Renal SNA as the primary mediator of slow oscillations in blood pressure during hemorrhage

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Malpas, Simon C. and Don E. Burgess. Renal SNA as the primary mediator of slow oscillations in blood pressure during hemorrhage. Am J Physiol Heart Circ Physiol 279: H1299–H1306, 2000.—Blood pressure contains a distinct low-frequency oscillation often termed the Mayer wave. This oscillation is caused by the action of the sympathetic nervous system on the vasculature and results from time delays in the baroreflex feedback loop for the control of sympathetic nerve activity (SNA) in response to changes in blood pressure. In this study, we used bilateral renal denervation to test the hypothesis that it is SNA to the kidney that contributes a large portion of the vascular resistance associated with changes in the strength of the slow oscillation in blood pressure. In conscious rabbits, SNA and blood pressure were measured during hemorrhage (blood withdrawal at 1.35 ml·min⁻¹·kg⁻¹ for 20 min). Spectral analysis identified a strong increase in power at 0.3 Hz in SNA and blood pressure in the initial compensatory phase of hemorrhage before blood pressure started to fall. However, in a separate group of renal denervated rabbits, although the power of the 0.3-Hz oscillation under control conditions in blood pressure was similar, it was not altered during hemorrhage. Wavelet analysis revealed the development of low-frequency oscillations at 0.1 Hz in both intact and denervated animals. In conclusion, we propose that changes in the strength of the oscillation at 0.3 Hz in arterial pressure during hemorrhage are primarily mediated by sympathetic activity directed to the kidney.

METHODS

Animal preparation. Experiments were performed on rabbits (weight 2.5–3.1 kg, n = 14) that underwent surgery, at least 7 days before the experiment, for implantation of a recording electrode around the left renal sympathetic nerve. With the rabbits under halothane anesthesia, the left renal nerve was exposed by a retroperitoneal approach. With the use of an operating microscope, an intact nerve was fed through a coiled electrode (14). The electrode was held in place and insulated from the surrounding tissue by Wacker Sil-Gel (Wacker-Chemie, Munich, Germany). The other end of the Sil-Gel was connected to a recording electrode around the left renal sympathetic nerve.

Current evidence favors the concept of time delays and lags in the baroreflex loop as the origin for the 0.1-Hz oscillation in blood pressure (4, 6, 8, 11, 20, 34). In this model, a change in blood pressure is sensed by the arterial baroreceptors, and the afferent signal to the central nervous system is altered. Subsequently, the mean SNA level is altered, which in turn changes vascular tone in the target organ. Fundamental to this model is that the combination of a series of time delays means that the change in blood pressure results in an output change in vascular resistance that is slightly phase shifted, and instead of buffering the initial change in blood pressure, it leads to the development of its own change in blood pressure. It is generally thought that the oscillation in blood pressure results from global changes in SNA. However, it is often overlooked that the pattern of SNA to various target organs is not uniform and not always modulated by baroreceptors (24–26), raising the possibility that it is SNA to a few key organs that dominates in the production of the 0.1-Hz oscillation in blood pressure.

In the present study, we used bilateral renal denervation to test the hypothesis that it is SNA to the kidney that contributes a large portion of the vascular resistance associated with changes in the power of the slow oscillation (0.3 Hz in the rabbit) in blood pressure during hemorrhage. SNA and blood pressure were measured in conscious rabbits during hemorrhage, which, as we have previously established (22), produces an increase in the strength of the oscillation at 0.3 Hz in renal SNA.

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of the electrode was tunneled under the skin for later retrieval on the day of the experiment. In a separate group of seven animals, instead of implantation of a renal electrode, bilateral renal denervation was performed. All animals also had a Silastic catheter (inner diameter 0.86 mm) inserted into the superior vena cava via the right jugular vein during this surgery for withdrawal of blood. This catheter was filled with heparin (1,000 IU/ml) with the other end sealed and was placed under the skin for retrieval on the day of the experiment.

