Background and Purpose—The role of the sympathetic nervous system in cerebral autoregulation remains poorly characterized. We examined cerebral blood flow responses to augmented arterial pressure oscillations with and without sympathetic blockade and compared them with responses in the forearm circulation.

Methods—An oscillatory lower body negative pressure of 40 mm Hg was used at 6 frequencies from 0.03 to 0.08 Hz in 11 healthy subjects with and without α-adrenergic blockade by phentolamine.

Results—Sympathetic blockade resulted in unchanged mean pressure and cerebral flow. The transfer function relationship to arterial pressure at frequencies >0.05 Hz was significantly increased in both the cerebral and brachial circulations, but the coherence of the relation remained weak at the lowest frequencies in the cerebral circulation.

Conclusion—Our data demonstrate a strong, frequency-dependent role for sympathetic regulation of blood flow in both cerebral and brachial circulations. However, marked differences in the response to blockade suggest the control of the cerebral circulation at longer time scales is characterized by important nonlinearities and relies on regulatory mechanisms other than the sympathetic system. (Stroke. 2010;41:102-109.)

Key Words: cerebral blood flow ■ hemodynamics ■ sympathetic nervous system ■ transcranial Doppler

Cerebral perfusion is maintained constant over a wide range of systemic pressures through counterregulatory changes in cerebrovascular resistance. Original studies of cerebral flow responses supported a counterregulation against changes in arterial pressure encompassing the time scale from minutes to hours. However, the recent ability to assess cerebral blood flow velocity on a beat-by-beat basis has allowed the observation that cerebral flow is regulated not just over minutes and hours, but also on shorter time scales of only a few beats. Data suggest that blood flow responses are dampened in response to pressure changes over periods as short as 15 seconds and that this dampening becomes progressively greater over longer time periods. Thus, the relationship between pressure and flow in the cerebrovasculature is a high pass filter wherein slower changes in pressure are effectively counterregulated, whereas faster oscillations pass through relatively unaffected.

Despite the fact that this autoregulatory capacity of the cerebral vasculature is of critical importance, the underlying physiology remains incompletely understood. A number of different, and possibly overlapping, physiological mechanisms such as the sympathetic nervous system, endothelial derived nitric oxide, and vascular myogenic responses could play some part in cerebral autoregulation, but the specifics of their respective involvement remain largely unknown. For example, the cerebrovascular bed is well innervated by sympathetic nerve fibers, but their role in autoregulation is poorly understood and highly controversial. However, when nerves along the arteries of the brain that have connections to the cervical sympathetic chain have been sectioned or stimulated, responses have been either absent or inconsistent. There are some inferential data suggesting that the sympathetic nervous system may play a role in cerebrovascular regulation, but only two studies have directly examined this possibility in humans. Zhang et al used complete ganglionic blockade by trimethaphan and found that the gain relation between cerebral flow and systemic pressure almost doubled, indicating that the degree of cerebral counterregulation to pressure fluctuations was reduced by removal of all autonomic neural effects. The other, more recent study used prazosin, an α-adrenoceptor antagonist, and found a modest attenuation of cerebral flow response to a single, brief (approximately 3 beats) hypotensive stimulus in 6 volunteers. These two studies suggest an autonomic, and perhaps primarily sympathetic, role in cerebral blood flow control.

If the sympathetic system is indeed involved in control of cerebral blood flow, it is critical to know its relative impor-
tance and the dynamics and magnitude of its effects. However, given the presence of redundant controllers, it is difficult to isolate the role of any one system by examining the cerebral circulation in isolation. By comparing the cerebral circulation with a vascular bed primarily under sympathetic control, we can use $\alpha$-adrenergic blockade to discern the relative role of the sympathetic nervous system in cerebrovascular control. If sympathetic control is prepotent at some time scales, but not others, it would profoundly affect our understanding of cerebral blood flow control and treatment of pathophysologies related to sympathetic dysregulation.

Materials and Methods

Subjects
Eleven volunteers aged 21 to 40 years (4 females) gave informed consent for this study. Volunteers were nonsmokers free from cardiovascular and neurological disorders and cardioactive medications. Participants were normotensive and refrained from alcohol, caffeine, and rigorous exercise at least 24 hours before study. This protocol was approved by the Institutional Review Boards of the Hebrew Rehabilitation Center for Aged and Spaulding Rehabilitation Hospital and conformed to the Declaration of Helsinki.

