Dynamic modulation of cerebrovascular resistance as an index of autoregulation under tilt and controlled $\text{PETCO}_2$

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Edwards, Michael R., J. Kevin Shoemaker, and Richard L. Hughson. Dynamic modulation of cerebrovascular resistance as an index of autoregulation under tilt and controlled $\text{PETCO}_2$. Am J Physiol Regul Integr Comp Physiol 283: R653–R662, 2002. First published April 4, 2002; 10.1152/ajpregu.00452.2001.—Transfer function analysis of the arterial blood pressure (BP)-mean flow velocity (MFV) relationship describes an aspect of cerebrovascular autoregulation. We hypothesized that the transfer function relating BP to cerebrovascular resistance (CVRi) would be sensitive to low-frequency changes in autoregulation induced by head-up tilt (HUT) and altered arterial $\text{PCO}_2$. Nine subjects were studied in supine and HUT positions with end-tidal $\text{PCO}_2$ ($\text{PETCO}_2$) kept constant at normal levels: +5 and −5 mmHg. The BP-MFV relationship had low coherence at low frequencies, and there were significant effects of HUT on gain only at high frequencies and of $\text{PCO}_2$ on phase only at low frequencies. BP → CVRi had coherence >0.5 from very low to low frequencies. Transfer function analysis of BP → CVRi was applied to a dynamic index of altered cerebrovascular autoregulation under HUT and higher levels of $\text{PCO}_2$.

CEREBROVASCULAR AUTOREGULATION describes the process that maintains cerebral blood flow close to a desired set point, even though the arterial blood pressure is fluctuating from the mean or normal value. Cerebral blood flow can remain relatively constant across a range of arterial blood pressure from 60 to 150 mmHg (25). Recent technological developments have permitted exploration of the dynamic nature of cerebrovascular autoregulation. Cerebral blood flow is estimated from transcranial Doppler ultrasound measurement of mean flow velocity (MFV) in the middle cerebral artery (MCA), while noninvasive blood pressure devices provide an estimate of the arterial blood pressure at the level of the MCA ($BP_{\text{MCA}}$). Thus cerebrovascular autoregulation has been characterized by frequency domain analysis of the interrelationships between MFV and $BP_{\text{MCA}}$ (2, 4, 8, 11–13, 15, 34, 38). The beat-by-beat values of MFV and $BP_{\text{MCA}}$ are processed by cross-spectral analysis to yield amplitude and phase relationships of the transfer function with $BP_{\text{MCA}}$ as the input and MFV as the output. With the cross-spectral approach, there is often little or no coherence between $BP_{\text{MCA}}$ and MFV in the very-low (<0.07 Hz)-frequency (VLF) regions (4, 38), yet it is especially in this region where changes in $BP_{\text{MCA}}$ have little effect on the MFV. At higher frequencies, greater amplitude of change in MFV for a given change in $BP_{\text{MCA}}$ indicates the high-pass filter characteristic of the cerebrovascular system. Interestingly, changes in MFV are observed to precede changes in $BP_{\text{MCA}}$ (2, 4, 12, 13, 15, 38). Many researchers have explicitly stated that a greater phase lead of MFV before $BP_{\text{MCA}}$ is an indication of better autoregulation (2, 4, 12). Recently, this phase lead was suggested as evidence that the autonomic nervous system actively regulates cerebral blood flow in advance of changes in arterial blood pressure (7, 8), but this is inconsistent with the notion of autoregulation (15, 23). Thus there is a need to define an index of dynamic cerebral autoregulation that can accurately reflect the changes in the cerebrovasculature that allow cerebral blood flow to be maintained within the limits defined by the concept of cerebrovascular autoregulation.

