Frequency Response of the Renal Vasculature in Congestive Heart Failure

Gerald F. DiBona, MD; Linda L. Sawin

Background—The renal vasoconstrictor response to renal nerve stimulation is greater in congestive heart failure (CHF) rats than in control rats. This study tested the hypothesis that the enhanced renal vasoconstrictor response to renal nerve stimulation in CHF is a result of an impairment in the low-pass filter function of the renal vasculature.

Methods and Results—In response to conventional graded-frequency renal nerve stimulation, the reductions in renal blood flow at each stimulation frequency were greater in CHF rats than control rats. A pseudorandom binary sequence pattern of renal nerve stimulation was used to examine the frequency response of the renal vasculature. Although this did not affect the renal blood flow power spectrum in control rats, there was a 10-fold increase in renal blood flow power over the frequency range of 0.01 to 1.0 Hz in CHF rats. On analysis of transfer function gain, attenuation of the renal nerve stimulation input signal was similar in control and CHF rats over the frequency range of 0.001 to 0.1 Hz. However, over the frequency range of 0.1 to 1.0 Hz, although there was progressive attenuation of the input signal (−30 to −70 dB) in control rats, CHF rats exhibited a flat gain response (−20 dB) without progressive attenuation.

Conclusions—The enhanced renal vasoconstrictor response to renal nerve stimulation in CHF rats is caused by an alteration in the low-pass filter function of the renal vasculature, resulting in a greater transfer of input signals into renal blood flow in the 0.1 to 1.0 Hz range. (Circulation. 2003;107:2159-2164.)

Key Words: kidney ■ blood flow ■ vasculature ■ nervous system, sympathetic

Congestive heart failure (CHF) is characterized by increased renal sympathetic nerve activity (RSNA) and activity of the renin-angiotensin system, which contribute to a decrease in renal blood flow (RBF).1–5 The regulation of the renal circulation in CHF has been studied by use of a variety of reflex stimuli. In contrast to normal dogs, in which RBF is not affected by exercise, CHF dogs show substantial vasoconstriction during exercise, which, as shown by its reversal after renal denervation, is mediated by RSNA.6,7 Similarly, patients with CHF have increased magnitude and duration of renal vasoconstriction during sustained hand-grip exercise.8 By contrast, CHF rats subjected to whole-body heating showed a smaller increase in RSNA and no decrease in RBF compared with control rats.9 It is not surprising that diverse reflex stimuli thought to result in a general sympathetic activation would have different effects on RSNA. It is well established that the reflex response of the sympathetic nervous system is regionally differentiated such that an increase in sympathetic nerve activity to one region (eg, kidney) could coexist with a decrease to another region and, furthermore, that this differential response might be opposite with a different reflex stimulus.5

However, an unanswered question emerges from these observations: is the response of the renal vasculature to RSNA altered in CHF? To answer this question, in CHF rats, we measured the steady-state RBF responses to direct renal nerve stimulation and the dynamic RBF responses to pseudorandom binary sequence (PRBS) renal nerve stimulation. Thus, this approach permitted an assessment of the overall renal vascular response to the transfer of an investigator-determined signal applied to the renal nerves.

Methods

Adult male Sprague-Dawley rats (weight, 275 to 325 g) allowed free access to normal-sodium rat pellet diet and tap water drinking fluid were used for all studies. All animal procedures were performed in compliance with the University of Iowa Policies and Guidelines Concerning the Use of Animals in Research and Teaching and the United States Public Health Service NIH Guide for the Care and Use of Laboratory Animals.

CHF was produced by left coronary artery ligation with subsequent myocardial infarction by use of a method established and validated in our laboratory.1–3 Control (sham) and CHF rats were studied 4 to 6 weeks later.

Rats were anesthetized with sodium pentobarbital (50 mg/kg IP); an oral endotracheal tube was inserted, and mechanical ventilation with room air was instituted. A jugular vein was catheterized for the administration of additional anesthetic (10 mg · kg⁻¹ · h⁻¹ IV) and isotonic saline at 0.05 mL/min. A carotid artery was catheterized for the measurement of arterial pressure (AP) and heart rate. Via a left flank incision, the left renal nerve bundle was dissected free and...
placed on a silver wire bipolar electrode to which it was fixed with Silgel (Wacker Chemie). The electrode was connected to an electrical stimulator (Grass S88) or the output of a computer-controlled stimulator, and the nerve bundle was sectioned between the electrode and the neuraxis, ensuring that the only activity passing to the left kidney derived from the stimulator. A noncannulating electromagnetic flow probe (1.5-mm circumference) was placed around the left renal artery and connected to an electromagnetic flowmeter (Carolina Medical Electronics).

