Effect of renal nerve stimulation on responsiveness of the rat renal vasculature

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DiBona, Gerald F., and Linda L. Sawin. Effect of renal nerve stimulation on responsiveness of the rat renal vasculature. Am J Physiol Renal Physiol 283: F1056–F1065, 2002.—When the renal nerves are stimulated with sinusoidal stimuli over the frequency range 0.04–0.8 Hz, low (<0.4 Hz)- but not high (≥0.4 Hz)-frequency oscillations appear in renal blood flow (RBF) and are proposed to increase responsiveness of the renal vasculature to stimuli. This hypothesis was tested in anesthetized rats in which RBF responses to intrarenal injection of norepinephrine and angiotensin II and reductions in renal arterial pressure (RAP) were determined during conventional rectangular pulse and sinusoidal renal nerve stimulation. Conventional rectangular pulse renal nerve stimulation decreased RBF at 2 Hz but not at 0.2 or 1.0 Hz. Sinusoidal renal nerve stimulation elicited low-frequency oscillations (<0.4 Hz) in RBF only when the basal carrier signal frequency produced renal vasoconstriction, i.e., at 5 Hz but not at 1 Hz. Regardless of whether renal vasoconstriction occurred, neither conventional rectangular pulse nor sinusoidal renal nerve stimulation altered renal vasoconstrictor responses to norepinephrine and angiotensin II. The RBF response to reduction in RAP was altered by both conventional rectangular pulse and sinusoidal renal nerve stimulation only when renal vasoconstriction occurred: the decrease in RBF during reduced RAP was greater. Sinusoidal renal nerve stimulation with a renal vasoconstrictor carrier frequency results in a decrease in RBF with superimposed low-frequency oscillations. However, these low-frequency RBF oscillations do not alter renal vascular responsiveness to vasoconstrictor stimuli.

ABUNDANT EXPERIMENTAL EVIDENCE in several mammalian species, including the rabbit, indicates that renal sympathetic nerve stimulation at low frequencies, ≤1.0 Hz, does not affect renal blood flow (RBF) or glomerular filtration rate (GFR) but is capable of increasing both renal release and renal tubular sodium reabsorption (2, 6, 7, 9, 11, 23, 24). Renal sympathetic nerve stimulation at higher frequencies (>1.0 Hz) decreases RBF and GFR and further increases both renal release and renal tubular sodium reabsorption (6). These studies used conventional single rectangular pulses of known duration, amplitude, and frequency.

With power spectral analysis, renal sympathetic nerve activity (RSNA) is seen to contain oscillations at several frequencies, both faster and slower than the frequency of pulsatile arterial pressure (i.e., cardiac cycle or heart rate). For the rabbit in the basal state, the RSNA power spectrum showed an oscillation centered at 0.3 Hz. This was intermittently associated with an oscillation at a similar frequency in the RBF power spectrum, being observed during some experiments (15–17) but not during other experiments (18, 19) in the same laboratory. This variability in the observation of a 0.3-Hz oscillation in the RBF power spectra may be related to the marked effect of anesthesia and surgical stress on an already highly variable level of RSNA in the rabbit, as reflected by the response of RBF to renal denervation. Renal denervation increased RBF in conscious rabbits by 55% (18) and 65% (12) at ~7 days after renal denervation in two studies but had no effect at >10 days after renal denervation in another study (1) from the same laboratory. Hemorrhage resulted in an increase in the 0.3-Hz oscillations in both the RSNA and RBF power spectra, with the latter being eliminated by renal denervation (18). In the rat in the basal state, the RSNA power spectrum showed an oscillation at 0.4 Hz (3), which was not associated with an oscillation at a similar frequency in the RBF power spectrum (8). This may be accounted for by the fact that RBF in the rat is little affected by renal denervation (6–8), reflecting a more stable and lower level of RSNA in the basal state compared with the rabbit. Compressing or application of heat to the rat tail led to increases in the oscillations at 0.3–0.4 Hz and 0.2 Hz, respectively, in both the RSNA and RBF power spectra, with the latter being eliminated by renal denervation (8).

Although such low frequencies do not decrease RBF when used in a conventional single rectangular pulse renal sympathetic nerve stimulation protocol, these power spectral analysis findings have prompted the development of different patterns of renal sympathetic nerve stimulation to reexamine...
the influence of these low-frequency RSNA oscillations on RBF.