Aaslid and colleagues (1, 36) evaluated cerebrovascular autoregulation by determining the rapidity of the response to a reduction in $BP_{\text{MCA}}$ by the sudden release of cuffs placed around the upper thighs. They calculated a dynamic index of cerebrovascular resistance (CVRi) from $BP_{\text{MCA}}$/MFV and observed the changes in this variable as a function of the decrease in $BP_{\text{MCA}}$ as the cuffs were deflated after 3 min of circulatory occlusion of the legs. The CVRi has been frequently evaluated from steady-state data in supine and head-up tilt (HUT) postures (17, 29) to reflect cerebrovascular autoregulation. In this study, we explored the utility of CVRi as a beat-by-beat variable that can be related to changes in $BP_{\text{MCA}}$ so that a dynamic indicator of cere-
brovascular autoregulation (15) can be studied under conditions of altered arterial P\textsubscript{CO\textsubscript{2}}.

In everyday life, the cerebrovascular system must adapt rapidly to the reduction in BP\textsubscript{MCA} that occurs on going from a supine to an upright posture. Coincident with the transition to an upright posture in many individuals is a reduction in arterial P\textsubscript{CO\textsubscript{2}} (3, 21). Because a decrease in arterial P\textsubscript{CO\textsubscript{2}} would increase CVR\textsubscript{i} (20) and modify autoregulation (1, 21, 33), we have controlled the end-tidal P\textsubscript{CO\textsubscript{2}} (PET\textsubscript{CO\textsubscript{2}}) to maintain constant arterial P\textsubscript{CO\textsubscript{2}} at hypo-, normo-, and hypercapnic levels in the supine to the tilt posture. We hypothesized that, consistent with the dynamic nature of cerebrovascular autoregulation, changes in CVR\textsubscript{i} would effectively follow the spontaneous modulation of BP\textsubscript{MCA}, especially in the lower-frequency ranges, to minimize the change in MFV. We further hypothesized that the dynamic indicator of cerebrovascular autoregulation derived from cross-spectral analysis of BP\textsubscript{MCA} to CVR\textsubscript{i} would detect reduced gain of autoregulation during hypercapnia compared with hypocapnia and during HUT compared with supine posture.

METHODS

Subjects. Nine healthy subjects (6 men and 3 women, mean age 24.8 yr, range 22–34 yr) volunteered to participate in the study after being fully informed of the experimental details. The women were tested between days 3 and 10 of their menstrual cycle (follicular phase). All procedures were approved by the Office of Research Ethics at the University of Waterloo.

Experimental protocol. Subjects reported to the laboratory 3 h after a meal and after caffeine ingestion. Subjects were instrumented and placed in the supine position. Once subjects were determined to be in a steady-state resting condition (by monitoring blood pressure, respiration, and gas exchange), resting baseline respiratory rate, tidal volume, PET\textsubscript{CO\textsubscript{2}}, mean arterial blood pressure (MAP), and MFV were measured over a 10-min period.

Subjects performed three separate HUT tests to 45° each with a different level of PET\textsubscript{CO\textsubscript{2}} presented in random order. A regulated breathing protocol was used across all conditions to control the effects of respiration on blood pressure and autonomic neural output (10, 31) and to allow us to achieve hypocapnic conditions. Respiratory frequency was fixed at 15 breaths/min by an auditory signal for the initiation of inspiration and expiration, while tidal volume was increased to 50% above baseline values by having the subjects reach specified end-inspiration and end-expiration points on an oscilloscope displaying the respiration signal. This breathing protocol resulted in a decrease in PET\textsubscript{CO\textsubscript{2}} of 8–10 mmHg compared with normal resting levels. PET\textsubscript{CO\textsubscript{2}} was then altered to one of three levels using a computer-controlled, dynamic end-tidal forcing system similar to that of Robbins et al. (27). Normocapnia (N-CO\textsubscript{2}) was maintained at resting PET\textsubscript{CO\textsubscript{2}} levels determined during the baseline collection. Hypocapnia (LoCO\textsubscript{2}) and hypercapnia (HiCO\textsubscript{2}) levels were 5 mmHg below and above N-CO\textsubscript{2}, respectively. Each of the three PET\textsubscript{CO\textsubscript{2}} conditions was maintained during 7 min of supine and 7 min of HUT, with a 5- to 10-min rest period between trials. Maximum deviation of PET\textsubscript{CO\textsubscript{2}} was less than ±0.5 mmHg within a test.

