Renal denervation improves cardiac function in rats with chronic heart failure: Effects on expression of β-adrenoceptors

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Zheng H, Liu X, Sharma NM, Patel KP. Renal denervation improves cardiac function in rats with chronic heart failure: Effects on expression of β-adrenoceptors. Am J Physiol Heart Circ Physiol 311: H337–H346, 2016. First published June 10, 2016; doi:10.1152/ajpheart.00999.2015.—Chronic activation of the sympathetic drive contributes to cardiac remodeling and dysfunction during chronic heart failure (HF). The present study was undertaken to assess whether renal denervation (RDN) would abrogate the sympathoexcitation in HF and ameliorate the adrenergic dysfunction and cardiac damage. Ligation of the left coronary artery was used to induce HF in Sprague-Dawley rats. Four weeks after surgery, RDN was performed, 1 wk before the final measurements. At the end of the protocol, cardiac function was assessed by measuring ventricular hemodynamics. Rats with HF had an average infarct area >30% of the left ventricle and left ventricular end-diastolic pressure (LVEDP) >20 mmHg. β1- and β2-adrenoceptor proteins in the left ventricle were reduced by 37 and 49%, respectively, in the rats with HF. RDN lowered elevated levels of urinary excretion of norepinephrine and brain natriuretic peptide levels in the hearts of rats with HF. RDN also decreased LVEDP to 10 mmHg and improved basal dP/dt to within the normal range in rats with HF. RDN blunted loss of β1-adrenoceptor (by 47%) and β2-adrenoceptor (by 100%) protein expression and improved isoproterenol (0.5 μg/kg)-induced increase in +dP/dt (by 71%) and −dP/dt (by 62%) in rats with HF. RDN also attenuated the increase in collagen 1 expression in the left ventricles of rats with HF. These findings demonstrate that RDN initiated in chronic HF condition improves cardiac function mediated by adrenergic agonist and blunts β-adrenoceptor expression loss, providing mechanistic insights for RDN-induced improvements in cardiac function in the HF condition.

renal nerve; sympathetic nerve activity; cardiac function; heart failure

NEW & NOTEWORTHY

This study shows in a comprehensive way that renal denervation initiated after a period of chronic heart failure enhances cardiac contractility and the responsiveness of hearts to isoproterenol stimulation, and these effects are due in part to renal denervation-induced increase in β-adrenoceptor protein and function.

WORLDWIDE, MORE THAN 100 MILLION people are afflicted with chronic heart failure (HF), imposing a significant burden on the health care systems throughout the world. Chronic overactivation of the sympathetic nervous system is one of the major pathophysiological soliloquy abnormalities leading to the progression of chronic HF (28, 29, 48). Activation of sympathetic outflow is initially a hemodynamic adaptation to compensate for decreased cardiac function and output. This adaptation also includes an increase in Na+ and fluid retention and activation of the renin-angiotensin-aldosterone system (11, 34). Additional adverse effects of elevated sympathetic drive directed toward the heart have been shown to result in the desensitization or downregulation of β-adrenoceptors (3, 42), myocyte injury (5), and a predisposition to ventricular arrhythmias (26). This overactivation of sympathetic outflow relates to a poor prognosis and mortality in patients with chronic HF (4). Therefore, any method of inhibiting this enhanced sympathetic drive might be an effective therapy for HF. The beneficial effects of sympathoinhibitory drugs in the treatment of HF are consistent with this contention (25). The source/cause for the increased sympathoexcitation associated with HF is not entirely clear. Although several lines of evidence point to a role for altered afferent input such as the baro and cardiac sympathetic afferent input, the role of other afferent inputs such as the renal afferents has not been examined in contributing to an increased sympathetic drive (32).