Experimental protocol. On the day of the experiment, catheters were inserted in a central ear artery for measurement of arterial pressure. The ends of the renal electrode and venous catheter were retrieved from under the skin under local anesthesia. SNA was amplified, filtered between 50 and 5,000 Hz, full-wave rectified, and integrated by using a low-pass filter with a time constant of 20 ms. This integrated SNA signal and arterial pressure were continuously recorded throughout the experiment and were sampled at 1,000 Hz by using an analog-to-digital data acquisition card (National Instruments, Austin, TX). Calibrated signals were displayed on a computer screen and saved to disk with the use of a program written in the LabVIEW graphic programming language (National Instruments). After the preparatory procedures were completed, rabbits were left for 60 min before the experimental protocol commenced. Animals were given 1,000 IU in 2 ml of heparin via the chronically indwelling superior vena cava catheter. After a 20-min period of control recording, blood was withdrawn with the use of a constant withdrawal pump at a rate of 1.35 ml/min. Blood was withdrawn with the use of a constant withdrawal pump at a rate of 1.35 ml/min. After a 20-min period of control record-

**Data processing.** The fluctuations in blood pressure and SNA were investigated in the frequency domain with the use of spectral analysis techniques. Although these variables display oscillations at frequencies >2 Hz, such as those occurring with each cardiac cycle, this has already been described in some detail (17) and was not the focus of the present study. Analysis of oscillations at frequencies <2 Hz was performed as previously described (22). Briefly, all beat-to-beat signals were replayed on screen for visual inspection, and data segments with artifacts caused by obstruction of the arterial catheter or movement were eliminated. The 20-min hemorrhage period was divided into sections of 5 min for analysis along with the 20-min prehemorrhage control period. Because equidistantly sampled data are required for a fast Fourier transform, the beat-to-beat data segments were resampled at 10.24 Hz and partitioned into segments of 100 s (1024 points) in length, overlapping by 50 s. Each segment was subjected to detrending to remove the underlying mean value and slow changes in the parameters and was then subjected to an overlapped fast Fourier transform according to methods described by Berger et al. (3). The resulting frequency resolution was 0.01 Hz.

To further clarify changes in the various rhythms during hemorrhage, we also undertook band-pass filtering and wavelet analysis. Wavelet analysis allows one to follow the variations in the oscillations continuously throughout hemorrhage without the need to conduct spectral analysis over set periods of time. Wavelet analysis complements the more standard spectral analysis by giving a picture of fluctuations as a function of both frequency and time. Wavelet analysis is particularly useful in problems like this one, where the dynamics contain several different frequency scales. To construct a filter, we multiplied the Fourier transform of a time series \( \hat{x}(f) \) by a smooth notch function \( H(f) \) built from hyperbolic tangent functions, namely

\[
H(f) = \frac{1}{2} \left( \tanh \left( \frac{f - f_1}{\sigma} \right) - \tanh \left( \frac{f - f_2}{\sigma} \right) \right) + \tanh \left( \frac{f + f_2}{\sigma} \right) - \tanh \left( \frac{f + f_1}{\sigma} \right)
\]

where \( \sigma = 103f \) is the smoothing interval and \( f_1 \) and \( f_2 \) give the frequency range of the filter. To obtain the filtered time series, one takes the inverse Fourier transform of the product. The wavelet transform \( \hat{x} \) is essentially a band-pass filter that allows one to study the signal on any chosen scale \( s \) (10). The continuous wavelet transform of a time series \( x(t) \) is defined as

\[
\hat{x}(s, t) = \frac{1}{\sqrt{s}} \int_{-\infty}^{\infty} x(t') \varphi \left( \frac{t' - t}{s} \right) dt'
\]