Thus the renal sympathetic nerves have been stimulated with a sinusoidal pattern that varied the amplitude of a basal rectangular pulse (5-Hz frequency, 5-ms duration) between 0 and +10 V at frequencies between 0.04 and 1.0 Hz (20). Superimposed on a background of renal vasoconstriction produced by the high frequency of the basal pulse, sinusoidal pattern frequencies of ≈0.4 Hz (but not >0.4 Hz) induced oscillations at the same frequencies in RBF. It was proposed that 1) the low-frequency (≈0.4 Hz) oscillations in the RBF signal, coherent with those in the sinusoidal renal sympathetic nerve stimuli, contribute to an increase in the responsiveness of the renal vasculature to stimuli; and 2) the higher-frequency (>0.4 Hz) oscillations, markedly attenuated in the RBF signal compared with those in the renal sympathetic nerve stimuli, contribute to the stability of RBF by ensuring a steady-state level of renal vasoconstriction (19, 20).

Although no data were presented concerning this hypothesis, consideration can be given to studies of the vascular responses to renal arterial injection of both vasoconstrictor and vasodilator substances. For the most part, such studies have been performed in animals whose basal level of RSNA is substantially increased above that seen in the conscious state because of the dual effects of anesthesia and surgical stress. Occasionally, the renal sympathetic nerves have been sectioned, reducing the basal level of RSNA to zero. If the basal level of RSNA and its frequency composition have a major effect on the responsiveness of the renal vasculature to stimuli, then it would be expected that the results from studies with enhanced basal levels of RSNA should be noticeably different from those in which renal denervation has reduced the basal level of RSNA to zero. The literature yields mixed evidence in this area. In anesthetized rabbits, both renal denervation and intrarenal prazosin (with and without intact renal innervation) attenuated the renal vasoconstrictor response to intravenous angiotensin II (4). However, in anesthetized dogs, the renal vasoconstrictor responses to angiotensin II were similar during renal denervation and renal sympathetic nerve stimulation (26).

However, to more directly test this hypothesis, the current studies were performed in anesthetized rats with denervated kidneys in which the renal vasoconstrictor responses to renal arterial injection of angiotensin II and norepinephrine were measured during control and renal sympathetic nerve stimulation with both conventional and sinusoidal stimulus patterns. Additionally, to test the inherent ability of the renal vasculature to constrict and dilate, the RBF responses to decreases in renal arterial pressure were examined during control and renal sympathetic nerve stimulation with both conventional and sinusoidal stimulus patterns.

## METHODS

Adult male Sprague-Dawley rats (275–325 g), allowed free access to a 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 NIH “Guide for the Care and Use of Laboratory Animals.”

Rats were anesthetized with pentobarbital sodium (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 or femoral artery was catheterized for the measurement of arterial pressure (AP) 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 (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).

For some experiments a tapered and curved PE-10 catheter was introduced into a femoral artery and advanced through the abdominal aorta and ~1 mm into the left renal artery. This was connected to a pump that delivered heparinized (30 U/ml) isotonic saline at 5 μl/min throughout the experiment. A rat was discarded if intrarenal injection of either norepinephrine or angiotensin II altered arterial pressure. A 10-μl bolus of test agent was introduced into the renal artery infusion line. One minute before administration of the test agent, the rate of renal artery infusion was increased to 144 μl/min, which allowed the bolus to be administered within 5 s. After recovery of RBF to baseline levels, the renal artery infusion rate was returned to 5 μl/min. After surgery, a 45-min 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 vasoconstrictor response to conventional rectangular pulse stimulation with pulses of 0.5-ms duration. Frequencies used were 0.2, 0.5, 1.0, 1.5, and 2.0 Hz; voltages used were 2, 4, 8, 12, and 16 V. Each 60-s period of renal nerve stimulation was preceded by a 5-min control period and followed by a 5-min recovery period. A variation of this experimental protocol was used to identify a supramaximal voltage for each rat. At a frequency of 2 Hz and 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 studies, rectangular pulses of 0.5-ms duration and supramaximal voltage (as determined for each rat) were used.

