Unilateral renal denervation improves autonomic balance in conscious rabbits with chronic heart failure

Alicia M. Schiller, Karla K. V. Haack, Peter R. Pellegrino, Pamela L. Curry, and Irving H. Zucker

Department of Cellular and Integrative Physiology, The University of Nebraska Medical Center, Omaha, Nebraska

Submitted 30 May 2013; accepted in final form 28 August 2013

Unilateral renal denervation improves autonomic balance in conscious rabbits with chronic heart failure. Am J Physiol Regul Integr Comp Physiol 305: R886–R892, 2013. First published September 4, 2013; doi:10.1152/ajpregu.00269.2013.—A hallmark of chronic heart failure (CHF) is an increased sympathetic tone resulting in autonomic imbalance. Renal denervation (DNx) in CHF patients has resulted in symptomatic improvement, but the protective mechanisms remain unclear. We hypothesized in CHF, unilateral renal DNx would improve cardiac autonomic balance. The present study used conscious, chronically instrumented New Zealand White rabbits undergoing renal DNx prior to pacing-induced CHF. Four treatment groups were used: nonpace, non-DNx [ Sham-Innervated (Sham-INVI)], nonpace DNx (sham-DNx), pace non-DNx (CHF-INV) or pace DNx (CHF-DNx). We examined several markers indicative of autonomic balance. Baroreflex sensitivity and time domain heart rate variability (HRV) were both decreased in the CHF-INV group compared with sham-INVI and were restored to sham levels by renal DNx. Power spectral analysis indicated an increase in low-frequency/high-frequency (LF/HF) ratio in the CHF-INV compared with the sham-INVI, which was normalized to sham levels by DNx. To assess whether this was due to a withdrawal of sympathetic tone or an increase in parasympathetic tone, the heart rate response was measured after an intravenous bolus of metoprolol or atropine. Bradycardia induced by intravenous metoprolol (indicative of cardiac sympathetic tone) was exacerbated in CHF-DNx rabbits compared with sham-INVI but was normalized in CHF-DNx. Conversely, the tachycardia in response to intravenous atropine (indicative of cardiac vagal tone) was not improved in CHF-DNx vs. CHF-INV animals. Renal DNx also prevented the increase in circulating plasma NE seen in CHF-INV rabbits. These results suggest renal DNx improves cardiac autonomic balance in CHF by a reduction of sympathetic tone.

Renal denervation can greatly influence renal function because the kidney is innervated by both effenter and afferent nerve fibers (8, 3). Activation of the renal efferent nerve fibers results in an increase in sodium reabsorption, renin secretion, and decreased renal blood flow and, therefore, may influence glomerular filtration rate (9). Activation of the renal afferent nerves has been implicated in the modulation of hypothalamic activity and the reflex influence of sympathetic outflow to organs, such as the heart and kidney (33). These mechanisms may be beneficial homeostatic measures during an initial decrease in cardiac output. However, a chronic decrease in cardiac output (such as in CHF) can lead to a vicious cycle, in which tonic increases in sympathetic outflow contribute to a state of autonomic imbalance and progression of the disease.

Markers of autonomic imbalance, such as a depressed baroreflex function, reduction of heart rate variability (HRV), and elevated plasma NE have been observed (6, 11, 30) in CHF and other diseases with excessive sympathetic tone such as hypertension.

Previous studies from our laboratory have shown that there is a decrease in renal blood flow and an increase in ANG II type 1 receptor expression in the renal cortex of rabbits with CHF. Unilateral renal DNx effectively restored both of these parameters to normal in the DNx kidney (5). Therefore, we utilized this model to examine whether unilateral renal DNx evoked global changes in sympathetic tone and autonomic imbalance. On the basis of previous studies involving bilateral renal DNx in hypertension and the high level of sympathetic tone present in CHF (30), we hypothesized that renal DNx would improve autonomic balance in CHF by a withdrawal of excessive sympathetic tone. We examined sham and CHF rabbits to determine whether unilateral renal DNx impacts autonomic function and balance.