where the analyzing wavelet \( \varphi \) has a width on the order of the scale \( s \) and is centered at time \( t \). We used the Mexican hat wavelet, which is the second derivative of a Gaussian function. For this wavelet, there is a conversion factor of approximately four between the scale \( s \) and the corresponding Fourier period (32). Thus, by applying a wavelet with a scale \( s = 0.5 \) to a time series, one picks out rhythms that have a period of ∼2 s. Using a standard Fourier transform routine, one can efficiently calculate the convolution integral in the frequency domain and then take the inverse Fourier transform to find the wavelet transform in the time domain. To compute the amplitudes of the wavelet transform, we used an analytic signal approach (16). Let \( x(t) \) represent an arbitrary signal. The analytic signal, a complex function of time, is defined by \( \tilde{x}(t) = x(t) + i\, y(t) \), where \( y(t) \) is the conjugate time series or Hilbert transform of \( x(t) \). The amplitude \( A(t) \) is then defined as \( A(t) = \sqrt{x^2(t) + y^2(t)} \). In the frequency domain, the Hilbert transform simply multiplies the positive frequency Fourier modes by \( i \) and the negative frequency modes by \( -i \). To visualize the wavelet transform of a time series, we made contour plots of the amplitude squared as a function of frequency and time.

**Statistical analysis.** With regard to the steady-state data, the influence of hemorrhage on the levels of each variable during hemorrhage was tested by repeated-measures analysis of variance (ANOVA), with the factors being neural status (intact or denervated) and time (1-min averages). The magnitudes of the power in different frequency bands before and during hemorrhage were compared in the intact and denervated groups by a split-plot ANOVA as described previously (18). The total sums of squares (SS) were partitioned into between-groups mean squares (state of innervation), with further partitioning of the SS of each group into between-animal mean squares. The significance of the difference between groups was assessed from the variance ratio \( F = \) between-groups mean square/within-groups mean square.

**RESULTS**

**Steady-state changes during hemorrhage.** The steady-state changes during hemorrhage have been reported in detail in our previous study, which followed the same hemorrhage protocol (22). Briefly, therefore, for the present group of renal nerve intact animals, hemorrhage was associated with a profound increase in SNA. After 5 min of blood withdrawal, the increase was \( 11 \pm 13\% \) (between-animal SE of mean) above control levels, at 10 min, 17 ± 9%, at 15 min, 90 ± 31%, and by...
the end of hemorrhage, SNA had reached 210 ± 115% of control levels. Arterial pressure did not significantly decrease until 9–11 min of blood withdrawal and reached 58 ± 4 mmHg after 20 min (control 83 ± 2 mmHg). The blood pressure responses to the withdrawal of blood were not significantly different between intact and denervated animals.

Oscillations in SNA and mean arterial pressure during hemorrhage. Under control conditions, five of seven animals showed a spectral peak at ~0.3 Hz in SNA (Figs. 1 and 2). Strong coupling between SNA and mean arterial blood pressure (MAP) at this frequency was indicated, because coherence was generally >0.5 (average 0.57 ± 0.13). This peak in SNA accounted for 22 ± 6% of total spectral power <2 Hz. In MAP, this same frequency band accounted for 14 ± 6% of total spectral power <2 Hz. This was not significantly different in renal denervated animals when measured either as a percentage of total power or as absolute spectral power.

Our previous study (22) reported the changes in the power of the oscillation in SNA during hemorrhage in detail. The new data and analysis in the present study relates to the comparison of the oscillation in MAP in renal nerve intact versus denervated rabbits. The first 10 min of hemorrhage were associated with pronounced increases in the power of oscillations centered at 0.3 Hz in SNA and in MAP (Figs. 1 and 2). In each rabbit, there was a significant increase in coherence between SNA and MAP in the initial 10 min of hemorrhage (range 0.48–0.95, average 0.79 ± 0.08). The phase delay between SNA and MAP under control conditions was positive, with the change in SNA leading the change in MAP by 1.37 ± 0.1 s at 0.3 Hz. This phase delay was not altered during hemorrhage. Although mean SNA continued to increase throughout the 20-min period, the power around 0.3 Hz did not

![Graphs showing spectral power changes in SNA and MAP during hemorrhage.](http://ajpheart.physiology.org/DownloadedFrom/H1301/)

**Fig. 1.** Examples of 0.3-Hz oscillations in 1 rabbit before (A) and during (B) hemorrhage. The top trace in each panel refers to the mean arterial pressure (MAP). SNA, sympathetic nerve activity.