### Sinusoidal Renal Nerve Stimulation

Sinusoidal signals were constructed in accordance with the theory of analog pulse modulation (10, 21) using purpose-written software (LabVIEW and Matlab). Modulation superimposes an information-bearing message signal on a carrier
signal for transmission, i.e., the carrier signal is multiplied by the message signal. Thus some parameter of the carrier signal is varied continuously (‘modulated’) in accordance with the message signal. In analog pulse modulation, the carrier signal is a constant-frequency pulse train so that some parameter (e.g., amplitude, duration) of the pulse train carrier signal is continuously varied in accordance with the message signal. In pulse amplitude modulation, the amplitudes of the regularly spaced pulses are continuously varied in proportion to the corresponding sample values of the message signal.

The carrier signal was a rectangular pulse of 0.5-ms duration with selected carrier signal frequencies ($f_c$) of 1 (non-renal vasoconstrictor) and 5 (renal vasoconstrictor) Hz. The message signal sinusoidally modulated the amplitude of the rectangular pulses of the carrier signal between 0 and $+10$ V (unipolar, rectified, or half-sinusoidal). The values of the message signal frequency ($f_m$) were 0.02, 0.05, 0.1, 0.2, 0.4, and 0.6 Hz. The total signal power over the frequency range of 0–1.0 Hz was constant for each value of $f_c$, irrespective of the value of $f_m$, i.e., the range was 9.9–10.4 (mV)$^2$/Hz for $f_c = 1$ Hz and 37.5–40.0 (mV)$^2$/Hz for $f_c = 5$ Hz.

Responsiveness of Renal Vasculature

Renal vasoconstrictor substances. Control measurements (no left renal nerve stimulation) of arterial pressure (measured from a carotid artery catheter) and renal blood flow were made during a 15-min control period. During the 15-min control period, two injections of both norepinephrine (20–40 ng) and angiotensin II (2–6 ng) were made into the left renal artery. In the subsequent experimental period, left renal nerve stimulation was performed with either the conventional or sinusoidal pattern of renal nerve stimulation.

In the conventional renal nerve stimulation protocol, three separate groups, identified by the frequency of left renal nerve stimulation, were studied. In the initial group, the frequency chosen (subthreshold) was that which was just below the frequency required to elicit a decrease in RBF; this averaged 1.0 ± 0.04 Hz ($n = 10$). In the second and third groups, the frequencies chosen were one-fifth (low) and twice (high) this subthreshold frequency, 0.2 ± 0.02 (low, $n = 6$) and 2.0 ± 0.07 (high, $n = 8$) Hz, respectively. Continuous measurements of AP and RBF were made during a 15-min left renal nerve stimulation period. The left renal artery injections of both norepinephrine and angiotensin II were repeated twice during the 15-min left renal nerve stimulation period, beginning 5 min after the onset of stimulation.

In the sinusoidal renal nerve stimulation protocol, extreme values of $f_c$ (1 and 5 Hz) and $f_m$ (0.1 and 0.6 Hz) were used to encompass the range of renal vasoconstriction (none at $f_c = 1$ Hz and maximal at $f_c = 5$ Hz) and the range of transfer of oscillations from the renal nerve stimulus into RBF (minimal at $f_m = 0.6$ Hz and substantial at $f_m = 0.1$ Hz). The duration of stimulation for each set of parameters was 10 min with a 5-min recovery period after each stimulation. The RBF responses to intrarenal administration of norepinephrine and angiotensin II were evaluated as noted above during both control and stimulation periods.

Alterations in renal arterial pressure. AP was measured from a catheter introduced into the femoral artery, advanced into the abdominal aorta so that its tip was below the level of the renal arteries, and was taken as renal arterial pressure (RAP). A snare was placed around the abdominal aorta above the level of the left renal artery. Recordings of RAP and RBF were made continuously and began with a 5-min control period. The suprarenal aortic snare was then tightened so as to reduce RAP by 20% for a 60-s experimental period, after which it was released for a 5-min recovery period. Another 5-min control period was then made. Thereafter, renal nerve stimulation was applied (both conventional and sinusoidal patterns) with parameters that were either subthreshold for the production of renal vasoconstriction or produced an ~20% decrease in RBF. When RBF was stable (within 1 min), the suprarenal aortic snare was tightened so as to reduce RAP to the same extent as previously for a 60-s experimental period, after which it was released for a 5-min recovery period.