Instrumentation
For each subject, a 20-gauge catheter was inserted into an antecubital vein for drug infusion. Subsequently, subjects were instrumented for electrocardiographic lead II (Dash 2000; General Electric), beat-by-beat photoplethysmographic arterial pressures (Finapres; Ohmeda), and oscillometric brachial pressures (DASH 2000; General Electric). Brachial pressures were a check for photoplethysmographic finger pressures throughout the study session. Subjects were instrumented for measurement of blood flow velocities in the middle cerebral and brachial arteries (2- and 4-MHz probes; Multidop T2, DWL). The transcranial Doppler ultrasonograph probe was positioned to measure cerebral flow velocity at the M1 segment of the middle cerebral artery at a depth of 50 to 65 mm. A custom probe fixation device held the probe in place. The brachial Doppler ultrasonograph probe was placed to measure brachial artery flow velocity at the antecubital fossa ipsilateral to the infusion site. Expired CO$_2$ was monitored by an infrared carbon dioxide analyzer (Vacumed) connected to a nasal cannula. All signals were digitized and stored at 500 Hz (Windaq; DATAQ Instruments and PowerLab, ADInstruments).

Protocols

Oscillatory Lower Body Negative Pressure
To create controlled blood pressure oscillations of varying frequencies, oscillatory lower body negative pressure (OLBNP) was applied similar to previously described. The subject’s lower body was sealed in a tank and a vacuum pump connected to a timing mechanism-controlled suction intervals. Suction was applied at 40 mm Hg across 6 frequencies: 0.03, 0.04, 0.05, 0.06, 0.07, and 0.08 Hz. These progressed from lowest to highest and with decreasing duration (Figure 1). The duration at each frequency provided 10 oscillations so that the range of frequencies encompassing the previously observed cerebral autoregulation could be studied reliably over a relatively short period of time.

Sympathetic Blockade
To effect $\alpha$-adrenergic sympathetic blockade, subjects received intravenous phentolamine as a 0.14-µg/kg bolus followed by 0.014-µg/kg/min infusion. This dosage effectively blocks sympathetic effects on the vasculature based on previously published data. After commencement of the phentolamine infusion, each subject rested quietly for approx-
Table 1. Mean Values at Each OLBNP Frequency With and Without Blockade

<table>
<thead>
<tr>
<th>Two-Way Analysis of Variance</th>
<th>Condition</th>
<th>OLBNP Frequency</th>
<th>0.03 Hz</th>
<th>0.04 Hz</th>
<th>0.05 Hz</th>
<th>0.06 Hz</th>
<th>0.07 Hz</th>
<th>0.08 Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-R interval, ms</td>
<td></td>
<td>Freq P=0.13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cond P&lt;0.01</td>
<td>1059±58</td>
<td>1035±53</td>
<td>1026±54</td>
<td>1031±51</td>
<td>1027±48</td>
<td>1007±47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Freq×Cond P=0.51</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td></td>
<td>Freq P=0.59</td>
<td>90.5±2.6</td>
<td>90.5±3.0</td>
<td>92.2±3.3</td>
<td>93.2±3.6</td>
<td>92.8±3.6</td>
<td>94.1±3.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cond P=0.76</td>
<td>91.7±3.6</td>
<td>92.4±3.0</td>
<td>91.5±2.9</td>
<td>91.0±2.7</td>
<td>90.5±3.0</td>
<td>91.9±2.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Freq×Cond P=0.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brachial flow, cm s⁻¹</td>
<td></td>
<td>Freq P=0.53</td>
<td>3.22±0.46</td>
<td>3.14±0.47</td>
<td>3.35±0.51</td>
<td>3.43±0.52</td>
<td>3.26±0.51</td>
<td>3.18±0.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cond P&lt;0.01</td>
<td>5.46±0.56</td>
<td>5.68±0.54</td>
<td>5.81±0.60</td>
<td>5.91±0.61</td>
<td>6.13±0.63</td>
<td>6.28±0.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Freq×Cond P=0.51</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebral flow, cm s⁻¹</td>
<td></td>
<td>Freq P=0.73</td>
<td>71.1±6.3</td>
<td>70.6±6.3</td>
<td>69.9±6.4</td>
<td>70.1±6.2</td>
<td>69.2±6.4</td>
<td>68.4±6.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cond P=0.81</td>
<td>70.1±6.2</td>
<td>69.0±6.2</td>
<td>69.0±6.4</td>
<td>69.2±6.5</td>
<td>69.3±6.7</td>
<td>68.3±6.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Freq×Cond P=0.92</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO₂, mm Hg</td>
<td></td>
<td>Freq P=0.13</td>
<td>37.3±2.05</td>
<td>36.5±2.16</td>
<td>35.8±1.98</td>
<td>35.6±2.13</td>
<td>33.0±2.38</td>
<td>35.6±2.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cond P&lt;0.01</td>
<td>32.8±2.25</td>
<td>31.9±2.01</td>
<td>31.3±1.83</td>
<td>31.3±1.88</td>
<td>30.6±1.92</td>
<td>30.4±2.07</td>
</tr>
</tbody>
</table>