METHODS

Animals. Experiments were carried out on 19 male New Zealand White rabbits ranging in weight from 3.7 to 3.9 kg (Charles River Laboratories, Wilmington, MA). All experiments were reviewed and approved by the University of Nebraska Medical Center Institutional Animal Care and Use Committee.

Animals underwent two separate surgeries and were included in two experimental groups when possible, as indicated in the experimental timeline shown in Fig. 1.

Surgical procedures. The model used to induce CHF and for renal DNx has been used previously in our laboratory (5). In brief, rabbits were instrumented with a radiotelemetry device placed in the right femoral artery (model TA11PA-C40; Data Sciences International, Minneapolis, MN) to measure pulsatile and mean arterial pressure (MAP) and heart rate (HR) in the conscious state. At the same time, a left thoracotomy was performed and a platinum wire-pacing electrode was placed on the left ventricle of the heart and a ground wire secured to the left atrial appendage. The chest was evacuated and closed. The pacing wires were tunneled beneath the skin and exited in
the midscapular region. The thoracotomy was closed in layers. No
earlier than 2 wk after this initial surgery, the renal DNx maneuver
was performed. Through a left flank incision, the left kidney was
identified, and the renal artery and vein were isolated. Using surgical
microscopy, we carefully removed all visible renal nerves from the
length of the artery and vein. The hilus of the kidney was cleared of
fat and any visible nerve fibers. The rabbits were allowed to recover
following an intravenous infusion of sodium nitro-
prusside (100 µg/kg) at a rate of 0.5 ml/min until MAP reached ~50
mmHg (~3–4 min). An infusion of phenylephrine (80 µg/kg) was
then started at the same rate and continued until the MAP reached
~100 mmHg (~4 min).

Arterial baroreflex curves were constructed by taking an average
HR every 5 s from the lowest MAP to the highest MAP achieved in
each experiment. The data were fit to a logistic regression, as previ-
ously described by Kent et al. (21) and used by our laboratory in
previous studies (15, 20). The following equation was used to fit the
data: \( HR = A/\left(1 + exp\left[B(MAP - C)\right]\right) + D \), where \( A \) is the HR
range, \( B \) is the slope coefficient, \( C \) is the pressure at the midpoint
of the range (BP50), \( D \) is the minimum HR, and MAP is mean arte-
trial pressure. The peak slope (or maximum gain) was determined
by taking the first derivative of the baroreflex curve as described
by \( Slope = A \times B \times \exp\left([B(MAP - C)]/(1 + \exp[B(MAP - C)]\right) \).

The mean values (see Table 1 and Fig. 4A) of each parameter
generated from individual curves in each group of rabbits were used
to derive the composite curves presented in Fig. 3, B and C.

*Autonomic blockade and HR change. On a separate day, after an
initial 15-min acclimation period, a bolus intravenous injection of
either 0.2 mg/kg atropine methyl bromide (25) or 1.0 mg/kg meto-
prolol bitartrate was given (15, 20). An additional 10 min of baseline
data was recorded. The last 5 min of this and the initial baseline
recording were used to calculate the change in HR following auto-
nomic blockade with atropine or metoprolol.

Heart rate variability. An ECG was recorded directly from the
cardiac pacing leads. The 5-min baseline period used for calculating
baseline HR and MAP was also used to calculate HRV. The analysis
was performed using the HRV module from LabChart 7 software
(ADInstruments, Colorado Springs, CO). Total power was calculated
as the integral over the entire power spectrum as an index of HRV.

The standard deviation of the normal R-R intervals (SDNN) was
calculated. Ectopic beats were excluded from the analysis using
cycle-length cutoff of <150 and >350 ms for ectopics and <100 and
>400 ms for artifacts (29). To perform spectral analysis of HRV the
normal R-wave–R-wave tachograms were linearly resampled and
transformed into the frequency domain using Welch’s method with
50% overlapping, 1,024-point Hann windows. The power of the
low-frequency (0.0625–0.1875 Hz) and high-frequency (0.1875–
0.5625 Hz) bands was then used to calculate the LF/HF ratio (24, 13).
Although some studies have cast doubt on the specificity of the
low-frequency (18) and high-frequency (36) spectral components of
HRV for the cardiac sympathetic and cardiovagal outflow, the LF/HF
ratio remains a widely used index of sympathovagal balance, with the
high-frequency component representing vagal activity and the low-
frequency component being more representative of sympathetic ac-
tivity (1).