Stimulation of the renal afferents in conscious rats increases blood pressure and heart rate (HR) that is mediated by over-activation of the sympathetic nervous system, suggesting an excitatory input from the kidney (44). Recent clinical trials have demonstrated that renal denervation (RDN) is effective in drug-resistant hypertension to reduce the renalhumoral drive and blood pressure (13, 19, 39). There have been a couple of studies that have examined the effect of RDN before, or just after, myocardial infarct on cardiac function and Na+ excretion (14, 15). Drug-resistant hypertensive patients with left ventricular hypertrophy and diastolic dysfunction show that RDN significantly reduces cardiac mass and improves diastolic function (2). Another recent study in patients with chronic systolic HF showed that RDN therapy produces improvements in symptoms and exercise capacity (1).

The underlying mechanisms for the observed improvement in cardiac function attributable to RDN are not entirely elucidated. One possibility for the positive results of the RDN in HF could be that RDN leads to a decrease in the central sympathetic drive, causing a reduction in sympathetic tone, thus reduced levels of plasma norepinephrine (NE). Suppression of sympathetic activity could reduce the downregulation of β-adrenoceptor drive to the heart and thus improve cardiac function. Thus we hypothesized that RDN has its beneficial effect on cardiac function by its sympathoinhibitory effect on the overall sympathetic drive and possibly to the heart as well. The present study was therefore designed to elucidate the effects of RDN on ventricular function and the changes in β-adrenoceptor subtype expressions in the hearts of rats with chronic HF.

METHODS

All procedures used for this study were approved by University of Nebraska Medical Center Institutional Animal Care and Use Com-
mittee and conducted according to the National Institutes of Health guiding principles for research involving animals. Male Sprague-Dawley rats weighing 220–250 g were purchased from Sasco Breeding Laboratories (Omaha, NE). Animals were housed with a 12-h:12-h light/dark cycle at ambient 22°C and 30–40% relative humidity. Laboratory chow and tap water were available ad libitum. After acclimatization for 1 wk, rats were assigned randomly to one of four groups: Sham, HF, Sham + RDN, HF + RDN (n = 10–13 rats/group).

Induction of HF. Rats were randomly assigned to either a Sham-operated control group or an HF group. HF was produced by left coronary artery ligation, as previously described (34, 46, 47). Rats were ventilated at a rate of 60 breaths/min with 2–3% isoflurane during the surgical procedure. The degree of left ventricular dysfunction and HF was determined by using both hemodynamic and morphological criteria. Left ventricular end-diastolic pressure (LVEDP) was measured by using a Mikro-Tip catheter (Millar Instruments, Houston, TX) at the time of the terminal experiments. To measure infarct size, the heart was dissected, and the atria and right ventricle were removed. A digital image of the left ventricle was captured using a digital camera. The percentage of infarct area to total left ventricle area was quantified using SigmaScan Pro (Aspire Software International, Ashburn, VA) (17). Rats with both LVEDP >15 mmHg and infarct size >30% of total left ventricular wall were considered to be in HF.

Renal denervation. Four weeks after coronary artery ligation surgery, rats underwent RDN under anesthesia (ketamine, 48 mg/kg; xylazine 12 mg/kg ip). Briefly, the kidneys were exposed through a retroperitoneal flank incision. Complete bilateral RDN was achieved by cutting all the visible renal nerves around both the renal artery and vein and painting the vessels with 70% ethanol. This method is known to ablate the afferent and efferent renal nerves (20, 30, 47). The final experiments were performed at 1 wk after RDN (5 wk after coronary artery ligation).

Kidney cortex NE content measurements. Renal tissue NE content was measured to confirm the completeness of RDN. Kidney cortex was homogenized using 1 mM ethylenediaminetetraacetic acid and 4 mM sodium metabisulfite in 0.1 N HCl. After centrifugation of the homogenates, NE concentration in the supernatants was measured using a commercially available ELISA kit (Labor Diagnostika Nord, Nordhorn, Germany), following the manufacturer’s instructions.

