Losartan corrects abnormal frequency response of renal vasculature in congestive heart failure

Gerald F. DiBona and Linda L. Sawin
Department of Internal Medicine and Physiology and Biophysics, University of Iowa College of Medicine and Veterans Administration Medical Center, Iowa City, Iowa 52242

Submitted 17 April 2003; accepted in final form 18 June 2003

DiBona, Gerald F., and Linda L. Sawin. Losartan corrects abnormal frequency response of renal vasculature in congestive heart failure. Am J Physiol Heart Circ Physiol 285: H1857–H1863, 2003. First published June 26, 2003; 10.1152/ajpheart.00364.2003.—In congestive heart failure, renal blood flow is decreased and renal vascular resistance is increased. This is associated with an abnormality in the low-pass filter function of the renal vasculature wherein higher frequencies (≥0.01 Hz) within renal sympathetic nerve activity are not normally attenuated and are passed into the renal blood flow signal. This study tested the hypothesis that excess angiotensin II action mediates the abnormal frequency response characteristics of the renal vasculature in congestive heart failure. In anesthetized rats, the renal vasoconstrictor response to graded frequency renal nerve stimulation was significantly greater in congestive heart failure than in control rats. Losartan attenuated the renal vasoconstrictor response to a significantly greater degree in congestive heart failure than in control rats. In control rats, the frequency response of the renal vasculature was that of a first order (−20 dB/frequency decade) low-pass filter with a corner frequency (3 dB, 30% attenuation) of 0.002 Hz and 97% attenuation (−30 dB) at ≥0.1 Hz. The functional effects of angiotensin II are reversed by acute AT1 receptor blockade. In chronic CHF, with sustained exposure to increased circulating concentrations of angiotensin II, there is structural remodeling of the cardiovascular system, including an increase in the wall thickness-to-lumen ratio of renal resistance vessels, which results in increased minimum vascular resistance and increased vasoconstrictor responsiveness. Such chronic structural alterations would not be expected to be affected by acute AT1 receptor blockade but are known to be reversed by chronic AT1 receptor blockade.

The current studies tested the hypothesis that the enhanced renal vasoconstrictor response to renal sympathetic nerve stimulation and the associated abnormality in the frequency response characteristics of the renal vasculature seen in congestive heart failure are mediated by the action of angiotensin II on renal angiotensin II AT1 receptors.

hemodynamic alterations occur in the setting of increased renal sympathetic nerve activity (RSNA) in addition to increased activity of the renin-angiotensin system (RAS), both of which contribute to the renal vasoconstriction.

In a previous study (4) using a well-validated model of CHF in the rat, it was shown that the renal vasoconstrictor response to graded frequency renal nerve stimulation was enhanced in CHF rats compared with control normal rats. This was characterized by an abnormality in the low-pass filter function of the renal vasculature wherein normally excluded higher RSNA frequencies were transmitted into the RBF signal.

Angiotensin II is known to stimulate angiotensin II AT1 receptors located on the renal vasculature to cause renal vasoconstriction and on the renal sympathetic nerve terminals to facilitate norepinephrine release (2, 10). The functional effects of angiotensin II are reversed by acute AT1 receptor blockade. In chronic CHF, with sustained exposure to increased circulating concentrations of angiotensin II, there is structural remodeling of the cardiovascular system, including an increase in the wall thickness-to-lumen ratio of renal resistance vessels, which results in increased minimum vascular resistance and increased vasoconstrictor responsiveness (2). Such chronic structural alterations would not be expected to be affected by acute AT1 receptor blockade but are known to be reversed by chronic AT1 receptor blockade (8).

The current studies tested the hypothesis that the enhanced renal vasoconstrictor response to renal sympathetic nerve stimulation and the associated abnormality in the low-pass filter function of the renal vasculature observed in CHF are dependent on the functional reversible actions of circulating angiotensin II on renal AT1 receptors.

METHODS

Adult male Sprague-Dawley rats (275–325 g) were used for all studies. The rats were allowed free access to a normal sodium rat pellet diet and tap water. 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 National Institutes of Health.
ABNORMAL FREQUENCY RESPONSE IN HEART FAILURE

Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, Revised 1985).