**Fig. 2.** Changes in spectral power for MAP and SNA in 1 rabbit in the control period (A) and between 5 and 10 min of hemorrhage (B). Mean SNA increased 32% in this rabbit by 10 min of hemorrhage. Coherence between SNA and MAP was over 0.5 in the frequency band from 0.25 to 0.35 Hz and was maximal at 0.3 Hz in each animal. Oscillations associated with respiration were also present between 0.75 and 1.40 Hz in all animals. These data are not presented because this finding has already been reported in a previous study (22).
Animals (Figs. 3 and 4). The wavelet spectrums showed significant decreases in power in this frequency band, although it still remained above control levels. Howev-er, in the renal denervated group, although absolute spectral power of MAP between 0.25 and 0.45 Hz was profoundly increased during the first 10 min of hemorrhage (Figs. 3–5). Subsequently, the next 10 min were associated with relative decreases in power in this frequency band, although it still remained above control levels. However, in the ren-aL denervated group, although absolute spectral power of MAP between 0.25 and 0.45 Hz was similar to that in the intact group in the control period, there were no significant changes in the power of MAP at any phase of hemorrhage in this frequency band (Fig. 5). The wavelet spectrums showed significant increases in spectral power of MAP oscillations <0.25 Hz during hemorrhage in both intact and denervated animals (Figs. 3 and 4).

**DISCUSSION**

The main finding of this study was that changes in the slow oscillation in blood pressure (0.3 Hz) occurring during hemorrhage were caused by the specific action of SNA on the renal vasculature. Rabbits in which both kidneys were denervated showed no significant change in the strength of the MAP oscillation at 0.3 Hz during hemorrhage, indicating that neural control of the renal vasculature plays a key role in generating the pattern of oscillations observed in hemorrhage.

It is often overlooked that the pattern of SNA to the various target organs is quite selective. In particular, SNA to the lungs, kidney, and spleen is highly baroreceptor sensitive (25, 28); however, SNA to the skin and gut is only weakly regulated by baroreceptor activity (24–26). With regard to the human, it appears that SNA to the skin is not dissimilar to that in animals, because previous studies have found little evidence of baroreflex modulation of skin SNA (12, 33). The 0.1-Hz oscillation in blood pressure in humans is proposed to result from time delays and lag in the baroreflex loop (8, 11), where a change in blood pressure is sensed by the arterial baroreceptors and the afferent signal to the central nervous system is altered. Subsequently, the mean SNA level is altered, which in turn changes vascular tone in the target organ. Thus, for vascular resistance in any particular organ to contribute to the occurrence of the oscillation, SNA to that organ must be baroreceptor sensitive.

Renal denervated rabbits were able to maintain arterial pressure during hemorrhage to the same extent as renal nerve intact animals. This does not necessarily indicate that the renal nerves do not contribute to the maintenance of arterial pressure during blood loss, because other factors, both hormonal factors and increased SNA to other organs, may compensate. Previously with regard to the kidney, we found that the onset for a reduction in renal blood flow during hemorrhage was delayed if the renal nerves were not present (22). However, this still raises the question that if the mean blood pressure response to hemorrhage was similar, regardless of the presence or absence of renal nerves, then why was the strength of the 0.3-Hz rhythm in blood pressure different? A possible explanation can be found by considering that SNA to a variety of organs contains common components and also components such as the 0.3-Hz oscillation that may be more specific to the kidney. Generalized SNA to the vasculature of many organs contributes largely to the maintenance of a mean level of blood pressure, i.e., the direct current (DC) gain level of blood pressure. Thus SNA to other organs increased in response to hemorrhage and assisted in maintaining blood pressure; however, the results of the present study indicate that this SNA did not contain a strong 0.3-Hz component. It should be noted that the oscillation at 0.3 Hz is a variation around a DC gain level. Given that the 0.3-Hz power is relatively low compared with that of the other frequencies present in renal SNA (between 15 and 20% of total power in the rabbit, with the balance being cardiac and respiratory related; Ref. 17), it is likely that it contributes in only a modest way to the DC gain level of blood pressure. The value of the 0.3-Hz rhythm lies in its indication of neural control of the circulation and the possibility that its analogous frequency in human blood pressure (0.1 Hz) may be used as an index of sympathetic tone/baroreflex control.

