard automated sphygmomanometry.25 Average clinic SBP (3 readings) was used as outcome BP for success of RDN. Patients were asked to lie supine for neuro-cardiovascular assessment. Beat-by-beat BP was measured using a Finometer (Finapres medical systems), calibrated to the BP for continuous monitoring of heart rate.

Multunit MSNA was measured from the right peroneal nerve at the fibular head using tungsten microelectrodes (FHC Inc, Bowdoin, ME). Cardiac baroreflex function was measured using the spontaneous sequential technique,26 and sympathetic baroreflex sensitivity was measured using the spontaneous threshold technique.27,28 For further methods, data, and statistical analyses regarding microneurography and baroreflex sensitivity, please see the online-only Data Supplement Methods.

Animal Study

Animal experiments were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986 and associated guidelines. Adult (12 weeks; n=7) SH rats bred in the University of Bristol Animal Services Unit were used. See online-only Data Supplement Methods for surgical procedures.

Data Collection

Arterial pressure and heart rate were recorded for 24 hours a day as described in the online-only Data Supplement Methods. To avoid movement artifact, lumbar SNA (LSNA) was recorded for an hour at the same time each day while the rats (undisturbed in their home cage) were observed to be quiescent. On days 2 and 7, rats were exposed to a brief burst of high frequency noise, which produces a stress increase in sympathetic outflow. The magnitude of the response to noise stress was used to check whether the LSNA signal remained stable and responsive throughout the experiment. On experimental days 1 and 8, baroreflex function measured using venous infusions of vasoactive solutions were used to produce increases and decreases in BP20 (see online-only Data Supplement Methods).

Results

Hemodynamic Response to RDN in SH Rats and Human Hypertensives

Surgical RDN in SH rats was associated with a >90% reduction in renal tissue noradrenaline content (31±7 pg/100 μg protein RDN versus 443±45 pg/100 μg protein; P>0.01). Mean SBP, diastolic BP, and mean arterial pressure decreased within 24 hours of RDN by an average of 10–2 mm Hg (P<0.05), and this level was maintained for the full 10 days of recording. Over a similar time course, moderate reductions in LSNA of 25±11% could be observed (values averaged over 3 days preprocedure and 6–9 days postprocedure; Table 3; P<0.05).

Figure 1 shows the group (rat) average arterial pressure and LSNA response to RDN across the full recording period. Table 2 outlines the group mean hemodynamic response to RDN in resistant hypertensive humans, 1 and 6 months postproced. In contrast to the SH rats, mean clinic BPs did not change after RDN (n=8; 1 and 6 months post-RDN; P>0.05). However, when individuals were considered, clear responders (decrease in clinic SBP >10 mm Hg) and nonresponders became evident (Figure 2). In fact, 50% of the patients responded with a >10 mm Hg drop in SBP at 1 and 6 months post-RDN. There was no change in mean stroke volume, cardiac output, heart rate, total peripheral resistance, and MSNA 1 month or 6 months post-RDN (P>0.05). Regarding interindividual responses in MSNA, 4 of 7 patients responded with a decrease (ie, >10% fall) in MSNA at 6 months. The individuals with a change in MSNA at 6 months post-RDN were not necessarily those with a change in SBP. One individual who had the highest decrease in SBP (~44 mm Hg) had an increase in MSNA (+28 bursts/100 heart beats; Figure 2, patient 6M). Changes in MSNA at 1 and 6 months postprocedure.

Table 3. Cardiovascular and Sympathetic Responses to Bilateral RDN in SH Rats (n=7)