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

Rats were anesthetized with pentobarbital sodium (50 mg/kg ip). An oral endotracheal tube was then 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; pulsatile, P, mean = M) and heart rate (HR). 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; Munich, Germany). The electrode was connected to an electrical stimulator (model S88, Grass) or the output of a computer-controlled stimulator, and the nerve bundle was sectioned between the electrode and the neuraxis, assuring that the only activity passing to the left kidney was derived from the renal nerves. A nonstimulating electromagnetic flow probe (1.5 mm circumference) was placed around the left renal artery and connected to an electromagnetic flowmeter (Carolina Medical Electronics). The flow probe was calibrated in situ by pumping heparinized rat blood at known flow rates through the cannulated rat renal artery (with the flow probe in place) at the end of the experiment. After surgery, a 45-min period was allowed for equilibration and stabilization.

Conventional renal nerve stimulation. For each rat, a supramaximal voltage was determined as follows. At a frequency of 2 Hz and at a rectangular pulse duration of 0.5 ms, stimulation voltage was progressively increased until further increases in stimulation voltage did not result in further decreases in RBF. For further study, rectangular pulses of 0.5-ms duration and supramaximal voltage (as determined for each rat) at frequencies of 0, 0.5, 1.0, 1.5, and 2.0 Hz were used. Each 60-s period of renal nerve stimulation was preceded by a 5-min control period and followed by a 5-min recovery period.

This experimental protocol was used in groups of both Control and CHF rats beginning 10 min after the administration of either losartan 10 μmol/kg iv or vehicle (0.2 ml isotonic NaCl). This resulted in four experimental groups (n = 6 rats/group): Control, Control-Losartan, CHF, and CHF-Losartan.

At the end of the experiment, the AP and RBF responses to a dose of angiotensin (100 pmol iv) were determined. Thereafter, the carotid artery catheter was advanced into the left ventricle for the measurement of LVEDP. The rats were euthanized with an overdose of pentobarbital sodium, and a 20-min recording of postmortem signals was made. The heart was removed and weighed.

Pseudorandom binary sequence stimulation. For each rat, a stimulus voltage was determined that produced the maximum decrease in RBF but was not supramaximal (i.e., a voltage that activated all nerve fibers). This was determined by stimulating the renal nerves at a 2-Hz frequency, a 0.5-ms duration, and voltages between 5 and 15 V in steps of 1 V. The maximum voltage determined was used for pseudorandom binary sequence stimulation (PRBS) in each rat. After a 10-min recovery period, a 30-min control recording of mean AP (MAP) and RBF was made. Ten minutes after the administration of either losartan (10 μmol/kg iv) or vehicle (0.2 ml isotonic NaCl), the renal nerves were then stimulated with a PRBS for 30 min. The PRBS (5–7) was composed of a basal pulse of frequency of 2 Hz and duration of 2 ms and voltage, which was switched between a low voltage (0.5 V) and the maximum voltage previously determined for each rat. Every 0.5 s, 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 interest, 0–0.7 Hz, a desirable feature of an input signal for systems analysis of frequency response (7).

This experimental protocol resulted in four experimental groups: Control (n = 6), Control-Losartan (n = 8), CHF (n = 6), and CHF-Losartan (n = 7).

At the end of the experiment, the MAP and RBF responses to a dose of angiotensin (100 pmol iv) were determined. Thereafter, the carotid artery catheter was advanced into the left ventricle for the measurement of LVEDP. The rats were euthanized with an overdose of pentobarbital sodium, and a 20-min recording of postmortem signals was made. The heart was removed and weighed.

Simulation. Whereas PRBS is a rigorously defined multi-frequency test input signal composed with uniform power over the frequency range of interest (5–7), it is not endogenous RSNA. To examine the effect of any alteration in the low-pass filter characteristics of the renal vasculature in CHF on normally occurring RSNA, the following analysis was made. An RSNA input signal (recorded in a control anesthetized rat; amplification 20,000; band-pass filtered from 30 to 1,000 Hz) was sampled at 1,000 Hz. With the use of the values of transfer function gain for both Control and CHF rats (from Fig. 3), the filter coefficients of the equivalent linear phase filters were determined. The Control and CHF linear phase filters were applied to the previously recorded RSNA input signal, which generated RSNA output signals, respectively, Control filter, and CHF filter RSNA output signals. The power spectra of the RSNA input signal (unfiltered) and the Control filter and CHF filter RSNA output signals were determined.