For subthreshold, non-renal vasoconstricting renal nerve stimulation, a frequency of 1 Hz was used in the conventional pattern and values of $f_c = 1$ Hz and $f_m = 0.2$ Hz were used in the sinusoidal pattern. For renal vasoconstricting renal nerve stimulation, a frequency of 2 Hz was used in the conventional pattern and values of $f_c = 5$ Hz and $f_m = 0.2$ Hz in the sinusoidal pattern. One group of eight rats received the subthreshold non-renal vasoconstricting renal nerve stimulation pattern, four with the conventional and four with the sinusoidal stimulation pattern. Another group of eight rats received the renal vasoconstricting renal nerve stimulation, four with the conventional and four with the sinusoidal stimulation pattern.

Data Analysis

AP, both pulsatile and mean, was recorded via an electronic pressure transducer (Statham). HR was determined via a tachometer (Grass 7P4) driven by the pulsatile arterial pressure waveform. RBF, both pulsatile and mean, was recorded via the electromagnetic flowmeter, the output of which was low-pass filtered below 10 Hz by the built-in analog filter; renal vascular resistance (RVR) = AP/RBF. The outputs of the pressure transducer, the tachometer, the electromagnetic flowmeter, and the renal nerve stimulator (RNS) 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.

Analog AP, RNS, and RBF signals were sampled from tape at 100 Hz, and each block of 100 data points was averaged to yield 1-s averages. To take into account differences in both magnitude and duration of renal vasoconstriction produced by norepinephrine, angiotensin II, and the various forms of renal nerve stimulation, the RBF responses were calculated as change in the area under the time curve (calculated using the trapezoidal rule; area units = RBF (ml/min) × time (seconds)) and expressed as a percentage of the respective control period values.

Spectral analysis was performed with Matlab software routines on analog data sampled from tape at 1,000 Hz. The control and stimulation periods were resampled (Matlab function: resample) at 10.24 Hz, and power spectral density was calculated by Welch's method (Matlab function: pwelch) on blocks of 1,024 points that overlapped by 50% and were subjected to a Hanning window. The periodograms for each windowed block were ensemble-averaged for the control and stimulation periods. For plotting, the spectra were smoothed via cubic spline interpolation. The units of power are millimeters of mercury squared for AP, millivolts squared per hertz for RNS, and (millimeters per minute)$^2$/hertz for RBF.

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 groups (25). A significance level of 5% was chosen. All data are expressed as means ± SE.

RESULTS

Conventional Renal Nerve Stimulation

Significant renal vasoconstriction was not observed at frequencies of 0.2, 0.5, or 1.0 Hz at any amplitude (Fig. 1, left). Above 1.0 Hz, the renal vasoconstrictor responses exhibited voltage dependence, with the responses at 16 V being not significantly different from those at 12 V. The responses at a frequency of 2.0 Hz were greater than those at 1.5 Hz. Over the entire range of amplitudes, renal nerve stimulation at 0.2, 0.5, and 1.0 Hz did not significantly affect RBF (Fig. 1, right). At each amplitude, renal nerve stimulation at 2.0 Hz produced greater renal vasoconstriction than at 1.5 Hz; however, the renal vasoconstrictor responses at 16 V were not significantly different from those at 12 V for both 1.5 and 2.0 Hz.

Responsiveness of Renal Vasculature

Conventional renal nerve stimulation. Basal RBF was 7.2 ± 0.3 ml/min for the entire group (n = 24). By experimental design, renal nerve stimulation at 0.2 and 1.0 Hz did not affect basal RBF, whereas 2.0 Hz decreased basal RBF by 25 ± 3%. Figure 2 shows that the renal vasoconstrictor responses to renal arterial administration of norepinephrine and angiotensin II were similar during the control period and during stimulation at 0.2, 1.0, and 2.0 Hz.

Sinusoidal renal nerve stimulation. The RBF power spectrum during sinusoidal renal nerve stimulation showed coherent oscillations at each value of \( f_m \) whose power was greater when \( f_c = 5 \) Hz than when \( f_c = 1 \) Hz. Figure 3 shows the total RBF power in the 0- to 1.0-Hz frequency band (Fig. 3, top) as well as the RBF power at each individual value of \( f_m \) (Fig. 3, bottom) for control (no stimulation) and stimulation periods at both \( f_c = 1 \) Hz (Fig. 3, left) and \( f_c = 5 \) Hz (Fig. 3, right). During stimulation at \( f_c = 1 \) Hz, which did not produce renal vasoconstriction, both the total RBF power in the 0- to 1.0-Hz frequency band and the RBF power at each individual value of \( f_m \) are similar during the control and stimulation periods. During stimulation at \( f_c = 5 \) Hz, which produced renal vasosconstriction, both the total RBF power in the 0- to 1.0-Hz frequency band and the RBF power at each individual value of \( f_m \) are greater during the stimulation than the control period.