Renal DNxs confirmation/plasma NE analysis. At the conclusion of
the study, kidneys from several rabbits were randomly selected for
renal cortical NE analysis. Our laboratory has previously used this
procedure and showed a significant decrease in tissue NE levels in the
DNx kidney (5). Samples of cortical tissue were homogenized and
processed to perform an ELISA (GenWay Biotech, San Diego, CA)

Table 1. Parameters used to generate composite baroreflex curves in Fig. 3, B and C

<table>
<thead>
<tr>
<th></th>
<th>Min HR, bpm</th>
<th>BP50, mmHg</th>
<th>Slope coefficient, 1/(s·slope)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-INV</td>
<td>183.99 ± 19.9</td>
<td>76.40 ± 2.5</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>CHF-INV</td>
<td>247.88 ± 15.1*</td>
<td>75.70 ± 7.9</td>
<td>0.11 ± 0.03</td>
</tr>
<tr>
<td>Sham-DNx</td>
<td>115.24 ± 29.7*</td>
<td>67.24 ± 6.0</td>
<td>0.06 ± 0.01*</td>
</tr>
<tr>
<td>CHF-DNx</td>
<td>39.52 ± 24.6†</td>
<td>93.01 ± 11.0†</td>
<td>0.05 ± 0.01*</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. CHF, chronic heart failure; INV, inner\nsed; Dnx, denervation; HR, heart rate; BP, blood pressure. *P < 0.05
vs. Sham-INV. †P < 0.05 vs. CHF-INV. n = 5–7/group.
for NE. To normalize the NE content to protein concentration, a protein assay kit (Pierce, Rockford, IL) was used according to the manufacturer’s directions. At the end of the study, NE tissue levels were compared between the left (DNx) and right (INV) kidney in three rabbits.

During the course of both sham and CHF experimental periods, blood samples were obtained by placing a catheter in a central ear artery. Approximately 3 ml of whole blood was collected and immediately placed on ice in a heparinized collection tube. No more than 30 min later, the blood was centrifuged at 1,000g for 15 min. The plasma was collected and stored at −80°C. Plasma NE levels were measured using the same ELISA procedure as described above.

Statistical analysis. Data are expressed as the means ± SE. All HR and MAP data were obtained with the use of a 16-channel Powerlab (model 16 SP; ADInstruments). Differences between groups were determined with a one-way ANOVA followed by a post hoc analysis using the Bonferroni correction. A paired t-test was used to determine differences in renal cortical NE content (GraphPad Prizm 4). A value of P < 0.05 was considered statistically significant.

RESULTS

Baseline hemodynamics. One to two weeks after rapid ventricular pacing, rabbits exhibited signs of impaired cardiac function, as validated by echocardiography. Left ventricular end-systolic dimensions and left ventricular end-systolic volume were increased in both CHF groups compared with their respective Sham group. EF and FS were also decreased in all CHF animals compared with their respective Sham group (Table 2). As reported previously in this model of CHF, the rapid ventricular pacing protocol produces an increased HR and decreased MAP in CHF compared with Sham rabbits (5, 20). Renal DNx did not alter any of the baseline hemodynamic parameters.

Renal cortical NE content. The renal DNx surgical maneuver performed in our laboratory has previously been validated to be an effective means of removing the renal nerves (5) by showing a significant decrease in renal cortical NE levels in the DNx kidney compared with the contralateral innervated side. In the current study, we found a pronounced decrease in renal cortical NE content of the left/DNx kidney compared with the contralateral kidney in the same animals, indicating complete and prolonged renal DNx (Fig. 2A).