Urinary NE excretion measurements. Urinary NE excretion was measured as an index of overall sympathetic activation. Five weeks after surgery, rats from all four groups were placed in metabolic cages, and urine was collected for 24 h. Urinary NE concentration of thawed samples was measured using a commercially available ELISA kit (Labor Diagnostika Nord, Nordhorn, Germany), following the manufacturer’s instructions. Urinary NE concentration multiplied by urine flow rate was measured to confirm the completeness of RDN. Kidney cortex NE content measurements. renal tissue NE content was measured to confirm the completeness of RDN. Kidney cortex was homogenized using 1 mM ethylenediaminetetraacetic acid and 4 mM sodium metabisulfite in 0.1 N HCl. After centrifugation of the homogenates, NE concentration in the supernatants was measured using a commercially available ELISA kit (Labor Diagnostika Nord, Nordhorn, Germany), following the manufacturer’s instructions.

Preparation of ventricular lysates. Ventricular tissues were homogenized in ice-cold buffer containing 20 mM Tris, 1 mM dithiothreitol (pH 8.0) with protease inhibitor cocktail from Cell Signaling Technology (Danvers, MA) and centrifuged at 14,000 revolutions/min for 15 min. The pellets were resuspended in chilled buffer containing 20 mM NaPO4, 10 mM MgCl2, 1 mM dithiothreitol (pH 7.4), and protease inhibitor cocktail. Protein concentration was measured using Pierce BCA protein assay kit (Pierce Biotechnology, Rockford, IL).

Density of β1- and β2-adrenoceptors, collagen 1, and brain natriuretic peptide in ventricular tissues. A 40-μg sample of ventricular tissue was loaded onto the 7.5% SDS-PAGE gel for electrophoresis. The fractionated proteins on the gel were electrochemically transferred onto the polyvinylidene difluoride membrane. Transferred membranes were blocked with 5% nonfat dried milk and incubated with primary antibody overnight at 4°C followed by the corresponding peroxidase-conjugated secondary antibody for 1 h. The primary antibodies used were β1-adrenoceptor (sc-47778), β2-adrenoceptor (sc-570) from Santa Cruz Biotechnology (Santa Cruz, CA), brain natriuretic peptide (BNP) (ab19645), collagen 1 (ab34710) from Abcam (Cambridge, MA), and β-actin (A2066) from Sigma (St Louis, MO). An enhanced chemiluminescence substrate (Pierce) was used to visualize the signals, which were detected by Worklab digital image system (UVP, Upland, CA). ImageJ (National Institutes of Health, Bethesda, MD) was used to quantify the signal. The expressions of β1- and β2-adrenoceptors, collagen 1, and BNP were calculated as the ratio of the intensity of the β1- and β2-adrenoceptors, collagen 1, and BNP band, respectively, relative to the intensity of the β-actin band.

It should be noted that, to get a good band for β1-receptors (in HF) and collagen 1 (in Sham), we had to load fairly high levels of protein. These relatively high levels of protein loading caused some “apparent bleeding over fluorescence signal” in the actin band. Reducing the amount of protein led to the β-actin band residing within the lane with no apparent bleeding over. However, with these lower levels of protein, the bands for β1-receptors and collagen 1 were too faint to detect. It should be noted that the β1-receptors and collagen 1 bands show the distribution within the lane, indicating that there was actually no spilling over of the sample from one lane to the other. For densitometry analysis, we used the same width of box (nonoverlapping) and then used the same size box to place over the actin band within the same gel. This then provided the ratio of “protein X:β-actin” being compared.

Masson’s trichrome staining of heart sections. Masson’s trichrome staining of heart sections was performed to differentiate between collagen and smooth muscle. Formalin-fixed samples were dehydrated in graded alcohol and embedded in paraffin. Transverse sections of paraffin-embedded hearts were cut into 4-μm-thick sections. Sections were then deparaffinized with xylene and rehydrated in stepwise decreasing ethanol. Masson’s trichrome staining was conducted according to the guideline of the reagent kit from Sigma. After incubation in Weigert’s iron hematoxylin solution, the slides were stained with Biebrich scarlet-acid fuchsin and aniline blue and dehydrated in ethanol and xylene. The collagen fibers were stained blue, the nuclei were stained black, and mycardium was stained red.