<table>
<thead>
<tr>
<th>Neural and Hemodynamic Variables</th>
<th>Baseline</th>
<th>RDN</th>
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</thead>
<tbody>
<tr>
<td>SBP, mm Hg</td>
<td>164±5</td>
<td>154±5*</td>
</tr>
<tr>
<td>MBP, mm Hg</td>
<td>135±3</td>
<td>127±3*</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>326±5</td>
<td>336±5</td>
</tr>
<tr>
<td>sBRS-HR, bpm/mm Hg</td>
<td>-1.08±0.07</td>
<td>-1.17±0.07*</td>
</tr>
<tr>
<td>sBRS-PI, ms/mm Hg</td>
<td>0.64±0.03</td>
<td>0.74±0.02*</td>
</tr>
<tr>
<td>LSNA, µV</td>
<td>1.58±0.092</td>
<td>1.20±0.034</td>
</tr>
<tr>
<td>LSNA, %</td>
<td>100</td>
<td>75.4±8.7*</td>
</tr>
<tr>
<td>SBP variability, mm Hg</td>
<td>13.72±0.98</td>
<td>12.99±0.79</td>
</tr>
<tr>
<td>DBP variability, mm Hg</td>
<td>12.42±0.70</td>
<td>12.17±0.79</td>
</tr>
<tr>
<td>HR variability, bpm</td>
<td>35.56±2.15</td>
<td>39.53±0.90</td>
</tr>
</tbody>
</table>

Data shown are mean of 3 baseline days and days 6 to 9 post-RDN (mean±SEM). DBP indicates diastolic blood pressure; HR, heart rate; MBP, mean blood pressure; PI, pulse interval; RDN, renal denervation; SBP, systolic blood pressure; sBRS, spontaneous baroreflex sensitivity; and SH, spontaneously hypertensive.

*P<0.05.
6 months did not correlate with changes in SBP at either 1 or 6 months after RDN (r = −0.14 and −0.23, respectively; P > 0.05). There was no relationship between baseline MSNA and change in SBP at 1 or 6 months (r = 0.05 and 0.07, respectively). In addition, we found no relationship between the number of ablations and the change in BP at 1 and 6 months (r = 0.17 and 0.20).

Cardiac Baroreflex Sensitivity
In SH rats, cardiac baroreflex sensitivity was assessed using 2 methods. First, automated examination of spontaneous ramps in SBP and heart rate across the 24-hour recording period showed a significant increase in spontaneous cardiac baroreflex sensitivity from 0.64±0.03 to 0.74±0.02 ms/mm Hg (see Table 2; P < 0.05), which again occurred very rapidly (within 24 hours) of the RDN surgery. Second, using vasoactive agents, the maximum gain of the baroreflex function curve increased (Figure 3A; −4.45±0.43 versus −5.77±0.56 bpm per mm Hg; P = 0.028), which was because of both an improvement in the lower plateau of the curve (314±9 versus 289±15 bpm; P = 0.036; Table S2) and a leftward shift in the BP50 (138±3 versus 131±4 mm Hg; P = 0.021). Without a significant change in heart rate, the operating point moved toward the center of the cardiac curve, thereby improving the ability to buffer hypotensive swings in arterial pressure.

The response of the cardiac baroreflex to RDN in resistant hypertensive humans mirrored that found in the SH rats. That is, the sensitivity (slope) of spontaneous baroreflex was increased at 6 months postprocedure compared with pre-RDN (pre, 6.7±1.9 ms/mm Hg versus post 6 months, 14.3±2.7 ms/mm Hg; Figure 3B). At 1 month, there was a trend of an increase in cardiac baroreflex sensitivity (+4.4±1.8 ms/mm Hg; P = 0.08). Figure 4B illustrates the change in cardiac baroreflex sensitivity in 1 patient (patient 6) who responded to RDN, with a decrease in SBP at 6 months postprocedure. Interestingly, 6 of 7 patients responded with an increase in cardiac baroreflex sensitivity even if SBP did not change as was the case in 4 patients. It should be noted that we did attempt to examine whether the cardiac baroreflex sensitivity changed in response to decreasing and increasing SBP separately. However, because there was a lack of cardiac sequences (≥4 heart beats) when either decreasing or increasing SBP was considered separately, we could not statistically examine whether the ability of the cardiac baroreflex to buffer either falling or rising BP improved after RDN. There was no relationship between the change in cardiac baroreflex sensitivity and the change in SBP at both 1 (r = 0.12) and 6 months (r = 0.20).