After surgery, a 45-minute period was allowed for equilibration and stabilization.

Conventional Renal Nerve Stimulation

The initial experimental series was designed to test the voltage and frequency dependence of the renal vasconstrictor response to conventional rectangular pulse stimulation with pulses of 0.5-ms duration and frequencies of 0.5, 1.0, 1.5, and 2.0 Hz. For each rat, a supramaximal voltage was determined. At a frequency of 2 Hz and a rectangular pulse duration of 0.5 ms, stimulation voltage was increased progressively until further increases in stimulation voltage did not result in further decreases in RBF. For additional studies, rectangular pulses of 0.5-ms duration and supramaximal voltage (as determined for each rat) were used. Each 60-second period of renal nerve stimulation was preceded by a 5-minute control period and followed by a 5-minute recovery period.

At the end of the experiment, the carotid artery catheter was advanced into the left ventricle for the measurement of left ventricular end-diastolic pressure (LVEDP). The rats were killed with an overdose of sodium pentobarbital, and a 20-minute recording of postmortem signals was made. The heart was removed and weighed.

Pseudorandom Binary Sequence

For each rat, a stimulus voltage was determined that produced the maximum decrease in RBF but was not supramaximal. This was determined by stimulating the renal nerves at a frequency of 2 Hz, a duration of 0.5 ms, and voltages between 5 and 15 V in steps of 1 V. The maximum voltage so determined was used for each PRBS in each rat. After a 10-minute recovery period, a 10-minute control recording of AP and RBF was made. Then the renal nerves were stimulated with a PRBS for 30 minutes. The PRBS \( z \) was composed of a basal pulse with a frequency of 2 Hz and duration of 2 ms and voltage that was switched between a low voltage (0.5 V) and the maximum voltage previously determined for each rat. Every 0.5 second, a decision was made to switch between the low voltage and the maximum voltage or to stay at the current voltage. This provided a signal with a flat power spectrum over the broad frequency range of 0 to 1.0 Hz, a desirable feature of an input signal for systems analysis of frequency response.

At the end of the experiment, the carotid artery catheter was advanced into the left ventricle for the measurement of LVEDP. The rats were killed with an overdose of sodium pentobarbital, and a 20-minute recording of postmortem signals was made. The heart was removed and weighed.

Data Analysis

The postmortem signals were subtracted from the recorded control and experimental period data. APs, both pulsatile and mean, were recorded via an electronic pressure transducer (Statham). Heart rate was determined via a tachometer (Grass 7P4) driven by the pulsatile AP waveform. RBFs, both pulsatile and mean, were recorded via the electromagnetic flowmeter, the output of which was low-pass filtered at <10 Hz by the built-in analog filter. The outputs of the pressure transducer, the tachometer, the electromagnetic flowmeter, and the renal nerve stimulator were led to a Grass model 7D polygraph recorder for graphic output and to VHS tape via a pulse code modulation adapter (Vetter Model 4000A PCM Recording Adapter) for later offline analysis.

For the conventional renal nerve stimulation data, the maximum change in RBF with each stimulation frequency was calculated as the percentage change from the mean value of the preceding 5-minute control RBF value. Renal vascular resistance (RVR) was calculated as RVR = AP/RBF.

For the PRBS stimulation data, analog AP, renal nerve stimulator, and RBF signals were sampled from tape at 500 Hz. Subsequent processing of the data were performed with Matlab software. The 500-Hz-data files were digitally low-pass filtered (3.5-Hz cutoff frequency, finite-impulse-response order 50) and then decimated to a rate of 5 Hz. These 5-Hz data were split into blocks of 8192 data points. The transfer spectra were calculated from AP (input) and RBF (output) during spontaneous autoregulation and from renal nerve stimulator (input) and RBF (output) during PRBS. The transfer function was taken as the quotient of the cross spectrum of input and output and the power spectrum of the input. The algorithm involved mean detrending and a Hanning window with 50% overlap of the blocks. After conversion of the gain values into decibels (20*log[gain]), a mean spectrum was calculated from the consecutive spectra and averaged for all rats.

A linear relationship between phase angle and frequency indicates the presence of a time delay whose magnitude may be calculated from the slope of the linear portion. The transfer function is the ratio of two polynomials whose constants are filter coefficients.

Statistical analysis was performed with ANOVA with the subsequent use of Scheffe’s method for simultaneous comparisons within and between groups. A significance level of 5% was chosen. Data in the text, tables, and figures are expressed as mean±SEM.