Freq indicates frequency; Cond, condition; Freq×Cond, frequency and condition interaction term.

immediately 5 minutes before the OLBNP protocol was repeated exactly as described previously.

Data Analysis

Data were analyzed using custom software written in Matlab (Version 7.1; Mathworks). The 500-Hz waveforms of arterial pressure and cerebral and brachial blood flows were decimated to 5 Hz and low pass filtered with a cutoff of 0.4 Hz to provide mean values. Filtering, as opposed to interpolated means, was used to provide signals that were independent of possible changes in the electromechanical delay from R-wave to generation of a pressure–flow pulse. These mean waveforms as well as breath-by-breath CO₂ and R-R intervals were subsequently averaged within each OLBNP frequency to provide overall means. Power spectral density estimates were calculated by Welch average modified periodogram method. For each OLBNP frequency, the filtered time series was divided into 5 segments of equal length that overlapped by 50%. This windowing was chosen to provide equal confidence in coherence across the range of OLBNP frequencies and so that an estimated squared coherence of >0.49 indicated a significant spectral relation. The signals in each segment were linearly detrended, smoothed through a Hamming window, and fast-Fourier transformed. Spectral power estimates were averaged across all windows. The product of the pressure signal with the complex conjugate of the cerebral or brachial flow velocity signal provided the cross spectrum from which coherence and transfer functions were derived. Confidence intervals and precision of estimate for the transfer function were derived based on the level of coherence from standard random process theory. We examined coherence and gain between arterial pressure and brachial flow and arterial pressure and cerebral flow at each OLBNP frequency. Gain was weighted by its precision to obtain the most accurate means for statistical analysis. In this way, unreliable estimates received appropriately small weights when group averages and statistics were computed.

Statistics

Log transformations were applied to spectral powers and the inverse hyperbolic tangent to coherence to provide estimates with asymptotically standard distributions. The Box-Cox transformation was applied to all other data to ensure normality. However, for ease of interpretation, values and confidence intervals presented here are standard units. To account for the precision of the transfer function estimates, a weighted two-way analysis of variance was used to determine the effects of frequency and sympathetic blockade on gain, and a standard two-way analysis of variance was used to determine the effects for all other variables. If a significant interaction between frequency and condition was observed, paired t tests (weighted t tests for gain) were performed to determine at which frequency a significant effect of sympathetic blockade occurred. Differences were considered significant when P<0.05. Values are reported as mean±SE.

Results

Figure 1 shows the effect of OLBNP in a representative subject. Note the consistency of the resultant arterial pressure oscillations and the differing response between vascular beds; cerebral blood flow tracks pressure only at higher frequencies, whereas brachial flow demonstrates the reverse. There was no effect of OLBNP frequency on mean values of any variable (Table 1), but arterial pressure fluctuations were greatest at the lowest frequencies (Table 2). Sympathetic blockade resulted in significant tachycardia but no change in arterial pressure. Cerebral flow was unchanged with blockade, whereas mean brachial flow increased (Table 1). Surprisingly, arterial CO₂ showed a consistent decrease across all frequencies of OLBNP after sympathetic blockade.

Arterial pressure and cerebral and brachial flow oscillations in response to OLBNP were increased with sympathetic blockade, although there were differing responses across frequency (Table 2; Figure 2). The greatest increases in arterial pressure fluctuations with sympathetic blockade occurred at the slowest frequencies, whereas the greatest increases in cerebral blood flow oscillations occurred at the highest. In fact, at 0.03 Hz OLBNP, there was no significant increase in the amplitude of cerebral blood flow oscillations.
Brachial flow oscillations, in contrast, showed no differential effect of sympathetic blockade at slower frequencies. An example of this can be seen in Figure 3. In the absence of sympathetic buffering, brachial flow closely tracks arterial pressure oscillations, whereas cerebral flow shows a biphasic response indicative of autoregulatory responses to both the pressure fall and rise with each pressure fluctuation.