<table>
<thead>
<tr>
<th>SNA Spectral Power, %</th>
<th>Control</th>
<th>0–5</th>
<th>5–10</th>
<th>10–15</th>
<th>15–20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low frequency (0.04–0.25 Hz)</td>
<td>25±4</td>
<td>27±4</td>
<td>30±5</td>
<td>38±7*</td>
<td>46±5*</td>
</tr>
<tr>
<td>Mid frequency (0.25–0.45 Hz)</td>
<td>22±2</td>
<td>23±2</td>
<td>30±3*</td>
<td>23±3</td>
<td>23±3</td>
</tr>
<tr>
<td>High frequency (0.75–1.40 Hz)</td>
<td>13±1</td>
<td>14±3</td>
<td>12±3</td>
<td>10±2</td>
<td>7±3</td>
</tr>
</tbody>
</table>

Because absolute microvolt levels varied between animals, data were calculated as the percentage of the total power (≤2 Hz) comprised by each frequency band. Values are means ± SE and do not summate to 100% because of power outside the frequency bands. SNA, sympathetic nerve activity. *Significantly different from control.
Further support for our results can be derived from analysis of the model proposed by DeBoer et al. (11) and further expanded in the rat by Burgess et al. (8). As has been previously noted, the 0.1-Hz oscillation in blood pressure in humans is analogous to 0.3 Hz in rabbits and 0.4 Hz in rats. This species difference can be accounted for by the larger physical distance between arterial baroreceptors and the brain and various target organs in man compared with that in rabbits and rats, which means that conduction times for the afferent and efferent signals will be longer. However, if all organs with sympathetic innervation contributed to the origin of the oscillations in blood pressure in proportion to their percentage of cardiac output, then it is suggested that no clear oscillation would be evident. The slow conduction velocities in postganglionic nerves [0.7 m/s (13)] would mean that SNA to the extremities would reach its target up to 1 s later than the innervation to major organs in the thoracic cavity. This would result in the vasoconstrictor response to the SNA signal being temporally nonuniform, and although there could still be blood pressure variability around this frequency, it is most likely that this would appear as a much wider band of variability than has been observed (e.g., Fig. 2).

Stauss and colleagues (30, 31) recently observed that the frequency-response characteristic of the mesenteric vasculature to SNA was different from that of the skin vasculature in rats. The vasculature delay and lag time is a large component of the feedback loop, which gives rise to the oscillation at 0.4 Hz in the rat (8). If blood flow to organs such as the skin played a significant role in the generation of the oscillation, one could reasonably expect more than one peak in the spectrum of blood pressure. The existence of a single peak between 0.2 and 0.45 Hz in rabbits and rats (e.g., Fig. 2)
supports the concept that it is SNA to organs with similar vasculature time delays and frequency response characteristics that gives rise to the oscillation.

If the 0.3-Hz oscillation is caused by baroreflex feedback, the possibility is raised that a number of factors known to alter baroreflex gain may alter the strength of the oscillation at 0.3 Hz. Such factors include increased circulating levels of angiotensin II, which may act via circumventricular organs such as the area postrema to further increase SNA and alter baroreflex gain. Previously, blockade of endogenous angiotensin II in the medulla produced increases in baroreflex gain (2). Thus increases in circulating angiotensin II might be expected to increase the strength of the 0.3-Hz rhythm. Although there is a lack of studies reporting changes in the 0.3-Hz rhythm in response to a variety of stimuli, if the model of baroreflex feedback via the vasculature proves correct, then any treatment, pathology, or stimuli that is known to alter gain, either between SNA and the vasculature or between blood pressure and SNA, could be expected to alter the strength of the 0.3-Hz rhythm in blood pressure. This possibility can be used in the present study to explain why the 0.3-Hz oscillation increased in strength in blood pressure before a change occurred in mean blood pressure and why this oscillation subsequently diminished in the later phase of hemorrhage despite continued increases in mean SNA levels. In the initial compensatory phase of hemorrhage, increased baroreflex gain between blood pressure and SNA would lead to an increase in power at 0.3 Hz; however, once blood pressure had begun to fall (from the 9th min) and the kidney vasculature was under a moderate level of vasconstriction, it is possible that the gain between SNA and the vasculature was reduced, thus diminishing the strength of the 0.3-Hz oscillation, independent of arterial baroreflex control over SNA.