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

For the conventional renal nerve stimulation data, the maximum change in RBF with each stimulation frequency was calculated as the percent change from the mean value of the preceding 5-min control RBF value. RVR = MAP/RBF.

For the PRBS stimulation data, analog AP, renal nerve stimulator, and RBF signals were sampled from the tape at 500 Hz. Because the voltage required to activate all nerve fibers differed for individual rats, the amplitude of all PRBS stimuli was normalized to unity by dividing by the maximum voltage. Subsequent processing of the data was performed with Matlab software. The 500-Hz data files were digitally low-pass filtered (3.5 Hz cut-off frequency, finite-impulse response, order 50) and then decimated to a rate of 5 Hz. These 5 Hz data were split into blocks of 4,196 data points. The transfer function spectra were calculated from PRBS (input) and RBF (output) during PRBS. The transfer function
was taken as the quotient of the cross spectrum of input and output divided by the power spectrum of the input. The algorithm involved mean detrending and a Hanning window with 50% overlap of the blocks. To permit comparison between rats, the transfer function gain (magnitude) values over the frequency range have been normalized to the value at 0 Hz frequency (DC). After conversion of the normalized transfer function gain values into decibels (20 log[gain]), a mean spectrum was calculated from the consecutive spectra and averaged for all rats. The time delay was calculated from the slope of the plot of phase (radian) of the transfer function versus frequency over its linear portion (0.05 to 0.6 Hz); time delay = change in phase angle (radian)/2π change in frequency (Hz).

Coherence is a frequency domain estimate of a linear correlation coefficient between two signals indicating the degree to which the variance in one signal can be explained by a linear operation on the other signal. The coherence spectra were calculated from PRBS (input) and RBF (output) during PRBS. The coherence function was taken as the quotient of the square of the cross spectrum of input and output divided by the product of the power spectrum of the input times the power spectrum of the output. The algorithm involved mean detrending and a Hanning window with no overlap of blocks of 256 data points.

Statistical analysis was performed with analysis of variance with the subsequent use of Scheffe’s method for simultaneous comparisons within groups and the subsequent use of the F ratio and modified statistic for nonsimultaneous comparisons between group (9). A significance level of 5% was chosen. Data are expressed as means ± SE.

RESULTS

Because values for body weight, heart weight, heart weight-to-body weight ratio, and LVEDP were similar in the rats used for both experimental protocols, they have been pooled (Table 1). CHF rats had significantly increased body weight, heart weight, heart weight-to-body weight ratio, and LVEDP compared with Control rats.

The MAP, RBF, and RVR responses to the test dose of angiotensin II are shown in Table 2. Because the results were similar in the rats used for both experimental protocols, they have been pooled. The test dose of angiotensin II produced pressor and renal vasoconstrictor responses, which were similar in Control and CHF rats. Administration of losartan to Control and CHF rats abolished these angiotensin II-induced pressor and renal vasoconstrictor responses. Thus losartan produced effective AT1 receptor blockade in both the systemic and renal vasculature of Control and CHF rats.

CONVENTIONAL RENAL NERVE STIMULATION. Before conventional renal nerve stimulation, MAP tended to be lower, whereas RBF was significantly lower and RVR was significantly higher in CHF rats compared with Control rats (Table 3). In Control rats (in the absence of losartan), 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 (Fig. 1). In CHF rats (in the absence of losartan), 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, which were significantly different (P < 0.05) from the responses observed in Control rats. In Control rats, following losartan administration, the renal vasoconstrictor responses at 0.5 and 1.0 Hz were not significantly altered while they were significantly attenuated at 1.5 Hz (−46.0 ± 3.0%) and 2.0 Hz (−46.3 ± 3.1%). In CHF rats, after losartan administration, the renal vasoconstrictor responses at 0.5 Hz were not significantly al-