Experimental measures. Heart rate was determined by standard electrocardiogram methods. Arterial blood pressure was determined from the finger using noninvasive arterial photoplethysmography (Finapres, Ohmeda, Englewood, CO). BP\textsubscript{MCA}, was estimated from the noninvasive arterial blood pressure corrected for the vertical displacement from the transducer to the Doppler probe. MFV of the MCA was determined by transcranial Doppler ultrasonography (Transpect TCD MedaSonics, Freemont, CA) as described by Aaslid et al. (1). Briefly, after the application of ultrasound gel, a 2-MHz probe was placed over the temporal window to insonate the right MCA. The probe was securely positioned with a headband for the duration of the tests. Breath-by-breath ventilatory data were collected continuously using an ultrasonic flowmeter (Kou Consulting, Redmond, WA) and mass spectrometry (model MGA-1100, Perkin-Elmer Medical Gas Analyzer, Pomona, CA).

Data analysis. Data were recorded on digital format tape (TEAC, Montebello, CA) and transferred for analysis by a computer-based system to yield a data set sampled at 100 Hz. MFV was determined from the outer envelope of the fast Fourier-transformed Doppler signal. Beat-by-beat values were then determined for PET\textsubscript{CO\textsubscript{2}}, MAP, BP\textsubscript{MCA}, and MFV by averaging the calibrated waveforms over each cardiac cycle. CVR\textsubscript{i} was calculated for each heartbeat as BP\textsubscript{MCA}/MFV. The beat-by-beat data were aligned sequentially and resampled at the mean frequency of the R-R interval for each data set. Spectral and cross-spectral analyses were performed using Welch’s averaged periodogram method (Matlab, Math Works, Natick, MA) between the input variable BP\textsubscript{MCA} and the output variable MFV or CVR\textsubscript{i} after removing the linear trends and filtering with an eighth-order low-pass Butterworth filter at 0.75 Hz. Gain values for the cross-spectral transfer functions are presented in absolute values (see Tables 3 and 4 and Figs. 3 and 4) as well as normalized values (see Figs. 3 and 4). Normalized gain was determined for each individual test for BP\textsubscript{MCA} → MFV by dividing absolute gain by the mean value of conductance (MFV/BP\textsubscript{MCA}) over that test. Likewise, for BP\textsubscript{MCA} → CVR\textsubscript{i}, normalized gain was obtained by dividing absolute gain at each frequency by the mean value of CVR\textsubscript{i}/BP\textsubscript{MCA}. By convention, a negative phase value indicates that the input preceded the output. Common practice is to accept a linear relationship between the input and output variables when squared coherence exceeded 0.5, permitting evaluation of transfer function gain and phase relationships (4, 37). This value was slightly greater than the exact value (0.45) in our study at which coherence was significantly different from zero (35). Frequency data were divided into three regions [VLF (0.003–0.007 Hz), low frequency (LF, 0.07–0.2 Hz), and high frequency (HF, 0.2–3 Hz)] to permit comparison with other studies (37) and on the basis of distinct regions of physiological response.

Statistics. Baseline data collected in the supine posture during normal breathing were compared with the N-CO\textsubscript{2} supine values by paired t-tests. The steady-state data of the three levels of PET\textsubscript{CO\textsubscript{2}} across two levels of tilt were analyzed with a three (HiCO\textsubscript{2}, LoCO\textsubscript{2}, and N-CO\textsubscript{2}) by-two (supine and upright) ANOVA with repeated measures on both factors for each of the primary dependent variables. The same statistical model was applied at each frequency (VLF, LF, and HF) for autospectral power and for averaged transfer function gain and phase using data only when the squared coherence exceeded 0.5 for the spectral relationship between BP\textsubscript{MCA} → CVR\textsubscript{i} and BP\textsubscript{MCA} → MFV. Because of many missing data points in the VLF region (i.e., squared coherence <0.5) between BP\textsubscript{MCA} and MFV, this frequency was deleted from the analysis. The significance level was set at P < 0.05. If differences were detected, a Student-Newman-Keuls post hoc test was used. Values are means ± SD.
Table 1. Steady-state responses during supine and HUT positions across all gas conditions