Several recent studies have begun to explore the possibility of measuring the 0.1-Hz oscillation in skin blood flow as an index of sympathetic activity or baroreflex gain (5, 27). Our results do not negate this approach. However, it is suggested that changes in the strength of this oscillation in skin blood flow are unlikely to be caused by SNA directed solely toward the skin vasculature, but such changes are more likely to reflect the effect of SNA to organs that are strongly baroreceptor related, i.e., the kidney. As a result, any change in the strength of the oscillation in the skin blood flow would be more likely to be driven by the oscillation in blood pressure in a direct pressure-flow relationship rather than through changes in vascular resistance.

We used hemorrhage as a simple technique to induce an increase in SNA. The advantage of this approach is that, in its initial phase, it has previously been shown to cause an increase in mean SNA to multiple target organs, not just to the kidney (19). Thus the absence of any significant increase in blood pressure variability in
the renal denervated animals suggests that the changes in SNA to other organs did not cause an oscillation in blood flow to those organs and thus did not contribute significantly to the oscillation at 0.3 Hz in blood pressure. We previously reported changes in the variability of renal SNA and its effect on renal blood flow during hemorrhage (22). We have presented those results from the present series of animals only where it is relevant for clarity. Previously, however, we made no analysis of MAP variability between renal nerve intact and denervated animals. We propose that this analysis (both wavelet and spectral analysis) and its interpretation provides novel insight into circulatory control. We undertook wavelet analysis because it allows one to follow the variations in the oscillations continuously throughout hemorrhage without the need to conduct spectral analysis over set periods of time. This is particularly useful if one wants to determine the precise onset of an oscillation. This analysis helped us to identify a slow frequency oscillation <0.25 Hz developing during the later phase of hemorrhage as blood pressure began to fall. Because this occurred in both intact and denervated animals, it is not likely to rely on renal SNA. Previously, we found that the renal vasculature displays an inherent ability to resonate between 0.1 and 0.2 Hz (23). We suggested that this was a property of the vasculature that was under both active and passive control. We showed that nitric oxide was likely to buffer this ability, because blockade of endogenous nitric oxide led to an increased ability to oscillate at 0.16 Hz. It is possible that changes in other mediators of vascular tone, such as angiotensin II, also play a role in its occurrence.

One factor that may limit the implication of our results is the possibility that the effect observed is confined to the sympathetic response occurring during hemorrhage. However, we suggest this may not be the case. Our previous research established that hypoxia in conscious rabbits results in an increase in the strength of oscillations in renal blood flow at 0.3 Hz, an effect ablated by prior renal denervation (17). If SNA to other beds had also induced a cycle of vasoconstriction and dilation at 0.3 Hz, then we would have expected there to be little difference between intact and renal denervated rabbits because the kidney would have still shown an oscillation at 0.3 Hz due to a direct pressure-flow relationship. In addition, examination of the data from the study by Janssen et al. (Table 2 in Ref. 17) indicates that the increase in the strength of the 0.3-Hz oscillation in blood pressure during hypoxia was less in renal denervated rabbits, although the effect was not specifically analyzed.

It has been proposed that measurement of blood pressure variability in humans at 0.1 Hz can be used as an index of mean SNA levels and/or baroreflex gain (4, 21). The results of the present study indicate that such measurements may not reflect generalized cardiovascular control but rather neural control of the renal vasculature under some stimuli. In the case of hemorrhage (and possibly other interventions), the increase in SNA triggers a special pattern of MAP variability for which neural control of a particular vascular bed, the renal vasculature, appears to be necessary. In general, the increase in power at one spectral peak should not be thought of in terms of a global oscillation but, rather, may involve underlying dynamics between different vascular beds.

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