Picro Sirus red staining of heart sections. Transverse sections of paraffin-embedded hearts (4 μm) as mentioned above were deparaffinized with xylene and rehydrated in stepwise decreasing ethanol concentrations. The slides were incubated with 0.1% Sirus red in saturated picric acid for 1 h at room temperature, which gives near-
equilibrium staining. Staining was followed by extensive washing with acidic water, dehydration, and mounting. The collagen fibers were stained red, the nuclei were stained black, and myocardium was stained pale brown.

For measurement of collagen content of Masson trichrome- and Picro Sirius red-stained heart sections, ×20 images were obtained and then analyzed using Fiji software (ImageJ, NIH) (37). Collagen content was presented as percentage (%) of total area in the left ventricle across the four groups.

Statistical analysis. Data are presented as means ± SE. Differences among groups were assessed with two-way ANOVA followed by Student-Newman-Keuls procedure to test for post hoc analysis of significance. \( P < 0.05 \) was considered statistically significant.

RESULTS

General characteristics of animals. The general characteristics of the four groups of rats used in this study are shown in Table 1. The data represent mean values from animals used for cardiac function experiments. The body weight, whole heart weight, and heart weight/body weight ratio were significantly increased in the HF group. However, RDN had no significant effects on these parameters in both Sham and HF groups. Only rats with >30% infarct of the left ventricular wall were included in the study. Five rats in the HF group had infarct sizes <30% and were excluded from data analysis. Sham rats had no visible myocardial damage. LVEDP was significantly increased in the HF rats compared with both Sham groups and the HF + RDN group. HF group of rats had significantly lower basal \( dP/dt \) compared with Sham rats, which was partially improved by RDN. Although LVEDP and \( dP/dt \) were only partially normalized by RDN, the values in the HF + RDN group were nonetheless significantly different from those in either of the Sham groups. Taken together, these data confirm that the rats in the HF groups were experiencing cardiac dysfunction and that RDN did not normalize cardiac function completely.

Urinary NE excretion measurements. Urinary NE excretion as an index of overall sympathetic activation was significantly greater in HF rats compared with Sham-operated controls (8.1 ± 0.3 vs. 3.6 ± 0.3 µg/day, \( P < 0.05 \)). RDN reduced the urinary excretion of NE in HF group (4.2 ± 0.8 vs. 8.1 ± 0.3 µg/day, \( P < 0.05 \)). There was no significant change in the Sham rats with RDN, suggesting that RDN per se did not modify the excretion of NE in the urine in control conditions (Fig. 1A).

Kidney NE content was significantly greater in HF rats compared with Sham-operated controls (290 ± 16 vs. 188 ± 42 ng/g, \( P < 0.05 \)). RDN reduced the NE content of the kidneys to almost undetectable levels in both Sham and HF rats, which confirmed the completeness of RDN.

Levels of BNP in left ventricles. BNP concentrations as a marker for the determination of left ventricular dysfunction was measured in the ventricular lysates. Results shown in Table 1B demonstrate higher BNP levels in the HF group compared with the Sham group (1.06 ± 0.14 vs. 0.64 ± 0.04, \( P < 0.05 \)). Furthermore, RDN significantly attenuated the increased levels in the HF group (0.67 ± 0.13 vs. 1.06 ± 0.14 with no RDN, \( P < 0.05 \)), suggesting a useful impact of RDN on the ventricular function in rats with HF.

In vivo left ventricular function. Mean peak LVPs were significantly higher in Sham group than they were in HF group (139.7 ± 4.6 mmHg vs. 91.5 ± 5.9 mmHg, \( P < 0.05 \)). RDN did not alter peak developed LVP in the Sham group, but it blunted the reduction induced by HF (104.6 ± 4.1 mmHg after RDN vs. 91.5 ± 5.9 mmHg for HF with no RDN, \( P < 0.05 \)) (Fig. 2, A and B). Mean LVEDP in the Sham group was significantly lower than that of HF group (2 ± 1 mmHg vs. 23 ± 3 mmHg, \( P < 0.05 \)). RDN greatly reduced LVEDP (9 ± 2 mmHg compared with 23 ± 3 mmHg for HF with no RDN, \( P < 0.05 \)) only in the HF groups (Fig. 2C).