Circulating plasma NE levels. To evaluate whether unilateral renal DNx could reduce global sympathetic tone, circulating plasma, NE was measured (Fig. 2B). As expected, the CHF-INV group showed an increase in plasma NE compared with Sham-INV rabbits. Renal DNx prevented the increase in plasma NE in CHF rabbits.

Arterial baroreflex function. We evaluated baroreflex function as it is known to be depressed in CHF and other states of autonomic imbalance (11, 27, 30). Representative tracings from arterial baroreflex experiments are shown in Fig. 3A. Composite arterial baroreflex curves for HR and MAP are shown in Fig. 3B. Interestingly, baroreflex function was enhanced by renal DNx treatment in both treatment groups (i.e., sham and CHF states). The Sham-DNx group (left) exhibited an increased baroreflex function compared with the Sham-INV. The depressed baroreflex function observed in the CHF-INV group (right) was enhanced in the CHF-DNx group. Mean data for HR range and maximal gain are shown in Fig. 4. Renal DNx increased baroreflex HR range in the Sham-DNx group compared with Sham-INV (Fig. 4A). As expected, the CHF-INV group exhibited a decrease in HR range compared with Sham-INV, however, this was restored to Sham levels in the CHF-DNx group. Similar results were observed for the maximal baroreflex gain (peak slope: Fig. 4B), indicating that renal DNx enhances baroreflex function and abolishes the blunted baroreflex response normally observed in CHF-INV animals.

Table 2. Baseline hemodynamic parameters in Sham-INV, CHF-INV, Sham-DNx, and CHF-DNx rabbits

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham-INV</th>
<th>CHF-INV</th>
<th>Sham-DNx</th>
<th>CHF-DNx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>3.7 ± 0.1</td>
<td>3.8 ± 0.2</td>
<td>3.9 ± 0.1</td>
<td>3.9 ± 0.1</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>216 ± 10</td>
<td>258 ± 11*</td>
<td>196 ± 3.0</td>
<td>247 ± 5.0*</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>75.4 ± 5.3</td>
<td>65.5 ± 1.9*</td>
<td>71.2 ± 5.2</td>
<td>67.9 ± 3.6</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>16.8 ± 0.3</td>
<td>17.7 ± 0.5</td>
<td>15.9 ± 0.5</td>
<td>18.2 ± 0.4</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>11.6 ± 0.3</td>
<td>14.2 ± 0.6*</td>
<td>8.6 ± 2.0</td>
<td>17.7 ± 0.5*</td>
</tr>
<tr>
<td>LVd Vol, ml</td>
<td>8.2 ± 0.5</td>
<td>9.5 ± 0.7</td>
<td>8.2 ± 0.5</td>
<td>9.9 ± 0.5</td>
</tr>
<tr>
<td>LVsVol, ml</td>
<td>3.22 ± 0.1</td>
<td>5.4 ± 0.6*</td>
<td>3.1 ± 0.2</td>
<td>6.1 ± 0.3*</td>
</tr>
<tr>
<td>FS, %</td>
<td>31.1 ± 1.1</td>
<td>19.8 ± 1.2*</td>
<td>32.0 ± 1.4</td>
<td>17.4 ± 1.2*</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>62.4 ± 1.0</td>
<td>38.9 ± 1.1*</td>
<td>62.4 ± 1.0</td>
<td>43.5 ± 2.0*</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. MAP, mean arterial pressure; LV, left ventricle; LVEDD, left ventricular end-diastolic diameter; LVEDD, left ventricular end-systolic diameter; LVd, left ventricle diastolic; LVs, left ventricle, systolic; FS, fractional shortening. *P < 0.05 vs. respective sham group. n = 7/group.
Heart rate variability. Time and frequency domain of HRV was used as a reflection of autonomic balance in the heart. Therefore, we examined the effects of renal DNx on this parameter in animals with and without CHF. Fig. 5A shows representative Poincaré plots visualizing beat-to-beat HRV generated from ECG recordings from all groups of rabbits, and Fig. 5B shows summary HRV (SDNN). In CHF-INV rabbits, there was a profound depression in HRV compared with Sham-INV. However, in the CHF-DNx group, HRV was restored to sham levels. Renal DNx in the sham group did not affect HRV. Power spectral analysis was also performed (Fig. 5C) to examine any changes in the frequency domain of HRV. Frequency domain analysis indicated an increase in the LF/HF power spectral density ratio in the CHF-INV group compared with both sham groups. This increase in ratio was prevented in the CHF-DNx group. While the total power of the HRV spectrum was not different between groups by one-way ANOVA \((P = 0.11)\), there was a trend toward lower total power in the CHF-INV group (36.8 ± 11.5 ms\(^2\)) compared with both their CHF-DNx counterparts (219.3 ± 51.1 ms\(^2\)), as well as both the Sham-INV (273.8 ± 65.0 ms\(^2\)) and Sham-DNx (381.8 ± 104.8 ms\(^2\)) groups.