Sympathetic Baroreflex Sensitivity
All SH rats responded with an increase in sympathetic baroreflex sensitivity (Δ 4.5±1.1%/mm Hg; P < 0.05). This increase in gain was because of an increase in the upper curvature fitting parameter (0.29±0.05 versus 0.59±0.08; P < 0.05; Table S2), which corresponds to the ability to respond to falling BP. The response to rising BP was not significantly different (ie,
no change in the lower curvature, 0.28±0.05 versus 0.38±0.07; \(P=0.43\) 7 days after RDN. Figure 3B shows the mean group sympathetic baroreflex curves for SH rats. Note (similar to the cardiac baroreflex) the left and downward shift in both the operating point and BP50 (centering point) of the SNA baroreflex curve after RDN, thereby ensuring that the baroreflex regulation of LSNA remained on the linear portion of the curve.

Analogous to the SH rats, sympathetic baroreflex sensitivity increased in the resistant hypertensive humans at 1 (\(\Delta -1.7\pm0.4%/\text{mm Hg}\); \(P<0.05\)) and 6 months (\(\Delta -0.9\pm0.3%/\text{mm Hg}\); \(P<0.05\)) after RDN (Figure 5). Figure 5B illustrates the change in sympathetic baroreflex sensitivity after RDN at 1 and 6 months in 1 patient (3F). Overall, there was an increase (\(>10\%\), \(P<0.05\)) in baroreflex sensitivity in 6 of 7 patients.

In humans, because baroreflex function is dependent on whether BP is increasing or decreasing,\(^{30}\) we measured the sensitivity of the sympathetic baroreflex during falling and rising BPs separately. Similar to SH rats, we found that sympathetic baroreflex sensitivity during falling arterial pressures was significantly improved at 1 and 6 months after RDN (\(\Delta -1.4\pm0.6%/\text{mm Hg}\) and \(\Delta -1.76\pm0.4%/\text{mm Hg}\); \(n=6/7\); both \(P<0.05\); Figure 5). However, sympathetic baroreflex sensitivity to rising pressure was unchanged at 1 and 6 months after RDN (\(\Delta -0.6\pm0.4%/\text{mm Hg}\) and \(\Delta 0.4\pm0.6%/\text{mm Hg}\), respectively; \(P>0.05\)). The change in total sympathetic baroreflex sensitivity and baroreflex sensitivity to rising BP were not related to the change in SBP at 1 or 6 months. There was a trend toward a correlation between the change in SBP at 6 months and the increase in spontaneous baroreflex sensitivity to falling BP at 1 month (\(r=0.56; P=0.09\)).

**Discussion**

The novel findings of the current study were that after RDN there was (1) a small but consistent fall in SBP in SH rats, whereas
only 4 of 8 human patients responded with a >10 mm Hg fall in SBP at 6 months; (2) a decrease in BP in SH rats was mirrored by a reduction in SNA. In contrast, in human resistant hypertensive patients who responded with a decrease in SBP in response to RDN, MSNA did not necessarily fall; (3) an increase in cardiac and sympathetic baroreflex sensitivity was observed in both SH rats and human patients, which was not dependent on whether BP changed. In particular, sympathetic baroreflex control of falling BP improved in both SH rats and humans.

Figure 3. Sympathetic (A) and cardiac (B) baroreflex function curve measured in spontaneously hypertensive rats (n=7) before and after renal denervation (RDN) using intravenous phenylephrine and sodium nitroprusside injections. Note the leftward shift in both curves after RDN (#P<0.05).

Figure 4. A, Group-average cardiac spontaneous baroreflex sensitivity (sBRS) in resistant hypertensive patients (n=7) before and at 1 and 6 months after renal denervation (RDN). B, An example of the change in baroreflex gain (slope of linear regression) before and after RDN in an individual resistant hypertensive patient. Cardiac sBRS was calculated using the sequential technique. RRI indicates R-R interval; and SBP, systolic blood pressure.