Cross-spectral analysis of the cerebral and brachial response to arterial pressure fluctuations showed profound differences between the two vessels (Figure 4). Before sympathetic blockade, cerebral flow showed increasing coherence with pressure as frequency increased and brachial flow showed uniform coherence across all frequencies. Although both vascular beds demonstrated a sharp increase in coherence with sympathetic blockade, the frequency-dependent nature of cerebral coherence was maintained. The gain relations of the two vascular beds also demonstrated distinct differences at baseline, but these were ablated by sympathetic blockade. Before blockade, only the gain relation between cerebral flow and arterial pressure demonstrated a frequency dependence with increasing gain as frequency increased, whereas the brachial flow relation to pressure was consistent across all OLBNP frequencies. Sympathetic blockade resulted in a marked increase in the gain relations for both vascular beds, but only at frequencies >0.05 Hz.

It is possible that the modest relative hypocapnia during blockade had some impact on the observed responses. Therefore, we performed an additional analysis with end-tidal CO₂ as a covariate in the two-way analysis of variance comparing frequency and blockade effects. Lower end-tidal CO₂ tended to relate to lower coherence (P=0.08) and lower gain (P=0.06). However, the observed levels of hypocapnia did not counteract the effects of sympathetic blockade, frequency, or their interaction (P<0.01 for all with and without CO₂ as a covariate).

**Discussion**

Our data clearly demonstrate the important role of the sympathetic system in regulating cerebral blood flow. This is the first work to identify the time scales on which this control operates, the magnitude of its effect, and how its relative contribution differs between vascular beds. Furthermore, these results are indicative of the uniquely powerful involvement of complimentary controllers (eg, nitric oxide release, myogenic mechanisms) in regulating cerebral perfusion.

Whether the sympathetic nervous system plays a significant role in regulation of cerebral blood flow has been a
Figure 3. Effect of sympathetic blockade on pressure and flow at 0.03 Hz OLBNP in a representative subject.
controversial topic for decades. Although it has long been
known that the cerebral arteries are innervated by sympa-
thetic fibers,16,17 convincing evidence for their neural control
of cerebral flow has been sparse. Part of the curious puzzle of
nerves with no clear function may derive from interspecies
differences; different animal models can produce sharply
divergent findings.18 Indeed, it seems perfectly plausible that
bipeds engage different autoregulatory mechanisms than
quadrupeds. However, in a classic human study, Skinhoj
showed no effect of sympathetic blockade on cerebral blood
flow unless cerebral autoregulation was otherwise impaired.19
This would seem to stand at odds with more recent studies
suggestive of a consistent sympathetic role,9,10 but method-
ologic limitations may be responsible for these seemingly
contradictory findings. Studies from the 1970s and earlier
used techniques such as13Xenon clearance that take ≥10
minutes for a single measurement, orders of magnitude
slower than instantaneous transcranial Doppler measure-
ments. Little attempt has been made to formally reconcile
these older findings with recent data, and instead these two
techniques are commonly considered to be measuring differ-
ent “types” of autoregulation (ie, static and dynamic). Al-
though this might be thought of as a Manichaean construct,
our findings suggest it is broadly accurate that autoregulatory
responses can be categorized as effective over either longer or
shorter time scales. We found that sympathetic blockade
increased in the gain relation between arterial pressure and
cerebral blood flow at faster frequencies (>0.05 Hz or 20
seconds) but left autoregulatory control largely intact at
slower frequencies. At even longer time scales represented by
mean values over approximately 2 to 6 minutes (Table 1),
there was no effect of blockade on cerebral flow. Thus, it
appears that “static” autoregulation studies that have shown
no effect of the sympathetic system on cerebral autoregula-
tion19 are in fact compatible with our current data that clearly
show sympathetic involvement at characteristically shorter
time scales.

Sympathetic blockade increased the gain relation of arterial
pressure changes to blood flow at frequencies >0.05 Hz in
not only the cerebral circulation, but the brachial as well. This
common response between the two vascular beds seems to
indicate that sympathetic activity is most effective in a fairly
narrow frequency range. This may not be surprising given
frequency-dependent effects of vascular sympathetic outflow
on vascular resistance were first described in the 1960s by
Rosenbaum and Race.20 More recently, frequency domain

Figure 4. Coherence and gain between pressure and flow in the middle cerebral and brachial arteries with and without sympathetic
blockade. Freq, Frequency; Cond, Condition; Freq x Cond, frequency and condition interaction term. *P<0.05 versus baseline at the
OLBNP frequency.
approaches have generally confirmed these characteristics in a variety of animal models and tissue beds.21–23 In a novel approach, Stauss24 measured hand skin blood flow in responses to sinusoidal median nerve stimulation and demonstrated that blood flow responded at frequencies between 0.075 and 0.10 Hz, but not at lower or higher frequencies. Although we did not examine frequencies >0.08 Hz, our findings support the hypothesis that the sympathetic nervous system selectively buffers flow against arterial pressure changes in this range.