Results

The values for LVEDP and ratio of heart weight to body weight were similar for the rats used for the two different protocols, so they have been combined. In Control rats (\( n = 14 \)), LVEDP was \( 2.9±0.2 \, \text{mm Hg} \) and heart weight/body weight ratio was \( 0.41±0.02\% \). In CHF rats (\( n = 14 \)), LVEDP was \( 12.2±0.7 \, \text{mm Hg} \) and heart weight/body weight ratio was \( 0.70±0.03\% \). Both LVEDP and heart weight/body weight were significantly greater in CHF than in Control rats.

Conventional Renal Nerve Stimulation

Before conventional renal nerve stimulation, AP and RBF were lower and RVR was higher in CHF rats than Control rats (Table 1). In Control rats, graded-frequency renal nerve stimulation did not affect RBF at either 0.5 or 1.0 Hz but produced frequency-dependent decreases in RBF at both 1.5 and 2.0 Hz (Figure 1). In CHF rats, graded-frequency renal nerve stimulation did not affect RBF at 0.5 Hz but produced frequency-dependent decreases in RBF at 1.0, 1.5, and 2.0 Hz that were significantly different (\( P<0.05 \)) from the responses observed in Control rats.

PRBS Renal Nerve Stimulation

The effect of PRBS renal nerve stimulation on renal hemodynamics is shown in Table 2. Control period AP and RBF were significantly lower and RVR was significantly higher in

| TABLE 1. Baseline Data for Control Rats and CHF Rats Before Conventional Renal Nerve Stimulation |
|-----------------|-----------------|-----------------|
|                  | Control Rats    | CHF Rats        |
|                  | (\( n = 6 \))    | (\( n = 6 \))    |
| AP, mm Hg        | 123±3           | 105±3*          |
| RBF, mL/min      | 7.0±0.4         | 5.4±0.2*        |
| RVR, mm Hg · mL⁻¹·min⁻¹ | 17.1±0.8   | 19.6±0.6*       |

\( *P<0.05, \) CHF rats vs control rats.
CHF rats than Control rats. PRBS did not affect AP. PRBS significantly decreased RBF in both Control rats (−19%) and CHF rats (−33%); the decrease in RBF was significantly greater in CHF than Control rats. PRBS significantly increased RVR in both Control rats (+22%) and in CHF rats (+49%); the increase in RVR was significantly greater in CHF in CHF than Control rats.

The mean power spectra for RBF during the control and PRBS renal nerve stimulation periods for both Control and CHF rats are shown in Figure 2. During the control period, the mean RBF power spectra were similar in Control and CHF rats. In Control rats, PRBS renal nerve stimulation did not affect the mean RBF power spectrum. However, in CHF rats, PRBS renal nerve stimulation resulted in a 10-fold increase in the mean RBF power over the frequency range of 0.01 to 1.0 Hz.

The transfer function between the spontaneous oscillations in AP and RBF represents the dynamic aspects of RBF autoregulation. In contrast, in the CHF rats, in the frequency range <0.01 Hz, the values for gain (−40 to −50 dB) were not as negative as in Control rats, reflecting lesser autoregulation. At frequencies >0.2 Hz, the gain values in CHF rats were not different from those in Control rats.

The transfer function spectra for AP to RBF during PRBS renal nerve stimulation for Control and CHF rats are shown in Figure 4. In the Control rats, the values for gain during PRBS were slightly but not significantly less negative than during control, because there was extensive overlap over the entire frequency range. In the CHF rats, the values for gain during PRBS were similar to those during control over the frequency range of 0.001 to 0.01 Hz. At frequencies >0.01 Hz, the values for gain during PRBS were significantly less negative than during control. Values for gain were −40 to −50 dB at 0.1 Hz and −30 to −40 dB at 1.0 Hz during control compared with values of −20 to −30 dB and −10 to −20 dB at similar frequencies during PRBS. Thus, spontaneous autoregulation

### TABLE 2. Summary Data for Effect of PRBS Renal Nerve Stimulation on Renal Hemodynamics in Control Rats and CHF Rats

<table>
<thead>
<tr>
<th></th>
<th>Control Rats (n=8)</th>
<th>CHF Rats (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control PRBS</td>
<td>Control PRBS</td>
</tr>
<tr>
<td><strong>AP, mm Hg</strong></td>
<td>121±1</td>
<td>119±1</td>
</tr>
<tr>
<td></td>
<td>107±2*</td>
<td>106±2</td>
</tr>
<tr>
<td><strong>RBF, mL/min</strong></td>
<td>7.2±0.2</td>
<td>5.8±0.3†</td>
</tr>
<tr>
<td></td>
<td>5.7±0.1*</td>
<td>3.8±0.1†</td>
</tr>
<tr>
<td><strong>RVR, mm Hg·mL⁻¹·min⁻¹</strong></td>
<td>16.9±0.5</td>
<td>20.7±0.9†</td>
</tr>
<tr>
<td></td>
<td>18.6±0.3*</td>
<td>27.8±1.1†</td>
</tr>
</tbody>
</table>

*P<0.05 vs same period in Control rats.
†P<0.05 vs control period within group.
‡P<0.05 vs same period in Control rats.
was not affected by PRBS in Control rats, whereas it was further impaired by PRBS in CHF rats.