Temporal Response of BP and SNA to RDN
Essential hypertension is associated with elevated SNA in both animal models and humans.31,32 It is estimated that SNA is elevated in ≈50% of hypertensive patients.12 In our cohort of hypertensive patients, MSNA was high in all patients compared with MSNA levels reported in normotensive individuals of a similar age/sex.33,34 Because elevated sympathetic activity is associated with target-organ damage,35 it is crucial that any new interventional antihypertensive treatment, such as RDN, reduces SNA, as well as SBP. In all SH rats, we found that LSNA and SBP were decreased after RDN and that these changes occurred very rapidly (within 24 hours of RDN surgery). In contrast, only 4 of 8 resistant hypertensive patients exhibited a decrease in SBP at 6 months after the procedure. In 3 of 7 patients, the decrease in SBP was accompanied by a fall in MSNA. However, in 1 patient we found that MSNA was increased, despite a decrease in SBP, whereas in another there was an increase in SBP with no change in MSNA. Importantly, we found no relationship between the change in MSNA at 1 month and the change in SBP at 6 months, suggesting that a reduction in MSNA at 1 month is not necessarily predictive of a reduction in SBP at 6 months. The patient with largest reduction in SBP had an increase in MSNA at 1 month and continued to increase at 6 months after procedure. This suggests that changes in MSNA and SBP after RDN in resistant hypertensive humans are not always linked.

Brinkmann et al18 recently reported similar results in which MSNA and SBP were not changed in parallel after RDN in resistant hypertensive patients. However, these patients had lower SBP and MSNA before RDN compared with patients in both our study and those included in the Symplicity trials.9,18,22 Despite this, the SBP outcome data at 3 to 6 months after RDN reported by Brinkmann et al18 (30% >10 mm Hg drop in SBP) are similar to that observed in the current study. In stark contrast to both our study and the recent study by Brinkmann et al.,18 Hering et al22 demonstrate that both group-average multiunit and single-fiber MSNA are reduced alongside a decrease in SBP at 3 months after RDN.

RDN in SH rats has been previously shown to reduce SBP5–7 as it has in other hypertensive models19,36,37 and provided much of the basis for targeting the renal nerves in hypertensive
Differences in the effectiveness of the RDN procedure between animals and humans might explain why BP reductions are more consistent in animals. The rare human studies that have assessed renal noradrenaline spillover of afferent denervation may cause improvements in baroreflex sensitivity but not resting BP. Improvements in baroreflex control of BP are important to prevent large surges and falls in BP. Interestingly, we found that increased sympathetic baroreflex sensitivity in rats and humans was because of an increase in sensitivity to falling arterial pressure. Therefore, falling BP will be more effectively buffered by increments in SNA after RDN. Importantly, low baroreflex sensitivity is an independent predictor of mortality and major adverse cardiovascular-related events in hypertensive patients.43 Improvements in baroreflex function after RDN may, therefore, directly improve mortality risk in these patients. This might be related to improvements in BP lability/variability,44 a known risk for target organ damage, including stroke incidence.45,46 We measured BP variability over a 10-minute quiet resting period, which was unchanged after RDN at 1 and 6 months. However, this may not be a reliable estimation of BP variability over a 24-hour period, which is highly correlated to prognostic outcome in cardiovascular disease. Furthermore, improved baroreflex function might relieve patients of the orthostatic effects of some antihypertensive medications. Interestingly, increased sympathetic baroreflex sensitivity in humans at 1 month post-RDN was associated with reduced SBP at 6 months; this may act as an early predictor of successful treatment and provide a key mechanism for the depressor response. In stark contrast to our data, Brinkmann et al18 reported no change in either cardiac or sympathetic baroreflex sensitivity after RDN. We offer several explanations: first, our patients were more hypertensive at baseline (SBP difference =11 mmHg). Second, Brinkmann et al,19 in their study, followed up once between 3 and 6 months, whereas we made 2 postprocedural measurements at 1 and 6 months. Third, group average cardiac and sympathetic baroreflex sensitivity seemed to be similar to that reported in normotensive individuals.20,47 Supporting our impression that patients selected were not severely hypertensive. All told, given the negative outcome of the study by Brinkmann et al,18 data are difficult to interpret and one cannot be sure of procedural success.
Limitations
We are aware that the small patient numbers included in the human study and the variation in comorbidities in this cohort may limit some of our conclusions. However, our data add to and support recent published data regarding the effect of RDN on BP and SNA. Furthermore, the addition of our animal cohort provides information regarding why RDN may not always be successful in humans, but is in animals. We suggest that the aggressive surgical denervation procedure possible in animal studies results in a more complete denervation, compared with the endovascular procedure used in humans.