During stimulation at \( f_c = 5 \) Hz, at increasing values of \( f_m \), there is progressive attenuation of both the total RBF power in the 0- to 1.0-Hz frequency band and the RBF power at each individual value of \( f_m \). However, for values of \( f_m \leq 0.4 \) Hz, both the total RBF power in the 0- to 1.0-Hz frequency band and the RBF power at each individual value of \( f_m \) are significantly greater (\( P < 0.05 \) or better) during stimulation at \( f_c = 5 \) Hz than at \( f_c = 1 \) Hz. Therefore, pulse amplitude modulation at \( f_c = 5 \) Hz augments RBF power compared with the effect of pulse amplitude modulation at \( f_c = 1 \) Hz and this augmentation becomes progressively less at higher values of \( f_m \).

Figure 4 shows an example of the effect of sinusoidal renal nerve stimulation on RBF at \( f_c = 1 \) Hz (Fig. 4, top) and \( f_c = 5 \) Hz (Fig. 4, bottom) with \( f_m = 0.02, 0.05, 0.1, 0.2, 0.4, \) and 0.6 Hz. With \( f_c = 1.0 \) Hz, there was no
renal vasoconstriction at any $f_m$ and, although a non-uniform oscillatory pattern may be discerned in this example, there were no RBF oscillations that were coherent with $f_m$. With $f_c = 5.0$ Hz, there was a similar magnitude of renal vasoconstriction at all $f_m$ values and there were RBF oscillations at frequencies that were coherent with $f_m$.

For the entire group ($n = 8$), basal RBF was $7.4 \pm 0.4$ ml/min. At the extremes, with $f_c = 1.0$ Hz, the decrease in basal RBF with $f_m = 0.1$ Hz was $1.0 \pm 0.2\%$, similar to that seen with $f_m = 0.6$ Hz, $1.7 \pm 0.4\%$. With $f_c = 5.0$ Hz, the decrease in basal RBF with $f_m = 0.1$ Hz was $26.3 \pm 3.2\%$, similar to that seen with $f_m = 0.6$ Hz, $26.2 \pm 3.9\%$. As seen in Fig. 5, the renal vasoconstrictor responses to renal arterial administration of norepinephrine and angiotensin II were similar during the control period, the various sinusoidal (pulse amplitude modulation) renal nerve stimulation periods, and the recovery period.

Fig. 2. Renal vasoconstrictor responses to renal arterial injection of norepinephrine (NE; 1st pair of bars in each group) and angiotensin (ANG II; 2nd pair of bars in each group) during control period and during conventional RNS with 0.5-ms-duration rectangular pulses at supramaximal amplitude and frequencies of $0.2 (n = 6), 1.0 (n = 10)$, and $2.0 (n = 8)$ Hz. Baseline RBF was not decreased at either 0.2 or 1.0 Hz but was decreased by $25 \pm 3\%$ at 2.0 Hz.
Alterations in Renal Arterial Pressure

The control and stimulation period values for RAP, RBF, and RVR for the four different groups of rats are shown in Table 1. At subthreshold intensity, neither conventional nor sinusoidal pattern renal nerve stimulation affected RAP, RBF, or RVR. At vasoconstricting intensity, both conventional and sinusoidal pattern renal nerve stimulation did not affect RAP but decreased RBF and increased RVR. The magnitudes of the decreases in RBF and the increases in RVR were similar for conventional and sinusoidal pattern renal nerve stimulation. Therefore, the results were pooled and both the decrease in RBF from 6.9 ± 0.2 to 5.4 ± 0.1 ml/min (22%) and the increase in RVR from 18.2 ± 1.0 to 22.7 ± 1.5 mmHg·ml⁻¹·min⁻¹ (25%) were significant (P < 0.05).

The effect of renal nerve stimulation on the RBF responses to reductions in RAP were similar with conventional and sinusoidal renal nerve stimulation. Figure 6 illustrates the experimental protocol and results for RBF in a rat in which the conventional pattern of renal nerve stimulation was used. RAP was decreased before and during both subthreshold and vasoconstricting intensities of renal nerve stimulation.