The basal rates of change in pressure per time (±\( dP/dt \)) were also lower in the hearts of HF group compared with the Sham group (4.596 ± 348 mmHg/s vs. 5.636 ± 238 mmHg/s for +\( dP/dt \) and -4.668 ± 328 mmHg/s vs. -6.024 ± 328 mmHg/s for -\( dP/dt \), \( P < 0.05 \)). RDN significantly increased basal +\( dP/dt \) (5.767 ± 642 mmHg/s vs. 4.596 ± 348 mmHg/s, \( P < 0.05 \)) and -\( dP/dt \) (-5.827 ± 477 mmHg/s vs. -4.668 ± 328 mmHg/s, \( P < 0.05 \)) in the HF groups (Table 1).

When injected with 0.5 µg/kg isoproterenol, there was reduced change in contractility (±\( dP/dt \)) in the HF group compared with the Sham group (7.149 ± 526 mmHg/s vs. 15,707 ± 460 mmHg/s for +\( dP/dt \) and -5.688 ± 278 mmHg/s vs. -8,274 ± 265 mmHg/s for -\( dP/dt \), \( P < 0.05 \)). A similar trend was also observed following injection with 0.1 µg/kg isoproterenol in the HF groups (Fig. 3, A and B).

One week after RDN surgery, there was enhanced change in contractility (±\( dP/dt \)) of HF hearts to isoproterenol stimulation (0.5 µg/kg) (change of +\( dP/dt \): 4,356 ± 477 mmHg/s vs. 2,553 ± 411 mmHg/s with no RDN; change of -\( dP/dt \): -1,654 ± 304 mmHg/s vs. -1,020 ± 184 mmHg/s with no RDN, \( P < 0.05 \)), indicative of improvement of \( \beta \)-adrenoceptors and their functions (Fig. 3C).

There was robust responsiveness in ±\( dP/dt \) of Sham hearts to isoproterenol stimulation, irrespective of the presence of renal nerves (Fig. 3). There were no significant differences in

Table 1. Effects of RDN on characteristics of Sham and HF rats

<table>
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<tr>
<th>Parameters</th>
<th>Sham</th>
<th>HF</th>
<th>Sham + RDN</th>
<th>HF + RDN</th>
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<td>462 ± 22*</td>
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<td>5636 ± 238</td>
<td>4596 ± 348*</td>
<td>5070 ± 361</td>
<td>5767 ± 642*</td>
</tr>
<tr>
<td>-( dP/dt ), mmHg/s</td>
<td>-6024 ± 328</td>
<td>-4668 ± 328*</td>
<td>-7627 ± 1252</td>
<td>-5872 ± 477*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *\( P < 0.05 \) vs. sham; †\( P < 0.05 \) vs. HF; \( n = 8–10/group \); RDN, renal denervation; HF, heart failure; LV, left ventricle; LVEDP, left ventricular end-diastolic pressure.
basal HR or changes in HR to isoproterenol stimulation among the four groups of rats (Fig. 3D).

Relative levels of β-adrenoceptors. To further characterize the specific β-adrenoceptor isoform/s involved in enhanced isoproterenol responses following RDN in HF rats, Western blot analyses were conducted. As shown in Fig. 4A, ventricular homogenates from hearts of HF group contained 37% less β1-adrenoceptor protein compared with homogenates from the Sham group (0.43 ± 0.02 vs. 0.68 ± 0.09, P < 0.05). Homogenates from hearts of rats with HF also contained 49% less β2-adrenoceptors compared with the Sham group (0.28 ± 0.08 vs. 0.55 ± 0.06, P < 0.05, Fig. 4B). RDN did not significantly alter expression of β1- or β2-adrenoceptors in hearts of the Sham rats. However, in rats with HF, RDN blunted the loss of expression of β1-adrenoceptors (0.63 ± 0.08 vs. 0.43 ± 0.02 with no RDN, P < 0.05) (Fig. 4A) as well as those for β2-adrenoceptors (0.56 ± 0.07 vs. 0.28 ± 0.08 with no RDN, P < 0.05) (Fig. 4B).