Table 1. Body weight, heart weight, heart weight/body weight, and left ventricular pressure data in four groups of rats used for conventional and pseudorandom binary sequence renal nerve stimulation (pooled)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Body Wt, g</th>
<th>Heart Wt, g</th>
<th>Heart/Body Wt, %</th>
<th>LVEDP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>356 ± 9</td>
<td>1.31 ± 0.07</td>
<td>0.37 ± 0.02</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>Control-Losartan</td>
<td>14</td>
<td>347 ± 8</td>
<td>1.22 ± 0.05</td>
<td>0.35 ± 0.02</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td>CHF</td>
<td>12</td>
<td>382 ± 9*</td>
<td>2.51 ± 0.12</td>
<td>0.65 ± 0.04*</td>
<td>15.9 ± 2.5*</td>
</tr>
<tr>
<td>CHF-Losartan</td>
<td>13</td>
<td>375 ± 8*</td>
<td>2.54 ± 0.11*</td>
<td>0.67 ± 0.05*</td>
<td>16.9 ± 2.4*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of rats. LVEDP, left ventricular end-diastolic pressure; CHF, congestive heart failure. *P < 0.05 for CHF vs. Control and for CHF-Losartan vs. Control-Losartan.

Table 2. MAP, RBF, and RVR responses to angiotensin II test dose in four groups of rats used for conventional renal nerve stimulation and the four groups of rats used for pseudorandom binary sequence renal nerve stimulation (pooled)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>MAP, mmHg</th>
<th>RBF, ml/min</th>
<th>RVR, mmHg·ml⁻¹·min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>+42 ± 2</td>
<td>−3.1 ± 0.6</td>
<td>+30.1 ± 2.3</td>
</tr>
<tr>
<td>Control-Losartan</td>
<td>14</td>
<td>+1 ± 1*</td>
<td>−0.1 ± 0.2*</td>
<td>+0.2 ± 0.2</td>
</tr>
<tr>
<td>CHF</td>
<td>12</td>
<td>+40 ± 2</td>
<td>−3.4 ± 0.5</td>
<td>+59.7 ± 3.7*</td>
</tr>
<tr>
<td>CHF-Losartan</td>
<td>13</td>
<td>+1 ± 1*</td>
<td>−0.2 ± 0.2*</td>
<td>+0.6 ± 0.3*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. MAP, mean arterial pressure; RBF, renal blood flow; RVR, renal vascular resistance; Δ, change. *P < 0.05 for Control-Losartan vs. Control and CHF-Losartan vs. CHF.

Table 3. Baseline (control period) hemodynamic data in the four groups of rats used for conventional renal nerve stimulation

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>MAP, mmHg</th>
<th>RBF, ml/min</th>
<th>RVR, mmHg·ml⁻¹·min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>105 ± 4</td>
<td>6.21 ± 0.24</td>
<td>16.9 ± 1.2</td>
</tr>
<tr>
<td>Control-Losartan</td>
<td>6</td>
<td>103 ± 3</td>
<td>6.34 ± 0.21</td>
<td>16.2 ± 1.1</td>
</tr>
<tr>
<td>CHF</td>
<td>6</td>
<td>98 ± 5</td>
<td>5.12 ± 0.25*</td>
<td>19.1 ± 1.1*</td>
</tr>
<tr>
<td>CHF-Losartan</td>
<td>6</td>
<td>95 ± 3</td>
<td>5.02 ± 0.21*</td>
<td>19.0 ± 1.2*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. *P < 0.05 for CHF vs. Control and for CHF-Losartan vs. Control-Losartan.

AJP-Heart Circ Physiol • VOL 285 • NOVEMBER 2003 • www.ajpheart.org
vasoconstrictor responses were no longer significantly greater in CHF than in Control rats at 1.0, 1.5, and 2.0 Hz. After losartan administration, the renal vasoconstrictor responses were no longer significantly different between CHF and Control rats.