Perspectives: One Size Does Not Fit All
Although we completed RDN in a small subset of patients, we found that only 50% of patients responded with a decrease >10 mm Hg in SBP at 6 months post-RDN. In addition, MSNA did not necessarily change in parallel to alterations in SBP after RDN in humans. The findings that the antihypertensive effect of RDN in SH rats was relatively small and the variable response observed in humans support the contention that the etiology of resistant hypertension is multifactorial. In our human study and that of others, successful RDN treatment did not lower SBP to target levels in the majority of patients (<140 mm Hg; 38% at 6 months in Symplicity-1) or reduce BP to levels that allowed removal of antihypertensive medication. Clearly, other factors are involved and may include poor cerebral perfusion, excessive carotid body activity, activation of the innate immune system, and alterations in arterial baroreceptor input. Thus, we believe that detailed autonomic and imaging (eg, renal anatomy, cerebral vessel anatomy) profiling of resistant hypertensive patients is required to provide an informed assessment of the likelihood that RDN will be successful and will lower SBP substantially toward target levels. Whether basal levels of MSNA (single and multunit) will help in predicting who will respond to certain therapies is unclear and should be tested in patients where procedural success has been confirmed.

Conclusions
We confirm that RDN clearly has benefit to resting BP and MSNA in some resistant hypertensive patients. In our patients, baroreflex sensitivity was increased, which is linked to improved prognosis and mortality risk. Before we can predict the phenotype of patients who will respond robustly to RDN, studies assessing procedural success are imperative to move this treatment forward. On-table measures of procedural success would enable the operator to optimize the ablation therapy, and noninvasive measures would enable temporal correlations of effective denervation to SBP and MSNA responses. The introduction of new catheters with multiple burn sites and different conformations may assist in this regard.

Acknowledgments
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Disclosures
J.F.R. Paton is a consultant for Cibiem Inc. The other authors report no conflicts.

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45. Rothwell PM. Limitations of the usual blood-pressure hypothesis and importance of variability, instability, and episodic hypertension. Lancet. 2010;375:938–948.


Novelty and Significance

What Is New?

- We show new translational evidence that cardiac and sympathetic baro-
reflex sensitivity are improved in both hypertensive rats and humans after renal denervation. The improvement in baroreflex sensitivity was evident at 1 month and remained sustained at 6 months after renal denervation in resistant hypertensive humans.

- The improvement in cardiac and sympathetic baroreflex sensitivity seemed to be because of increased baroreflex sensitivity to falling blood pressures. This was found in both the rats and resistant hypertensive humans.

- The decrease in blood pressure and sympathetic activity in rats was more consistent than that in humans (all animals responded with a decrease in both). In humans, blood pressure was not reduced in all patients. In addition, alterations in sympathetic nerve activity did not appear to be in sync with changes in blood pressure. That is blood pressure reduced in some patients 1 and 6 months after renal denervation but sympathetic nerve activity increased progressively.

What Is Relevant?

- Improvements in baroreflex function after renal denervation in resistant hypertensive humans may help in improving the ability to buffer blood pressure during hypotensive episodes caused by some antihypertensive medications.

- Increased baroreflex sensitivity has been linked to improved cardiovas-
cular risk and reduced rates of mortality.

- Differences between rat and human blood pressure reductions and changes in sympathetic activity support the contention that hypertension is multifactorial in humans. Thus, renal denervation may not help to decrease sympathetic activity (a significant contributor to end-organ damage) and blood pressure in all resistant hypertensive patients.