The transfer function spectra for PRBS to RBF (ie, frequency response of the renal vasculature) for Control and CHF rats are shown in Figure 5. In Control rats, after a rapid transition from amplification (positive gain values) to attenuation (negative gain values) near 0.001 Hz, gain is little changed, save for a notch at 0.05 Hz, until 0.1 Hz. Thereafter, there is a steep negative slope of gain (ie, attenuation) up to 1.0 Hz. In CHF rats, the pattern is similar up to 0.1 Hz. Thereafter, in contrast to the steep attenuation seen in Control rats, gain remains flat, with a slight increase up to 1.0 Hz. Gain was significantly less negative in CHF than Control rats for the frequency range of 0.1 to 1.0 Hz. At frequencies >0.1 Hz, the slope of the plot of phase angle versus frequency was linear in 6 of 8 Control rats and in 5 of 8 CHF rats. The time delay values were 593 ± 29 ms for Control rats and 512 ± 30 ms for CHF rats.

Figure 4. Transfer function (TF) spectra for AP to RBF during PRBS renal nerve stimulation period in Control rats (left) and CHF rats (right). Data are mean for 8 Control and 8 CHF rats; SEM bars have been omitted for clarity.

Figure 5. Transfer function (TF) spectra for PRBS stimuli to RBF during PRBS renal nerve stimulation period in Control rats (left) and CHF rats (right). Data are mean for 8 Control and 8 CHF rats; SEM bars have been omitted for clarity.

A recursive infinite impulse response digital filter was designed as a least-squares fit to the observed frequency response for PRBS renal nerve stimulation (PRBS - RBF) in Control and CHF rats. With weighting on the frequency range of greatest divergence, 0.1 to 1.0 Hz, a best fit was achieved with order 2 in Control rats and order 3 in CHF rats. In Figure 6, it is seen that, as with the observed frequency responses, there is little difference between the gain of Control and CHF rats at frequencies <0.1 Hz. However, at frequencies >0.1 Hz, gain becomes more negative in Control rats, reflecting the low-pass filter characteristics with marked attenuation, whereas gain becomes less negative in CHF rats, with less attenuation allowing the passage of signals in this frequency range into RBF.

Figure 6. Frequency response of a recursive infinite impulse response digital filter modeled as a least-squares fit to observed frequency response (TF) for PRBS renal nerve stimulation (PRBS - RBF) in Control rats and CHF rats (data from Figure 5). With weighting on frequency range of greatest divergence, 0.1 to 1.0 Hz, a best fit was achieved with order 2 in Control rats and order 3 in CHF rats.

Discussion
There are several important findings in this study, as follows: (1) the renal vasoconstrictor responsiveness to renal nerve stimulation is increased in CHF rats compared with Control rats; (2) spontaneous autoregulation of RBF is less efficient in CHF rats than Control rats; (3) PRBS renal nerve stimulation, although it does not affect RBF autoregulation in Control rats, worsens RBF autoregulation in CHF rats; and (4) the normal low-pass filter function of the renal vasculature is impaired in CHF such that input oscillations in the frequency range of 0.1 to 1.0 Hz are not normally attenuated. It should be emphasized that these findings apply to the situation of general anesthesia and mechanical ventilation, with the caveat that it is not known whether they are applicable to the conscious state.

On the basis of the increase in both LVEDP and heart weight/body weight ratios and the decrease in both resting AP and RBF, these 4- to 6-week CHF rats had advanced disease.1–5
At the same frequency of conventional renal nerve stimulation, RBF decreased more in CHF rats than in Control rats. Some possible explanations for this increased responsiveness include presynaptic facilitation of norepinephrine release by increased angiotensin II, diminished norepinephrine uptake and clearance at the renal sympathetic nerve terminal, increased number of renal vascular postsynaptic $\alpha_1$-adrenoceptors and/or increased efficiency of signal transduction, among others.