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>N-CO₂</th>
<th>LoCO₂</th>
<th>HiCO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>57.8 ± 7.4</td>
<td>60.5 ± 7.4</td>
<td>75.7 ± 10.8</td>
<td>58.8 ± 9.9</td>
</tr>
<tr>
<td>MFV, cm/s</td>
<td>60.5 ± 11.1</td>
<td>61.0 ± 13.5</td>
<td>58.3 ± 12.9</td>
<td>53.4 ± 10.9</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>94.7 ± 12.6</td>
<td>96.6 ± 13.7</td>
<td>95.9 ± 12.9</td>
<td>98.1 ± 12.9</td>
</tr>
<tr>
<td>BP&lt;sub&gt;MCA&lt;/sub&gt;, mmHg</td>
<td>94.7 ± 12.6</td>
<td>96.6 ± 13.7</td>
<td>77.5 ± 13.3</td>
<td>98.1 ± 12.9</td>
</tr>
<tr>
<td>CVR&lt;sub&gt;i&lt;/sub&gt;, mmHg·cm&lt;sup&gt;-1&lt;/sup&gt;·s</td>
<td>1.6 ± 0.2</td>
<td>1.7 ± 0.4</td>
<td>1.4 ± 0.2</td>
<td>1.9 ± 0.5</td>
</tr>
<tr>
<td>Pet&lt;sub&gt;CO₂&lt;/sub&gt;, mmHg</td>
<td>39.7 ± 1.5</td>
<td>39.8 ± 1.9</td>
<td>39.9 ± 1.9</td>
<td>35.1 ± 1.6</td>
</tr>
</tbody>
</table>

Values are means ± SD. HUT, head-up tilt; N-CO₂, normocapnia; LoCO₂, hypocapnia; HiCO₂, hypercapnia; HR, heart rate; MFV, mean flow velocity; MAP, mean arterial pressure; BP<sub>MCA</sub>, blood pressure at the level of the middle cerebral artery; CVR<sub>i</sub>, cerebrovascular resistance index; Pet<sub>CO₂</sub>, end-tidal PCO₂. Statistical significance (P < 0.05) is as follows: supine vs. HUT for HR, BP<sub>MCA</sub>, and CVR<sub>i</sub>; between arterial PCO₂ levels (N-CO₂, LoCO₂, and HiCO₂) for MFV, CVR<sub>i</sub>, and Pet<sub>CO₂</sub>; between baseline supine and N-CO₂ supine for HR.

RESULTS

Steady-state and baseline averaged data. Mean data for spontaneous baseline data and for supine and HUT positions for all three levels of PetCO₂ are presented in Table 1. There were no differences between baseline and supine N-CO₂ for any variable except heart rate, where the difference was <3 beats/min. The measured PetCO₂ from the three different gas trials was significantly different (P < 0.05), indicating that we were successful in lowering and elevating PetCO₂ compared with N-CO₂ levels.

There were small reductions in MFV on going from supine to HUT, but this was not significant (P > 0.05). There was a main effect of PetCO₂ on MFV (P < 0.01), with higher values in HiCO₂ and lower values in LoCO₂ than in N-CO₂ (Table 1). MAP was not different between supine and HUT, nor was it affected by the different levels of PetCO₂ (P > 0.05). BPMCA was significantly reduced during HUT (P < 0.001).

CVR<sub>i</sub> was significantly different across the PetCO₂ gas conditions (P < 0.001). Post hoc analysis indicated that CVR<sub>i</sub> was greater under LoCO₂ and less under HiCO₂ than with N-CO₂. CVR<sub>i</sub> was also significantly decreased during HUT (P < 0.001). Heart rate was significantly increased during HUT (P < 0.001) but was not affected by the different levels of PetCO₂ (P > 0.05).