Summary

This is the first translational study that compares the response of blood pressure, sympathetic activity, and baroreflex sensitivity in spontaneously hypertensive rats after renal denervation to that in resistant hypertensive humans. Although renal denervation was of clear benefit to baroreflex function in rats and more importantly hu-
mans, not all humans responded with a decrease in blood pressure and sympathetic activity. The disparity between changes in blood pressure and sympathetic nerve activity in humans suggests that we do not fully understand the mechanism underlying reduction in blood pressure after renal denervation in humans. Furthermore, we provide additional evidence that renal denervation does not work to decrease blood pressure in all resistant hypertensive men and women.
Translational Examination of Changes in Baroreflex Function After Renal Denervation in Hypertensive Rats and Humans

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TRANSLATIONAL EXAMINATION OF CHANGES IN BAROREFLEX FUNCTION AFTER RENAL DENERVATION IN HYPERTENSIVE RATS AND HUMANS

Supplementary Methods and Tables (S1 and S2)
Human Study

Microneurography

A muscle sympathetic fascicle was identified when taps on the muscle belly or passive muscle stretch evoked mechanoreceptive impulses, in the absence of neural activation associated with sensory stimuli (Sundlof and Wallin, 1977). The recorded signal was amplified 80,000 fold, band pass filtered (700 to 5000 Hz), rectified and integrated (time constant 0.1 s) by a dedicated amplifier (Absolute Design and Manufacturing Services, Iowa).

Following instrumentation, 10 minutes of resting baseline data were collected in all patients. Heart rate, blood pressure and MSNA were measured and recorded continuously using a data acquisition program on a study laptop (LabChart, AD Instruments).

Data analyses

Data were sampled at 1000 Hz (LabChart, AD Instruments) and stored on a personal computer for off-line analysis. Mean arterial pressure was calculated as the time integral over the pressure pulse. Sympathetic bursts in the integrated neurogram were identified using a custom-manufactured automated analysis program (Kienbaum et al., 2001); burst identification was then corrected by inspection by a single observer. The program then compensated for baroreflex latency and associated each sympathetic burst with the appropriate cardiac cycle and DBP.

Spontaneous baroreflex sensitivity

The spontaneous cardiac baroreflex was assessed using the sequence technique (Bertinieri et al., 1988). Briefly, sequences of four or more successive heart beats in which there were simultaneous increases or decreases in SBP and RR interval were selected using Hemolab Software Suite (http://www.haraldstauss.com/HemoLab/HemoLab.php). A linear regression was applied to each of the sequences and an average regression slope was calculated for the sequences detected during each recording period. This slope represents the spontaneous cardiac baroreflex sensitivity. Data regarding the slope of the cardiac baroreflex were averaged across falling and rising SBP.

Spontaneous sympathetic baroreflex sensitivity was calculated by associating spontaneous fluctuations in diastolic blood pressure (DBP) to the occurrence of bursts of MSNA. The analysis that we used has been termed “threshold analysis” and has been described in detail (Kienbaum et al., 2001, Hart et al., 2010). In brief, DBP for each cardiac cycle (recorded during the baseline period) were grouped into blood pressure bins of 1 mmHg. The % of heart beats associated with a burst in each of these blood pressure bins was calculated and associated with the mean DBP in the corresponding bin. The slope of the relationship between the mean DBP and mean MSNA for each DBP bin was calculated using linear regression. The slope of this relationship has been previously shown to agree with the baroreflex sensitivity calculated during a modified Oxford baroreflex test (Hart et al., 2010).
We completed this analysis on all DBP values over the full 10 minute baseline to obtain a baroreflex sensitivity which was not dependent on whether DBP was increasing or decreasing (this reflected total baroreflex sensitivity). We then completed the same analysis specifically during spontaneous increases or decreases in DBP. More specifically, to calculate spontaneous baroreflex sensitivity to rising arterial pressure, we used cardiac cycles that were preceded by a cardiac cycle with a lower DBP. For spontaneous sympathetic baroreflex sensitivity to falling arterial pressures we used cardiac cycles which were preceded by cardiac cycles with a higher DBP. In total this meant that 2 cardiac cycles were included in our analysis. Linear regression analyses were weighted for the number of cardiac cycles within each DBP bin, and a minimum $r$ value of 0.5 was used as the criteria for accepting slopes.