When renal nerve stimulation was subthreshold and did not decrease basal RBF at control RAP, there was no effect on the RBF response to reductions in RAP (Fig. 6, left). When RAP was decreased, the time course and pattern of RBF responses were similar before and during subthreshold renal nerve stimulation.

When renal nerve stimulation was vasoconstrictor, the RBF response to reduction in RAP was significantly affected (Fig. 6, right). When RAP was decreased before vasoconstrictor renal nerve stimulation, the RBF response was similar to that observed before and during subthreshold renal nerve stimulation (Fig. 6, left). Vasoconstrictor renal nerve stimulation decreased basal mean RBF at control RAP from 6.9 to 6.3 ml/min. When RAP was decreased during vasoconstrictor renal nerve stimulation mean RBF decreased from 6.3 to 5.3 ml/min (16%), and when RAP was returned to normal there was an initial transient increase in RBF to the prestimulation level followed by a decrease in RBF below both the pre- and poststimulation levels.

The absolute area under the RBF curve beginning with the last RBF value before RAP reduction (after nerve stimulation) and ending when RBF returned to that value after return of RAP to normal was calculated with the trapezoidal rule. When renal nerve stimulation was subthreshold (Fig. 6, left), these values were 400 and 416 ml·min⁻¹·s for the RBF responses to RAP reduction before and after non-renal vasoconstricting renal nerve stimulation, respectively, a difference of 16 ml·min⁻¹·s or 4%. When renal nerve stimulation decreased basal RBF at control RAP (Fig. 6, right), these values were 398 and 482 ml·min⁻¹·s for the RBF.

Fig. 4. Effect of sinusoidal RNS on RBF at $f_s = 1$ Hz (top) and at $f_s = 5$ Hz (bottom) with $f_m = 0.02, 0.05, 0.1, 0.2, 0.4$, and 0.6 Hz.
Fig. 5. Renal vasoconstrictor responses to renal arterial injection of norepinephrine (NE) and ANG II during preceding control period, during sinusoidal (pulse amplitude modulation) RNS with \( f_c \) = 1 and 5 Hz and \( f_m \) = 0.1 and 0.6 Hz, and after recovery period. When \( f_c \) = 1 Hz, basal RBF was not significantly decreased at \( f_m \) = 0.1 (\(-1.0 \pm 0.2\%\)) or 0.6 (\(-1.7 \pm 0.4\%\)) Hz. When \( f_c \) = 5 Hz, basal RBF was similarly and significantly decreased at \( f_m \) = 0.1 (\(-26.3 \pm 3.2\%\)) and 0.6 (\(-26.2 \pm 3.9\%\)) Hz.

Responses to RAP reduction before and after vasoconstricting renal nerve stimulation, respectively, a difference of 84 ml·min\(^{-1}\)·s or 21%.

For the entire group, when subthreshold renal nerve stimulation did not decrease basal RBF (\( n = 8 \); 4 conventional and 4 sinusoidal renal nerve stimulation), the magnitude of the decrease in RBF (measured as area under the curve) during the reduction in RAP was not significantly affected. The percent difference between the RBF responses to RAP reduction before and during non-renal vasoconstrictor renal nerve stimulation was 2 ± 3%. When vasoconstrictor renal nerve stimulation decreased basal RBF at control RAP (\( n = 8 \); 4 conventional and 4 sinusoidal renal nerve stimulation) by 21 ± 3% (\( P < 0.05 \)), the magnitude of the decrease in RBF (measured as area under the curve) during the reduction in RAP was significantly affected. The RBF decreases in response to RAP reduction were 24 ± 4% (\( P < 0.05 \)) greater during vasoconstricting renal nerve stimulation than before.

**DISCUSSION**

The major findings in this study are that 1) conventional rectangular pulse renal nerve stimulation in the rat at frequencies \( \leq 1.0 \) Hz, although resulting in identifiable coherent oscillations in the RBF power spectrum, does not decrease RBF; 2) conventional or sinusoidal renal nerve stimulation either at low frequencies (subthreshold for renal vasoconstriction) or at high frequencies (renal vasoconstriction) does not increase the responsiveness of the renal vasculature to stimuli as reflected by unchanged renal vasoconstrictor responses to intrarenal norepinephrine and angiotensin II; and 3) conventional or sinusoidal renal nerve stimulation at renal vasoconstrictor (but not subthreshold) intensities significantly augments the decrease in RBF associated with a reduction in RAP.