Autospectral data. Representative time series data from the supine N-CO₂ tests for a single subject are presented in Fig. 1. Group mean autospectral powers at the VLF, LF, and HF regions for MFV, BPMCA, or CVR<sub>i</sub> within the VLF and LF regions (Table 2). In the HF region, power tended to be greater in the N-CO₂ trials than baseline because of the concentration of spectral power at the fixed breathing frequency, but this was significant only for CVR<sub>i</sub>. Across the controlled breathing trials, an effect of tilt was observed only in the LF region and only for MFV and BPMCA, but not for CVR<sub>i</sub>. An effect of PetCO₂ was found only for CVR<sub>i</sub> spectral power with greater amplitude in LoCO₂ than in N-CO₂ and HiCO₂, but this was significant only in the LF region (P < 0.05), and not in the VLF region (Table 2).

Cross-spectral data: BP<sub>MCA</sub> → MFV. The BP<sub>MCA</sub> → MFV cross-spectral data for all frequencies and gas conditions are shown in Table 3. Transfer function gain, phase, and coherence for the group mean responses for LoCO₂ and HiCO₂ during HUT are shown in Fig. 3. Comparisons between baseline and N-CO₂ could be made only at LF and HF because of the dropout of subjects in the N-CO₂ trial at VLF due to low coherence for the BP<sub>MCA</sub>-MFV relationship. Between-subject variations were quite large, and there were no significant differences between baseline and N-CO₂ for gain or phase (Table 3). A significantly
The primary finding of this study was that transfer function analysis for the input variable BP_{MCA} to the output variable CVRi provided a sensitive indicator of dynamic cerebrovascular autoregulation within the VLF and LF regions in the face of changes in arterial P_{CO2} and BP_{MCA}. The gain for BP_{MCA} → CVRi was reduced with HiCO_2 and increased with LoCO_2 compared with N-CO_2. This method also detected a reduced gain in the HUT position that was significant within the LF range for BP_{MCA} → CVRi. An overall main effect of P_{CO2} on the phase relationship for BP_{MCA} → CVRi was observed in the VLF range. The observations of improved dynamic cerebrovascular autoregulation in LoCO_2 or impaired autoregulation in HiCO_2 were anticipated, inasmuch as they were con-

**DISCUSSION**

The primary finding of this study was that transfer function analysis for the input variable BP_{MCA} to the output variable CVRi provided a sensitive indicator of dynamic cerebrovascular autoregulation within the VLF and LF regions in the face of changes in arterial P_{CO2} and BP_{MCA}. The gain for BP_{MCA} → CVRi was reduced with HiCO_2 and increased with LoCO_2 compared with N-CO_2. This method also detected a reduced gain in the HUT position that was significant within the LF range for BP_{MCA} → CVRi. An overall main effect of P_{CO2} on the phase relationship for BP_{MCA} → CVRi was observed in the VLF range. The observations of improved dynamic cerebrovascular autoregulation in LoCO_2 or impaired autoregulation in HiCO_2 were anticipated, inasmuch as they were con-

**Table 2. Average autospectral power during supine and HUT across all gas conditions**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>N-CO_2</th>
<th>LoCO_2</th>
<th>HiCO_2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Supine</td>
<td>Supine</td>
<td>HUT</td>
<td>Supine</td>
</tr>
<tr>
<td><strong>MFV, (cm/s)^2/Hz</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF</td>
<td>0.98 ± 0.56</td>
<td>2.55 ± 2.56</td>
<td>10.24 ± 16.19</td>
<td>2.21 ± 2.00</td>
</tr>
<tr>
<td><strong>BP_{MCA}, mmHg^2/Hz</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLF</td>
<td>42.48 ± 36.27</td>
<td>39.04 ± 20.60</td>
<td>39.17 ± 24.04</td>
<td>35.26 ± 14.34</td>
</tr>
<tr>
<td>HF</td>
<td>0.72 ± 0.76</td>
<td>4.52 ± 9.05</td>
<td>16.97 ± 33.97</td>
<td>4.69 ± 8.09</td>
</tr>
<tr>
<td><strong>CVRi, CVRU^2/Hz × 10^{-3}</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLF</td>
<td>24.8 ± 17.3</td>
<td>20.8 ± 9.6</td>
<td>23.8 ± 18.6</td>
<td>29.7 ± 15.5</td>
</tr>
<tr>
<td>LF</td>
<td>11.7 ± 8.3</td>
<td>9.8 ± 4.5</td>
<td>12.3 ± 8.7</td>
<td>14.5 ± 7.5</td>
</tr>
<tr>
<td>HF</td>
<td>0.4 ± 0.3</td>
<td>0.6 ± 0.4</td>
<td>0.6 ± 0.8</td>
<td>1.0 ± 0.8</td>
</tr>
</tbody>
</table>