**Statistics**

Data were analysed statistically using commercially available software (Sigma Stat 2.03, SPSS Inc, Chicago, IL). Group data are expressed as means ± SEM. A one-way analysis of variance (ANOVA) was used to measure differences in baseline hemodynamic variables pre-, one month and 6 months post-RDN. Two-way repeated measures ANOVA was used to examine whether mean baroreflex sensitivity (slope of linear regression) was different in response to increasing and decreasing arterial pressures within and between each group. To measure whether there was a relationship between changes in hemodynamic variables and changes in baroreflex sensitivity to increasing following RDN, linear regression analysis was performed and Pearson’s correlation coefficients calculated. The alpha-level was set at 0.05.

**Spontaneously Hypertensive Rat Study**

**Surgical Procedures**

Rats were anaesthetised with ketamine (60 mg kg$^{-1}$, I.M.) and medetomidine (250 μg kg$^{-1}$, I.M.) and strict aseptic techniques employed. A blood pressure catheter was introduced and fixed into the abdominal aorta as described previously. Following the blood pressure surgery, the aorta was gently and non-occlusively retracted to expose the left lumbar sympathetic trunk and the nerve carefully placed over the recording electrodes (Model TR46SP, Telemetry Research Ltd, Auckland, New Zealand) and isolated from surrounding tissue with a silicone elastomer. Finally, the femoral vein was catheterised for continued access as described in the supplementary methods. At least 7 days were allowed for recovery before a 3 day baseline period was recorded.

On Experimental Day 0 the animals were anesthetised and underwent bilateral RDN. Through a retroperitoneal incision, the nerves and adventitia were stripped from the renal artery and renal plexus, and painted with a dilute (10% in ethanol) phenol solution. The wounds were closed and animals returned to their home cage. Blood pressure and heart rate monitoring continued for a further 9 days. On day 9 the animals were euthanized (sodium pentobarbital 40mg/kg i.p.) and the kidneys extracted, weighed and frozen in liquid nitrogen for a subsequent renal tissue noradrenaline assay (Alpco Diagnostics, USA, No 17-NORHU-E01-RES)
Lumbar Nerve surgery
Through the abdominal incision the aorta was gently and non-occlusively retracted to expose the left lumbar sympathetic trunk. Using a fine round-tipped glass hook the nerve was freed from the surrounding connective tissue, and a small patch of parafilm slipped underneath to prevent fluid ingress. Electrodes from the SNA telemeter (Model TR46SP, Telemetry Research Ltd, Auckland, New Zealand) were carefully fixed in place using sutures and tissue adhesive, and the nerve passed over the hooks. The hooks and nerve were then isolated from surrounding tissue with silicone elastomer (Kwik-Sil, WPI Inc.). Finally, a polyurethane catheter (0.033 in (~0.84 mm) OD; 0.014 in (~0.36 mm) ID; Micro-Renathane, Braintree Scientific) was advanced into the left femoral vein so the tip was just above the femoral bifurcation. The catheter tip was coated with a heparin complex (Polysciences Inc., Warrington, PA, USA). The catheter was tunneled subcutaneously and connected to a custom-built stainless-steel connector (23G) exteriorized between the scapulas, as described previously (Abdala et al., 2006). The catheter was filled with saline containing heparin (100 U ml\(^{-1}\)) and penicillin G (2000 U ml\(^{-1}\)) solution. Indwelling catheters were flushed with the latter solution once a week.

Renal Noradrenaline Content
Briefly, the left kidneys were crushed on liquid nitrogen and homogenised in an extraction buffer (0.01M HCl, 1mM EDTA, 4mM sodium matabisulfate). Samples were then centrifuged (8000rpm for 30 min) and the supernatant protein concentration determined by DC-Protein assay (BioRad, UK, No 500-0112). Tissue noradrenaline content was assessed by ELISA (Alpco Diagnosics, USA, No 17-NORHU-E01-RES) and normalised to protein concentration.