It has been suggested (19, 20) that the finding of oscillations in the RBF power spectrum, coherent with low-frequency (\( \leq 1.0 \) Hz) components of complex forms

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**Table 1. Baseline and steady-state stimulation values for RAP, RBF, and RVR during control and stimulation periods with subthreshold and constricting intensities of conventional and sinusoidal pattern renal nerve stimulation**

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<thead>
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<th>Conventional-Constricting</th>
<th>Sinusoidal-Subthreshold</th>
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<td>RBF</td>
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<td>RVR</td>
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<td>17.6 ± 1.1</td>
<td>17.9 ± 1.0</td>
<td>22.1 ± 1.4*</td>
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Values are means ± SE. Each of the 4 groups of intensities and patterns of renal nerve stimulation contained 4 rats. RAP, renal artery pressure (mmHg); RBF, renal blood flow (ml/min); RVR, renal vascular resistance (mmHg·ml\(^{-1}\)·min\(^{-1}\)). Constricting intensities combined (\( n = 8 \); *\( P < 0.05 \) vs. corresponding control value.

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of renal nerve stimulation stimuli (e.g., sinusoidal), indicates that RBF is affected at these low frequencies, even in the absence of reductions in volumetric RBF. In the case of conventional rectangular pulse renal nerve stimulation, although oscillations coherent with each of the renal nerve stimulation frequencies used (0.2, 1.0, and 2.0 Hz) can be readily observed in the RBF power spectrum (data not shown), decreases in RBF were produced only by the 2.0-Hz frequency and not by the 0.2- and 1.0-Hz frequencies. Thus, although such RBF power spectrum oscillations may have some as yet unidentified functional significance, they are neither uniform nor ubiquitous indicators of decreases in RBF.

These results are in accord with previous studies in rats, rabbits, dogs, sheep, and monkeys, demonstrating that low frequencies of renal nerve stimulation (generally ≤1.0 Hz) do not affect RBF but are capable of increasing renin release and decreasing urinary sodium excretion (2, 6, 7, 9, 11, 23, 24).

Similar results were seen with sinusoidal renal nerve stimulation when a comparison was made between the renal vascular responses to stimulation with \( f_c \) values of 1 and 5 Hz over a wide range of \( f_m \) values. The RBF power spectrum showed coherent oscillations at each value of \( f_m \), whose power (for \( f_m \leq 0.4 \) Hz) was greater when \( f_c = 5 \) Hz than when \( f_c = 1 \) Hz. However, a reduction in RBF in association with superimposed coherent RBF oscillations was only seen when \( f_c = 5 \) Hz and not when \( f_c = 1 \) Hz.

These observations uncouple any fixed relationship between identifiable oscillations at certain frequencies in the RBF power spectrum and decreases in RBF. Thus certain patterns of renal nerve stimulation may result in identifiable oscillations at "low" frequencies in the RBF power spectrum but are not associated with renal vasoconstriction, i.e., decreases in RBF. It is not known whether these nonvasoconstrictor RBF power spectrum oscillations have any effect on overall renal function. Furthermore, although sinusoidal renal nerve stimulation at \( f_c = 5 \) Hz produced renal vasoconstriction with superimposed coherent (to \( f_m \)) RBF oscillations, it is not clear what physiological role may be assigned to these low-frequency oscillations in RBF. It was speculated that they might serve to increase the responsiveness of the renal vasculature to stimuli (19, 20). However, as assessed in this study, the renal vasoconstrictor responses to renal arterial administration of norepinephrine and angiotensin were not influenced at either \( f_c = 1 \) Hz (low-frequency oscillations in RBF power spectrum but no renal vasoconstriction or RBF oscillations) or \( f_c = 5 \) Hz (low-frequency oscillations in RBF power spectrum with renal vasoconstriction and RBF oscillations).