Vagal and sympathetic components of resting heart rate. To further investigate whether the changes observed in baroreflex function and HRV were due to a withdrawal of sympathetic tone, an increase in parasympathetic tone or both, we evaluated the change in resting HR in response to autonomic blockade (Fig. 6). In CHF-INV animals, the bradycardia in response...
metoprolol was enhanced compared with the Sham-INV group (Fig. 6A). However, this exaggerated response was attenuated in the CHF-DNx animals and restored to the response seen in the Sham-INV group. There were no differences between the Sham-INV and Sham-DNx groups. When the same protocol was performed following atropine (Fig. 6B), we observed a decreased tachycardia in the CHF-INV compared with Sham-INV group. This same trend was observed in the CHF-DNx groups, indicating no differences in the vagal contributions to resting HR in the CHF-DNx group.

**DISCUSSION**

The results of the present study show that unilateral renal DNx is sufficient to reduce local and global sympathetic outflow (Fig. 2), as evident by a reduction in both renal cortical NE levels and plasma NE levels and improved autonomic balance (Figs. 3–5) in CHF. Our data suggest that renal DNx evokes these protective effects mainly by mediating a reduction in sympathetic tone (Fig. 6).

Over the past several years, a growing body of literature has focused on the use of renal DNx as a therapy for drug-resistant hypertension (12, 23, 28, 32). These studies point to a profound and prolonged reduction in arterial pressure, and there is also some evidence to suggest a reduction in sympathetic outflow (31). Because increased sympathoexcitation characterizes both hypertension and CHF, the efficacy of this procedure in the setting of CHF has begun to be evaluated more extensively. Recent studies have shown benefit for using bilateral renal DNx in both animal and patient models of CHF (7, 19). The REACH pilot study provides evidence that in patients with chronic systolic heart failure, bilateral renal DNx increases exercise tolerance and provides patients with symptomatic improvement. Surprisingly, MAP in these CHF-DNx patients was maintained and did not decrease over a period of 6 mo (7). Given, however, that previous studies in hypertensive patients indicated that bilateral renal DNx continually lowered arterial pressure for up to 24 mo, additional studies will have to be performed in the CHF cohort to confirm that blood pressure is...
maintained for a longer time frame post-renal DNx (34). Previous studies from our laboratory have shown that in CHF, there is an increased level of ANG type 1 receptor and a deceased level of ANG type 2 receptor in the renal cortical vasculature of CHF rabbits (5). Unilateral renal DNx was able to locally restore this imbalance in the DNx kidney. Therefore, we investigated whether these local changes produced by unilateral renal DNx were also producing global changes in autonomic balance in CHF. The current study provides evidence that DNx of a single kidney restores autonomic balance and improves baroreflex function; future studies comparing the potential additive effects of unilateral and bilateral renal DNx, as well as unilateral renal DNx, as an alternative to bilateral renal DNx should be explored.