Baroreceptor Reflex Analysis
These data were then used to generate (5-parameter sigmoidal regression) baroreflex function curves, which define the baroreceptor reflex curves in terms of the range, lower plateau (ie the minimum heart rate attained with increases in BP), BP50 and fitting parameters which reflect the steepness of the upper and lower curvature (which correlates with the upper and lower gain; see Ricketts and Head 30 for full description). Following the vasoactive drug infusions (rats were given an i.v. bolus of the ganglionic blocker hexamethonium tartrate (10mg/kg) to examine global sympathetic vasomotor tone and noise level in the neurogram.

Data Analysis
Arterial pressure and lumbar sympathetic nerve activity (LSNA) signals were sampled at 500Hz using an analog-digital data acquisition card (PCI 6024E National Instruments, Austin, Texas) and continuously displayed by a data acquisition program (Universal Acquisition 11, University of Auckland, Auckland, New Zealand). Heart rate (HR) was derived from the arterial pressure waveform. The original SNA signal was amplified, filtered between 50-5000 Hz, full-wave rectified and integrated using a low pass filter with a 20ms time constant. Arterial pressure and heart rate were recorded for 24 hours a day, while to avoid movement artefact, lumbar SNA was recorded for an hour at the same time each day while the rats (undisturbed in their home cage) were observed to be quiescent. On days -2 and 7 rats were exposed to a brief burst of high frequency noise, which produces a stress increase in
sympathetic outflow. The magnitude of the response to noise stress was used to check that the LSNA signal remained stable and responsive throughout the experiment.

**Statistical Analysis**
Data was analysed using a one-way repeated measures ANOVA. Where appropriate, post-hoc comparisons were made between Baseline and RDN time points, using the Holm-Sidak Procedure. For the Baroreflex curves, the individually fitted curve parameters were averaged across the group statistically compared before and after RDN as above, and averaged group curves drawn.
**Supplementary Table 1 (S1):** Co-morbidities in resistant hypertensive patients

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<th>Sleep apnoea</th>
<th>Smoker</th>
<th>LVH</th>
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<td>Y</td>
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<td>N</td>
<td>N</td>
<td>Ex</td>
<td>Y</td>
<td>Controlled T2</td>
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</table>

AMI; acute myocardial infarction, TIA; trans-ischemic attack, COPD; chronic obstructive pulmonary disorder (includes sleep apnoea), Ex; ex-smoker, LVH; left ventricular hypertrophy, T2; type 2 diabetes mellitus.
**Supplement Table 2 (S2):** Averaged fitting parameters for 5p sigmoid baroreflex function curves derived from phenylephrine and sodium nitroprusside-induced ramps in arterial pressure (Oxford method) before and after RDN.

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<tr>
<th>Baroreflex curve Parameters</th>
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<th>Sympathetic</th>
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<td></td>
<td>BL</td>
<td>RDNX</td>
<td>p-value</td>
<td>BL</td>
<td>RDNX</td>
<td>p-value</td>
</tr>
<tr>
<td>P1 (lower Plateau)</td>
<td>314±9</td>
<td>289±10*</td>
<td>0.0360</td>
<td>34±8</td>
<td>33±8</td>
<td>0.6533</td>
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<tr>
<td>P2 (Range)</td>
<td>86±11</td>
<td>103±26</td>
<td>0.4528</td>
<td>118±8</td>
<td>107±7*</td>
<td>0.0175</td>
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<td>P3 (upper gain)</td>
<td>-0.16±0.08</td>
<td>-0.23±0.09</td>
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<td>-0.59±0.08*</td>
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<tr>
<td>P4 (BP50)</td>
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<td>128±2*</td>
<td>0.0318</td>
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<td>p5 (lower gain)</td>
<td>-0.20±0.06</td>
<td>-0.24±0.11</td>
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<td>Rest BP (mmHg)</td>
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<td>131±6**</td>
<td>0.0072</td>
<td>137±5</td>
<td>130±6*</td>
<td>0.0108</td>
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<td>Rest HR (beats/min)</td>
<td>335±17</td>
<td>347±11</td>
<td>0.2053</td>
<td>107±2</td>
<td>83±3*</td>
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