Given that the decrease in resting RBF is related to an increase in RSNA, the finding of an impairment in spontaneous autoregulation in CHF rats is not unexpected. Previous studies have indicated that when renal nerve stimulation is sufficient to produce a modest decrease in resting RBF, stepwise autoregulation of RBF is impaired, as manifested by an increase in the level of AP at which RBF autoregulation ceases (autoregulatory breakpoint). In response to neurogenic vasoconstriction in proximal segments of the renal vasculature, the decrease in downstream luminal pressure elicits myogenic vasodilation, thus encroaching on the reserve capacity for further autoregulatory vasodilation in response to changes in renal perfusion pressure. In the present study, the 23% difference in RBF between CHF rats and Control rats, most likely because of heightened RSNA in CHF rats, was sufficient to enable this RBF autoregulatory defect to be detected under spontaneous conditions.

During PRBS renal nerve stimulation, the relative decrease in RBF was greater in CHF rats ($-33\%$) than in Control rats ($-19\%$). In addition, the level of absolute RBF achieved in CHF rats was $34\%$ lower than in Control rats. The magnitude of the decrease in RBF in Control rats is approximately the threshold for impairment in stepwise autoregulation of RBF as observed during experiments using conventional renal nerve stimulation. In agreement with this, the alteration in the dynamic measurement of spontaneous RBF autoregulation was small and not significant. Conversely, the much greater decrease and lower level of RBF in CHF rats resulted in the consumption of a larger fraction of RBF autoregulatory reserve, which accounts for the more marked alteration in the dynamic measurement of spontaneous RBF autoregulation. PRBS renal nerve stimulation provides a wide frequency range forcing at uniform input signal power. This permits assessment of the frequency response of the renal vasculature in a relatively short time interval. The transfer function gain between the input signal, PRBS, and the output signal, RBF, provides a measure of either amplification (+ gain) or attenuation (− gain) that is applied to the input signal by the renal vasculature. In this way, the frequency response of the renal vasculature serves to quantitatively describe the filter characteristics of the renal vasculature. In the rat, the renal vasculature functions as a low-pass filter with a cutoff frequency in the vicinity of 0.2 to 0.4 Hz, depending on the method of assessment. In this situation, input signals are passed by the renal vasculature into the RBF signal with little attenuation up to the cutoff frequency, after which there is steep attenuation, so that input signals greater than the cutoff frequency are filtered out from the RBF signal. This is evident in the RBF power spectra (Figure 2), in which the decrease in RBF power over the frequency range from 0.1 to 1.0 Hz is greater in Control rats ($\approx 99\%$) than in CHF rats ($\approx 60\%$). This is further reflected in the transfer function gain (Figure 4), in which the decrease in gain over the frequency range from 0.1 to 1.0 Hz is greater in Control rats ($-40$ dB) than in CHF rats, which actually show a small increase ($+10$ dB). Thus, compared with the Control rats, it is clear that the low-pass filter function of the renal vasculature is impaired in CHF rats in a manner that allows passage of input signals into the RBF signal in the frequency range of 0.1 to 1.0 Hz. This is further manifested as an increased responsiveness of the renal vasculature to renal nerve stimulation in CHF rats. This contributes to their lower resting RBF and poorer spontaneous autoregulation of RBF at rest and during PRBS renal nerve stimulation.

Several possible explanations were proposed (see above) for the increased renal vasoconstriction to conventional renal nerve stimulation in CHF rats. It would be expected that these proposed explanations would result in a broad frequency alteration in the transfer function between PRBS and RBF, with increased gain over the entire frequency range of 0 to 1.0 Hz. In contrast, transfer function gain was affected primarily in the frequency range of 0.1 to 1.0 Hz. This finding seems more compatible with the view that in CHF, there are structural and/or functional alterations of the renal vasculature, which produces a change in its frequency-response characteristics that is selective for a limited frequency range. These structural and/or functional alterations may be related to chronic exposure to increases in both RSNA and circulating angiotensin II.

In summary, the renal vasoconstrictor response to conventional renal nerve stimulation is enhanced in CHF rats compared with Control rats. CHF rats have lower resting values of RBF and poorer spontaneous RBF autoregulation at rest. With PRBS renal nerve stimulation, CHF rats exhibit a greater renal vasoconstrictor response, and their spontaneous RBF autoregulation worsens. Transfer function analysis of PRBS to RBF indicates that the low-pass filter function of the renal vasculature in CHF rats is impaired in the frequency range of 0.1 to 1.0 Hz, allowing the passage of normally excluded nerve stimulation signals into the RBF signal with greater renal vasoconstriction.

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References
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