**PRBS stimulation.** The effects of PRBS renal nerve stimulation at 2 Hz on steady-state values of MAP, RBF, and RVR are shown in Table 4. During the control period (before PRBS, absence of losartan), the values were similar to those in the separate groups of rats used for the conventional renal nerve stimulation protocol (see Table 3). In the Control period, MAP tended to be lower, whereas RBF was significantly lower and RVR tended to be higher in CHF rats compared with Control rats. In the PRBS period (presence of losartan or vehicle), MAP was unchanged in Control rats and CHF rats but was significantly decreased in Control-Losartan rats and CHF-Losartan rats. RBF was significantly decreased in all groups with the decrease being greater in CHF rats than in Control rats (−30 ± 4% vs. −20 ± 3%, *P* < 0.05). Losartan administration resulted in a greater attenuation of the decrease in RBF in CHF rats (−30 ± 4 vs. −13 ± 3%) compared with Control rats (−20 ± 3 vs. −10 ± 2%). RVR was significantly increased in Control and CHF rats with the increase being greater in CHF rats (+42 ± 5 vs. +22 ± 3%). In Control-Losartan and CHF-Losartan rats, RVR showed a slight decrease, −9 ± 2 and −4 ± 1%, respectively, likely due to the combined actions of losartan to decrease MAP and to attenuate the PRBS-induced reductions in RBF.

Coherence serves as a linear correlation coefficient in the frequency domain between the input (PRBS) stimulus and the output (RBF) response. The values range from 0 (no correlation) to 1 (complete correlation). The coherence values for Control and Control-Losartan rats (Fig. 2, left) are generally > 0.5 over the frequency range of 0 to 0.6 Hz. However, coherence values for CHF and CHF-Losartan rats (Fig. 2, right) exceeded 0.5 only over the frequency range of 0 to 0.2–0.3 Hz and were significantly less than in Control and Control-Losartan rats over the frequency range 0.2–0.6 Hz. While it appears that coherence values were higher over a broader frequency range after losartan administration, there were no significant differences between Control and Control-Losartan rats or between CHF and CHF-Losartan rats.

The transfer function gain represents the degree to which the input signal PRBS is either attenuated (negative gain values) or amplified (positive gain values) in the output signal RBF. In Control rats (Fig. 3, left), the steep attenuation is characteristic of a low-pass filter. The corner frequency (~3 dB, 30% attenuation) was 0.002 Hz, and there was 85% attenuation (~16.5 dB) at 0.01 Hz and 97% attenuation (~30 dB) at 0.1 Hz. Thus frequencies ≥0.01–0.1 Hz contained in RSNA are effectively prevented from influencing RBF. The steep linear portion of the frequency response yields a slope of approximately −20 dB per frequency decade, consistent with the expected performance of a first order low-pass filter. The pattern in the Control-Losartan rats was not significantly different from that in the Control rats.

In CHF rats (Fig. 3, right), the pattern is markedly altered with limited attenuation, less than or equal to −5 dB (~44% attenuation) over the entire frequency range.

**Table 4. Effect of pseudorandom binary sequence renal nerve stimulation on steady-state values of MAP, RBF, and RVR during Control and PRBS periods.**

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP, mmHg</th>
<th>RBF, ml/min</th>
<th>RVR, mmHg·ml⁻¹·min</th>
<th>MAP, mmHg</th>
<th>RBF, ml/min</th>
<th>RVR, mmHg·ml⁻¹·min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>115 ± 3</td>
<td>6.53 ± 0.24</td>
<td>17.6 ± 1.3</td>
<td>112 ± 3</td>
<td>5.23 ± 0.17</td>
<td>21.4 ± 1.5</td>
</tr>
<tr>
<td>Control-Losartan</td>
<td>113 ± 3</td>
<td>6.64 ± 0.21</td>
<td>17.0 ± 1.2</td>
<td>93 ± 3</td>
<td>6.00 ± 0.20</td>
<td>15.5 ± 1.8</td>
</tr>
<tr>
<td>CHF</td>
<td>100 ± 6</td>
<td>5.21 ± 0.24</td>
<td>19.2 ± 1.5</td>
<td>99 ± 5</td>
<td>3.63 ± 0.16</td>
<td>27.3 ± 1.9§</td>
</tr>
<tr>
<td>CHF-Losartan</td>
<td>101 ± 2</td>
<td>5.30 ± 0.29</td>
<td>19.1 ± 1.2</td>
<td>85 ± 3</td>
<td>4.61 ± 0.18</td>
<td>18.4 ± 1.7</td>
</tr>
</tbody>
</table>