In addition to a similar increase in gain between pressure and flow, both vessels demonstrated an increased coherence in the relation after sympathetic blockade. That is, both vessels reacted more passively or linearly to changes in pressure than in the intact state. This broad increase in linearity in both vascular beds with blockade indicates that the sympathetic nervous system is active in regulation at rest and that the observed phenomena do not reflect the characteristics of a compliance vessel in the transmission of arterial blood pressure to flow velocity. However, the increase in coherence was much more striking in the brachial bed, wherein a substantial increase in linearity of the pressure–flow relationship was observed across all frequencies. The remaining low gain despite the linearization suggests that another mechanism continued to buffer against the slowest pressure changes in the functionally denervated state. The two most likely mechanisms for this buffering would be a myogenic response in resistance vessels and/or endothelial-derived nitric oxide release. Although either or both mechanism(s) could be involved, recent work by Pyke et al25 suggests that nitric oxide-dependent flow-mediated dilation responds with a roughly 0.03-Hz “dynamic” time constant (ie, 28 seconds) and has a proportional (ie, linear) response to increased shear stress. Given the strongly linear relationship, it seems likely that the nitric oxide system may be playing a role in regulating flow to the forearm vascular bed in the face of large, relatively slow arterial pressure changes during sympathetic blockade.

The cerebral vasculature, in contrast to the brachial, continued to demonstrate markedly reduced coherence in the pressure–flow relationship at the lowest frequencies after sympathetic blockade. This suggests a mechanism distinct from that regulating flow in the brachial vascular bed. Indeed, most evidence suggests that nitric oxide plays a negligible role in cerebral autoregulation; global nitric oxide synthase blockade has no effect on the spontaneous relationship between arterial pressure and cerebral blood flow26 or on the “dynamic autoregulatory index” derived from the bilateral ischemic thigh cuff response.27 Vascular myogenic mechanisms, although poorly understood in both animals and humans, have long been thought to be important in maintenance of cerebral perfusion.28 In fact, the ability to buffer against extrinsically induced fluctuations in cerebral perfusion pressure is blocked by nifedipine in rats and preferentially so at frequencies <0.1 Hz (10 seconds).29 Although no comparable human data exist, work suggests that the autoregulatory index is attenuated by the calcium channel blocker nicardipine.30 Our data provide no direct evidence for vascular myogenic mechanisms, but the contrasts between the brachial and cerebral vascular response to sympathetic blockade and our current understanding of regional vascular control strongly suggest an important myogenic role in maintenance of cerebral blood flow across longer time periods.

Limitations
One possible limitation for interpretation of these data are that arterial CO2 was significantly decreased with sympathetic blockade during OLBNP. This unexpected effect may be due to the combination of orthostatic stress and vasodilation. Because decreased end-tidal CO2 could lead to a decrease in cerebral blood flow,28 it is possible that an increase in cerebral blood flow with sympathetic blockade was masked. However, our results suggest that the hypocapnia decreased coherence and gain, and thus, at worst, our results underestimate the sympathetic nervous system’s role in the cerebral circulation. In addition, although our data demonstrate a clear role of the sympathetic system in regulating cerebral flow, they also underscore the limitations of linear methods for characterizing autoregulation. The dramatic increases in coherence between pressure and both cerebral and brachial flows demonstrate that sympathetic control may operate, at least in part, in a nonlinear fashion. Indeed, low coherence at longer time scales in the cerebral vasculature before sympathetic blockade highlights the need for robust nonlinear approaches to understand the relationships present in the unblocked state.

Summary
The current work clearly demonstrates the role of the sympathetic nervous system in cerebral autoregulation and provides further evidence of different regulatory systems active at different time scales in the cerebral and brachial circulations. Future work should isolate the role of the vascular myogenic and nitric oxide systems in these regions while characterizing important nonlinearities to provide more complete understanding of the physiological mechanism(s) unique to cerebral autoregulation.

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None.

References


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