The rapid ventricular pacing protocol resulted in reduced cardiac function validated by a significant decrease in EF by echocardiography. It is of interest that renal DNx in our model did not lead to improvements in cardiac function (Table 1), even though it appears that cardiac and global sympathetic tone is decreased. These results are consistent with previous data from our laboratory using this model (4). The reasons for this are not readily apparent; however, these effects may be model specific, as cardiac pacing was continued throughout the duration of the study by an external stimulus. Therefore, improvements in cardiac function are unlikely to be observed unless the pacing paradigm is discontinued. Secondly, it is possible that the effects of renal DNx on cardiac function take a longer period of time to manifest. This aspect of renal DNx was not evaluated in this study. For future studies, adaptations to our model protocol could be implemented to better observe changes in cardiac function. We are also planning future studies in which renal DNx will be performed after the development of CHF to further increase the clinical relevancy. Technical challenges in reanesthetizing sick CHF rabbits to perform renal DNx prevented collection of these data at the current time.

We and others have consistently shown that baroreflex function is impaired in the setting of CHF and may contribute to the sympathoexcitatory state (11, 22, 26). In the current study, the baroreflex HR range and maximal gain were increased in CHF animals following renal DNx. These data point to important differences in baroreflex-evoked changes in HR compared with resting baseline HR measurements, as renal DNx did not influence resting HR values in either the Sham or CHF groups. This may also reflect the fact that animals were continuously paced throughout the duration of the study.

In the present study, renal cortical NE measurements taken at the conclusion of the study or ~6 wk after renal DNx show virtually no NE present in the renal cortex, indicating little or no sympathetic innervation and no efferent reinnervation over this period of time (Fig. 2). We did not evaluate the presence of afferent innervation and the conclusion of the study and, therefore, cannot definitively say that afferent function was not restored over this period of time or make conclusions regarding the effect of efferent or afferent input separately.

If some of the effects of renal DNx are mediated by interruption of renal afferent nerves, this may lead to a reflexive decrease in sympathetic outflow, as increases in afferent nerve activity can evoke increases in efferent renal sympathetic nerve activity (10). It is not known whether there are compensatory changes in contralateral efferent renal nerve activity in the time frame studied in our protocol. Removal of afferents from one kidney is likely to affect a sympathetic excitatory reflex that may be more active in the setting of CHF, as has been shown for cardiac sympathetic afferents (37).

We observed an increase in baroreflex function in the Sham-DNx group compared with the Sham-INV group when we used a pharmacological approach to assess baroreflex function (Fig. 3B). Therefore, it is possible that there is a withdrawal in sympathetic tone or an increase in vagal tone in the sham state following DNx. However, changes in resting HR in response to atropine and metoprolol do not support this notion. Further investigation is required to determine whether renal DNx changes autonomic balance under nonpathophysiological conditions.

**Perspectives and Significance**

The current study shows that three independent measures of sympathovagal balance indicate that unilateral renal DNx prevents cardiac autonomic imbalance and decreased baroreflex function in rabbits with pacing-induced CHF. Autonomic blocker data demonstrate that this is through a sympatholytic effect. Unilateral renal denervation also had no effect on MAP in our model. This study raises the possibility that unilateral renal DNx may be sufficient to observe beneficial therapeutic effects in CHF. This is an important clinical application as a reduction in MAP in CHF, even if accompanied with a reduction in sympathetic tone, would not be a desirable outcome. The clinical implications of this finding suggest that unilateral renal DNx therapy may be effective in reducing both global and local sympathetic tone in CHF patients.

**ACKNOWLEDGMENTS**

We thank Ms. Johnnie F. Hackley for her excellent technical assistance in performing the echocardiographic measurements.

**GRANTS**

This work was supported by a National Institutes of Health National Heart, Lung, and Blood Institute grant PO1 HL-62222. A. M. Schiller was partially supported by an American Heart Association predoctoral fellowship, Midwest affiliate.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

Author contributions: A.M.S. and P.L.C. performed experiments; A.M.S. and P.R.P. analyzed data; A.M.S., K.K.V.H., P.R.P., and I.H.Z. interpreted results of experiments; A.M.S. prepared figures; A.M.S. and I.H.Z. drafted manuscript; A.M.S., K.K.V.H., and P.R.P. contributed to the preparation of the manuscript; A.M.S. and I.H.Z. approved final version of manuscript; I.H.Z. conceived and designed the study.

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RENAL DENERVATION AND AUTONOMIC BALANCE IN HEART FAILURE