Values are means ± SE; *n*, no. of rats. PRBS, pseudorandom binary sequence. *P* < 0.05 for CHF vs. Control and for CHF-Losartan vs. Control and for CHF-Losartan vs. Control-Losartan during the Control period. †*P* < 0.05 for each group during PRBS period vs. same group during Control period; ‡*P* < 0.05 for each group during PRBS period vs. same group during Control period; §*P* < 0.05 for each group during PRBS period vs. same group during Control period.
range. Thus frequencies $\geq 0.01-0.1$ Hz contained in RSNA are only 50% attenuated with respect to their influencing RBF. In the CHF-Losartan rats, it is evident that the pattern is similar to that in Control and Control-Losartan rats, representing the effect of losartan to normalize the low-pass filter capacity of the renal vasculature in CHF rats.

The plots of phase angle versus frequency were similar in all four groups. As shown for the Control rats (Fig. 4), there was an initial steep decrease in phase angle between 0 and 0.05 Hz. Thereafter, phase angle decreased linearly with increasing frequency indicating the presence of a pure time delay. The calculated values for time delay were similar in all four groups: Control, $700 \pm 15$ ms; Control-Losartan, $711 \pm 16$ ms; CHF, $708 \pm 18$ ms; and CHF-Losartan, $719 \pm 17$ ms.

**Simulation.** The Control filter RSNA output signal power is reduced by 1,000 times from the RSNA input.

![Fig. 2. Coherence during pseudorandom binary sequence (PRBS) renal nerve stimulation in Control, Control-Losartan, CHF, and CHF-Losartan rats. The lines represent mean values, and SE has been omitted for clarity.](image)

![Fig. 3. Frequency response characteristics of the renal vasculature as assessed by determination of transfer function gain (normalized to gain at frequency $= 0$ Hz, DC) during PRBS renal nerve stimulation in Control, Control-Losartan, CHF, and CHF-Losartan rats. The heavy weighted continuous and dashed lines represent the mean values, and the light weighted continuous and dashed lines represent means $\pm$ SE.](image)
signal power in a uniform fashion over the entire frequency range (see Fig. 5). A similar result was observed for the Control-Losartan filter RSNA output signal power (data not shown). This is the effect expected when a first-order low-pass filter with a low corner frequency is applied to an input signal. In contrast, the CHF filter RSNA output signal power is reduced by only 50% from the RSNA input signal over the same frequency range. The overall result is that a much larger fraction of the RSNA input signal power passes the CHF filter than the Control filter and is thus able to exert a greater vasoconstrictor effect on the renal vasculature.

**DISCUSSION**

These results demonstrate that the enhanced renal vasoconstrictor responses to renal nerve stimulation and the associated abnormality in the frequency response characteristics of the renal vasculature seen in CHF are mediated by the action of angiotensin II on renal angiotensin II AT1 receptors.

Although losartan was administered systemically, it was demonstrated that renal angiotensin II AT1 receptor blockade was achieved as the renal vasoconstrictor response to intravenous angiotensin II was completely inhibited. In regard to these studies, it is known that there are angiotensin II AT1 receptors located presynaptically on the renal sympathetic nerve terminals, which facilitate the release of norepinephrine from the sympathetic nerve terminal in response to renal nerve stimulation (10) and on the renal vasculature, which mediate vasoconstriction (2). The frequency range of renal nerve stimulation used has been shown to increase renin secretion rate, resulting in increased angiotensin II (3).

In both the Control and CHF rats, losartan administration decreased but did not abolish the renal vasoconstrictor responses to renal nerve stimulation. The dose of losartan used was sufficient to block the renal vasoconstrictor response to a dose of angiotensin II, which caused a 60% decrease in resting RBF. Thus if the renal vasoconstrictor responses to renal nerve stimulation (≤50% decrease in RBF herein) were entirely due to an increase in angiotensin II, then the renal vasoconstrictor responses would have been expected to be completely inhibited by losartan. Because this was not the case, this suggests that a substantial portion of the action of losartan action was to inhibit presynaptic angiotensin II AT1 receptors resulting in a lesser (but not absent) release of norepinephrine from renal sympathetic nerve terminals during renal nerve stimulation. This interpretation is consistent with previous studies (10) demonstrating that losartan dose dependently decreased the renal vasoconstrictor responses to renal nerve stimulation without affecting the renal vasoconstrictor responses to exogenous norepinephrine.