Levels of collagen in the left ventricle. To investigate the effects of RDN on tissue fibrosis and collagen deposition, we stained transverse heart sections from four similar groups of rats with Masson’s trichrome and Picro Sirius red staining. Compared with Sham group, we observed more diffuse interstitial deposition of collagen in the peri-infarct zone in HF group, and RDN attenuated this increased collagen deposition (Fig. 5, A–D). To further corroborate the staining data, we checked the expression of collagen 1 in ventricular lysates using Western blot. As shown in Fig. 6A, we found an approximately twofold increase in the collagen 1 level in the peri-infarct zone in the HF group compared with the Sham group (0.63 ± 0.08 vs. 0.32 ± 0.02, P < 0.05), whereas RDN significantly attenuated the increased collagen 1 level in HF group (0.39 ± 0.06 vs. 0.63 ± 0.08 with no RDN, P < 0.05). In contrast to these data, ventricular lysates from a site away from the myocardial infarcted region do not show significant differences in collagen 1 level in the four groups of rats (Fig. 6B).

Fig. 2. A: representative left ventricular pressure (LVP) recordings from Sham, HF, Sham + RDN, and HF + RDN rats. B: mean values of LVP in the 4 groups of rats. C: mean values of left ventricular end diastolic pressure (LVEDP) in the 4 groups of rats. *P < 0.05 vs. Sham, #P < 0.05 vs. without RDN.
Urinary flow rate and Na⁺ excretion measurements. Urinary flow rate and Na⁺ excretion were significantly lower in the HF group compared with the Sham group. Although RDN increased urine flow rate, as well as Na⁺ excretion, in both HF and Sham groups, it did not alter the difference between the Sham and HF groups (Fig. 7).

DISCUSSION

The present study shows that RDN performed after the onset of HF can reduce overall sympathetic outflow, as evident by reduced levels of urinary excretion of NE. Concomitantly, there is a decrease in levels of BNP in the left ventricle, improvement in LVEDP, and contractile function of the left ventricle to the adrenergic challenge. Consistent with these observations, the expression of β-adrenoceptors is enhanced after RDN. Markers of fibrosis were also improved in peri-infarcted regions of the myocardium. Taken together, these observations demonstrate that RDN prevented many of the pathological indices of HF and may offer a therapeutic intervention to improve cardiac function in the HF condition.

Targeting the renal sympathetic nerves using catheter-based therapeutic RDN is an attractive therapeutic approach for the treatment of hypertension or complications of HF (39, 41). One hypothesis suggests that renal afferent nerve input may be important in the establishment and maintenance of certain forms of experimental hypertension (12). Renal afferent nerve signals are integrated centrally and can result in an enhanced sympathetic drive, which is directed toward, not only the kidneys, but also other organ systems that have a dense sympathetic innervation, such as the heart and the peripheral vasculature, resulting in a rise in blood pressure (12, 24, 38).

The present study is unique in that RDN was initiated 4 wk after the onset of HF (chronic), making it relevant to patients who may be administered RDN as a therapeutic modality after diagnosis of the chronic HF condition. Prior experimental animal studies initiated RDN protocols either before or at the onset of myocardial infarct and measured only one of the above parameters in a rudimentary way (14).

It is well known that the mammalian kidney contains several distinct classes of sensory mechanoreceptors and chemoreceptors (18, 36) that transmit information to the central nervous system. Stimulation of afferent renal nerves has been shown to evoke alterations in sympathetic nerve activity and arterial blood pressure (31, 44). The excitatory influence may represent an activation of afferent renal afferents in response to a variety of stimuli (35, 36). There are multiple triggers in the HF condition that have the potential for increased activation of the renal afferent nerve activity, including reduced perfusion pressure, increased venous pressure, increased inflammation, and increased oxidative stress to name a few (18). It is possible that such excitatory influence mediated by renal afferents in the HF condition may also contribute to the underlying mechanism/s for the sympathoexcitation seen in HF. Under these circumstances, removal of these afferents by RDN may contribute to the sympathoinhibitory effects (32).