On the other hand, the renal vascular responses to reductions in RAP are more complex and involve an autoregulatory myogenic vasodilation that is limited by a lower level of RAP, i.e., the autoregulatory break point at which renal vasodilation is maximal. Those patterns of renal nerve stimulation associated with nonvasoconstrictor RBF power spectrum oscillations had no effect on the RBF response to reduction in RAP. With patterns of renal nerve stimulation that elicit renal vasoconstriction, there is a constant renal vasoconstrictor tone that raises the autoregulatory break point (i.e., reduces overall vasodilatory or autoregulatory capacity), resulting in a greater reduction in RBF for a given amount of reduction in RAP. Previous studies also showed that the effect of direct or reflex renal nerve stimulation on the RBF response to RAP reduction (i.e., autoregulatory capacity) is graded and dependent on the degree of reduction in basal RBF produced by the renal nerve stimulation (5, 14, 22). When renal nerve stimulation decreased basal RBF by more than ~15%, RBF autoregulatory capacity was
impaired as reflected by an increase in the autoregulatory break point. When renal nerve stimulation induced lesser decreases in basal RBF, RBF autoregulatory capacity was not affected.

In systems analysis, the input signal is often designed so as to provide a wide frequency range forcing at relatively uniform input signal power (13). The pulse amplitude modulation pattern of sinusoidal renal nerve stimulation achieves this aim because total signal power over the frequency range of 0–1.0 Hz was similar for all values of $f_m$ (0.02–0.6 Hz) for each value of $f_c$.

However, it is apparent that it is the ability of the carrier frequency, $f_c$, to induce renal vasoconstriction that determines whether the various values of the message frequency, $f_m$, produce superimposed oscillations in RBF at the same frequency. Even though the RBF power spectrum contained small identifiable oscillations at each value of $f_m$ when $f_c = 1$ Hz, neither renal vasoconstriction nor RBF oscillation at $f_m$ was observed. However, when $f_c = 5$ Hz, the RBF power spectrum contained greater oscillations at each value of $f_m$ and both renal vasoconstriction and RBF oscillations at $f_m$ were observed.

The presence of discrete oscillations in the RBF power spectrum does not necessarily indicate the presence of renal vasoconstriction, i.e., a decrease in volumetric renal blood flow. In the current case where $f_c = 1$ Hz, making the assumption that oscillations in the RBF power spectrum at frequencies $<1$ Hz (i.e., $f_m$) indicate that renal nerve stimulation at frequencies $<1$ Hz decreases RBF produces an erroneous conclusion. Similarly, in the case where $f_c = 5$ Hz, a frequency that produces substantial renal vasoconstriction, the finding of oscillations in RBF at frequencies coherent with $f_m$ superimposed on an established background renal vasoconstriction does not indicate that low-frequency ($f_m \leq 1.0$ Hz) renal nerve stimulation causes renal vasoconstriction. First, it is evident that the renal vasoconstriction is produced by the high carrier frequency, $f_c = 5$ Hz. This is seen as the initial downward deflection in RBF with institution of sinusoidal renal nerve stimulation. This is because $f_c = 5$ Hz yields a period of 0.2 s between pulses compared with a period of 2.5 s between pulses at $f_m = 0.4$ Hz and a period of 50 s between pulses at $f_m = 0.02$ Hz. Second, the observed RBF oscillations do not represent further renal vasoconstriction, i.e., RBF does not decrease below the reduced level established by high carrier frequency, $f_c = 5$ Hz. Rather, the oscillations are seen to be periodic migrations in which RBF increases toward the control level followed by RBF decreases toward but not below the level of renal vasocostruction established by $f_c = 5$ Hz. Third, in the absence of any initial vasoconstriction produced by the carrier frequency, as is the case when $f_c = 1$ Hz, no value of $f_m$, resulted in either renal vasoconstriction or RBF oscillations.

In summary, renal sympathetic nerve stimulation at frequencies $\leq 1.0$ Hz does not affect RBF but is capable of increasing renin secretion and renal tubular sodium reabsorption, resulting in decreases in urinary sodium excretion (6, 7). Low-frequency oscillations in RBF, whether produced by conventional rectangular pulse or sinusoidal renal nerve stimulation, do not affect the response of the renal vasculature to either norepinephrine or angiotensin. Renal vascular responses to reductions in RAP are affected only when either conventional or sinusoidal renal nerve stimulation produces renal vasoconstriction. Although both conventional and sinusoidal forms of renal nerve stimulation produce low-frequency oscillations in the RBF power spectrum, they can occur in the absence of any measurable change in volumetric RBF.

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