The finding that losartan attenuated the renal vasoconstrictor responses to renal nerve stimulation to a greater extent in CHF than in Control rats is consistent with the increased activity of the RAS and increased angiotensin II known to be present in CHF (2). It is also possible that for a given intensity of renal nerve stimulation, there is a greater increase in renin secretion rate and angiotensin II effect in CHF than in Control rats. This would contribute to there being a greater angiotensin II AT1 receptor-dependent presynaptic facilitation of norepinephrine release from renal sympathetic nerve terminals in CHF than in Control rats. The fact that the renal vasoconstrictor responses to renal nerve stimulation after losartan administration were similar in CHF and Control rats provides further support for this view.

As previously noted, the enhanced renal vasoconstrictor response to conventional renal nerve stimulation in CHF was associated with an abnormality in the frequency response of the renal vasculature.
The abnormality in the frequency response characteristics of the renal vasculature in CHF rats was related to the effect of angiotensin II to decrease baseline RBF and increase baseline RVR, i.e., a smaller diameter of the renal vascular lumen. It is possible that the presence of a tonic increase in renal vascular constrictor tone altered the ability of the renal vasculature to effectively discriminate, i.e., filter, frequencies within RSNA. However, there does not appear to be close coupling between the ability of losartan to affect the steady-state renal vasoconstrictor responses to either conventional renal nerve stimulation (Fig. 1) or PRBS (Table 2) and its ability to correct the abnormality in the frequency response characteristics of the renal vasculature in CHF (Fig. 3).

Furthermore, in the anesthetized rabbit, when angiotensin II was infused into the renal artery in amounts sufficient to decrease basal RBF by 33% (i.e., decreased diameter of the renal vascular lumen), the renal vasoconstrictor responses to graded frequency conventional renal nerve stimulation and the frequency response characteristics of the renal vasculature were not affected (6). It was concluded that decreases in basal RBF of ~30% appear to have little impact on the renal vascular responsiveness to renal sympathetic nerve stimulation or on the ability of the different frequencies within RSNA to modulate RBF. However, it was acknowledged that 1) these conclusions apply only to the effects of acute administration of exogenous angiotensin II and may not apply to the situation of chronic increases in endogenous angiotensin II as is the case here in CHF rats; 2) additional important information is likely to be derived from the use of angiotensin II AT1 receptor antagonists to more physiologically inhibit the effect of endogenous angiotensin II as used herein. It should be noted that, in the current studies, induction of CHF produced a decrease in basal RBF of ~20%, which was contributed to by the increase in both RSNA and angiotensin II as well as other likely factors (increased vasopressin and insufficient renal vasodilator factors, e.g., prostaglandins).

At frequencies <0.2 Hz, coherence values were similar in all four groups of rats. However, coherence decreased <0.5 at a lower frequency in CHF rats (0.2 Hz) than in Control rats (0.7 Hz) and this was not affected by losartan treatment. This indicates a lesser correlation between the input PRBS signal and the output RBF signal in CHF rats at frequencies >0.2 Hz. Thus in this frequency range, PRBS may be of lesser significance than some of the multiple other factors (e.g., dilator, constrictor substances) thought to be involved in the abnormal renal circulatory dynamics of CHF. The failure of this to be reversed by losartan would suggest that actions mediated via angiotensin II AT1 receptors are not a major determinant of the coherence values at frequencies >0.2 Hz in CHF.

In summary, the enhanced renal vasoconstrictor response to renal nerve stimulation and the associated abnormality in the frequency response characteristics of the renal vasculature seen in CHF are mediated by the actions of angiotensin II on renal angiotensin II AT1 receptors.

DISCLOSURES

This study was supported by National Institutes of Health Grants DK-15843 and HL-55006 and by a Department of Veterans Affairs Merit Review Award.

REFERENCES