Specifically, increased sympathetic activation of the cardiac innervation has been shown in rats with HF (43, 45). Consistent with these observations, cardiac NE spillover is also reported to be increased in patients with HF (10). This increased NE release is in conformity with the common observation of reduced expression of β-adrenoceptor in the HF condition (3). The decrease in expression of β-adrenoceptor in the HF condition is likely due to downregulation of adrenergic receptors in response to increased circulating levels of catecholamines. Similarly, we also observed a decrease in expression of β-adrenoceptors in rats with HF that are known to have elevated sympathetic drive and thus elevated plasma levels of catecholamines (16, 17). RDN, while reducing the global marker of sympathetic outflow, resulted in elevated expression of β-adrenoceptors in the heart. We interpret these results as an upregulation of β-adrenoceptors in response to a decrease in circulating levels of catecholamines in HF rats with RDN. In hemodynamic studies, we found that cardiac systolic function measured as change in pressure over time (±dP/dt) was improved under basal conditions as well as in response to β-adrenoceptor stimulation with isoproterenol after RDN. These data suggest...
that RDN improved cardiac contractile function as well as cardiac contractile reserve. This is consistent with clinical data indicating that RDN during systolic HF slows the progression of dysfunction and improves walk time (2, 7). A possible explanation for these observations is that -adrenoceptors, which are downregulated in the HF condition because of the overactivated sympathetic drive (i.e., increased circulating catecholamines), are restored after RDN because of restoration of the adrenergic tone as well as the adrenergic reserve for activation (i.e., expression of -adrenoceptor expression).

It should be recognized that cardiac cell surface -adrenoceptor density and affinity are critically important in the link between -adrenoceptors and cardiac function. Therefore, measurements of total -adrenoceptor protein levels may not directly parallel changes in cardiac function under all conditions. Furthermore, serial measurements of changes in cardiac

![Fig. 5. A: Masson’s trichrome staining of heart sections from Sham, HF, Sham + RDN, and HF + RDN rats. The collagen fibers were stained blue, the nuclei were stained black, and myocardium was stained red. B: Picro Sirius red staining of heart sections from similar 4 groups rats. The collagen fibers were stained red, the nuclei were stained black, and myocardium was stained pale brown. C and D: collagen contents in Masson’s trichrome (C) and Picro Sirius (D) staining were presented as percentage (%) of total area in the left ventricle across the 4 groups. Values are means ± SE (n = 3–6). *P < 0.05 vs. Sham, #P < 0.05 vs. without RDN.](#)

![Fig. 6. Top: representative Western blots showing steady-state levels of collagen 1 and corresponding -actin in the peri-infarcted zone (A) and noninfarcted area (B) of the left ventricular tissues from Sham, HF, Sham + RDN, and HF + RDN rat hearts. Bottom: mean values (n = 4) of collagen 1 protein in the 4 groups of rats. *P < 0.05 vs. Sham, #P < 0.05 vs. without RDN.](#)
function accompanied with alterations in cardiac β-adrenergic receptor density and affinity correlated with sympathetic activity in these experiments would provide a more definitive causal relation between alteration in the β-adrenergic system and impaired cardiac function.

Our study also indicated that there were higher levels of BNP in the rats with HF compared with Sham rats. RDN reduced these levels to within the normal range. BNP is rapidly synthesized in response to ventricular stretch and pressure overload (27). Increased levels would indicate deceleration of the myocardium, whereas decreasing levels would indicate improvement in cardiac function. Increasing levels of BNP in patients with HF may be indicative of patients at a higher risk of mortality associated with HF (23). A reduction of BNP levels by RDN in rats with HF was indicative of recovery of cardiac function.

In the progression of the HF condition, persistent ischemia and inflammation result in extracellular matrix formation and increased levels of collagen disposition (40). Consistent with this observation, we observed an increased level of collagen I in peri-ischemic regions of the left ventricle in rats with HF. RDN ameliorated the deposition of collagen in the perinfarcted zone of the left ventricle in rats with HF. RDN may also play an important role in this cardiac remodeling process, resulting in a protective effect in the HF condition. The precise mechanisms underlying this cardiac remodeling process remain unknown. Considering that excessive β-adrenergic stimulation has been shown to produce remodeling in HF and that RDN prevents the enhanced sympathetic tone in HF, it is reasonable to speculate that RDN may ameliorate this remodeling by the alterations in the adrenergic tone/effect. RDN-induced suppression of overexpressed collagen I localized in perinfarcted but not far from the infarcted zone may well be due to the fact that the sympathetic nerves are irreparably damaged in the infarcted tissue and are not available for rescue, as are those in the tissue away from the infarcted zone. Secondly, the amount of collagen I in the healthy tissue away from the infarct is very minimal to begin with, so the effect of RDN would be expected to be negligible and thus undetectable in these tissues.

Another mechanism for the improved cardiac function after RDN is improved preload, secondary to reduced peripheral sympathetic activation to the kidney and general vasculature. Impaired renal function and consequent fluid retention are also hallmarks of late congestive HF. RDN in animal models of HF have been shown to improve the ability of the kidneys to excrete a given load of Na⁺ load (8, 47). It should be noted that the improvement in excretion of Na⁺ and water attributable to RDN was not restricted to animals with HF (33). These studies showed that RDN improved renal excretory ability equally in HF and Sham animals. The efficacy of RDN to improve cardiac function may also be tied to improved renal function (increased Na⁺ and water excretion and decreased blood volume) because excessive cardiac preload critically contributes to the progressive decompensating of the failing left ventricle (9). Relieving fluid retention due to impaired renal function in the HF condition (cardio-renal syndrome) has been a predominate focus for general treatment for chronic congestive HF (6). The results in this study show that RDN also increased 24-h urine flow and Na⁺ excretion in both Sham as well as HF rats. It is conceivable that elimination of this excess volume in rats with HF had a potentially positive influence on cardiac function. Increased levels of BNP are consistent with the decompensating of myocardium and fluid retention during HF, whereas decreasing levels with RDN would indicate elimination of excess fluid and normalization of BNP. These observations may be particularly pertinent to provide insight into the improvement in LVEDP that is observed with RDN in rats with HF. It may well be that this reduction in overall volume improves the LVEDP in rats with HF. Another potentially important component is likely to involve endogenous fluid shifts triggered by amelioration of increase in sympathetic vasoconstriction in the major vascular beds throughout the body, such as improving the hemodynamic blood flow in the HF condition. Although these concepts have generated recent interest, the precise role of RDN in blood volume regulation and distribution remains to be examined experimentally.

Finally, RDN also affects the level of renin-angiotensin-aldosterone system activation because the renal nerves are one of the primary mediators of renin release within the kidneys. In rats with HF, there is an elevated level of angiotensin II in the circulation (17, 22). Elevated levels of angiotensin II have also been shown to increase sympathetic drive and thus contribute to the increased circulating levels of catecholamines (4). It is possible that RDN reduced the circulating levels of angiotensin II and thus the angiotensin II-mediated increase in circulating catecholamines in HF. It has been shown that RDN attenuates the direct cardiotoxic, detrimental effects on the myocardium attributable to excessive activation of neurohumoral response. Taken together, these effects represent the inhibitory nature of RDN on the involvement of sympathetic nerve activity and the renin-angiotensin-aldosterone system (21).

In conclusion, we have shown for the first time in a comprehensive way that RDN initiated after a period of chronic HF enhances cardiac contractility and the responsiveness of hearts.
to isoproterenol (catecholamine) stimulation, and these effects are likely due to an RDN-induced increase in β-adrenoceptor protein expression in the heart.

REFERENCES


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