Values are means ± SD. CVRU, cerebral vascular resistance units; VLF, very low frequency (0.03–0.07 Hz); LF, low frequency (0.07–0.2 Hz); HF, high frequency (0.2–0.3 Hz). Statistical significance (P < 0.05) is as follows: supine vs. HUT at LF for MFV and BP_{MCA}; between arterial P_{CO2} levels (N-CO_2, LoCO_2, and HiCO_2) for CVRi at LF; between baseline supine and N-CO_2 supine for CVRi at HF.
Table 3. Transfer function gain and phase for BP_MCA-MFV relationship

<table>
<thead>
<tr>
<th>Phase, degrees</th>
<th>VLF</th>
<th>LF</th>
<th>HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLF</td>
<td>0.547 ± 0.089 (6)</td>
<td>0.999 ± 0.251 (9)</td>
<td>1.266 ± 0.476 (9)</td>
</tr>
<tr>
<td>LF</td>
<td>0.795 ± 0.433 (6)</td>
<td>0.789 ± 0.195 (9)</td>
<td>0.983 ± 0.414 (9)</td>
</tr>
<tr>
<td>HF</td>
<td>0.638 ± 0.365 (3)</td>
<td>0.953 ± 0.368 (9)</td>
<td>1.033 ± 0.457 (9)</td>
</tr>
</tbody>
</table>

Values are means ± SD of number of observations in parentheses. See Table 2 legend for definition of abbreviations. Statistical significance (P < 0.05) is as follows: supine vs. HUT for gain at HF; between arterial PCO2 levels (N-CO2, LoCO2, HiCO2) for phase at LF.

Consistent with the rates of recovery of MFV on release of the leg cuff under conditions of altered arterial PCO2 (1).

In contrast to the positive findings from the BP_MCA-CVRi relationship, the transfer function analysis based on the BP_MCA-MFV relationship had low coherence in the VLF range as expected (4, 38). It was thus unable to identify critical changes in cerebrovascular control within this frequency range. The BP_MCA-MFV relationship failed to detect an effect of arterial PCO2 on gain, and it was able to detect an effect of supine vs. tilt on gain only in the HF range. The normalization process did identify an unexpectedly greater gain in LoCO2 than in HiCO2 in the LF range (Fig. 3; see Dynamic cerebrovascular autoregulation). A significant effect of arterial PCO2 on the phase relationship for BP_MCA → MFV was found in the LF range as a reduction in phase for HiCO2 and an increase for LoCO2. Overall, these results from BP_MCA → MFV spectral analysis revealed changes only at higher frequencies. Yet the autoregulatory process is referred to as a high-pass filter (4, 12, 38). Even though BP_MCA and MFV are the measured variables, they do not provide insight into cerebrovascular autoregulation, inasmuch as changes in BP_MCA are met by changes in cerebrovascular resistance to achieve relatively constant cerebral blood flow.

Methodological considerations. We calculated CVRi from the measured variables BP_MCA and MFV. This index has been widely used in studies of cerebrovascular autoregulation (1, 21, 29). True vascular resistance is defined as the ratio of pressure gradient across a vascular bed to the flow through this bed. The three components, pressure gradient, flow, and vascular resistance, are not and cannot be independent variables (5). The pressure drop across the cerebral circulation is unknown, because we are unable to measure venous or intracranial pressure. However, for any body position, CVRi accurately reflects changes in cerebrovascular resistance. CVRi has limitations during transitions between body positions, inasmuch as the arterial pressure is modified by the hydrostatic component (BP_MCA), while the intracranial and/or venous pressures are affected differently by posture (28). In the present study, the focus was on cross-spectral analysis of the BP_MCA-CVRi relationship. In spectral analysis, the mean values are removed, and only the variations from the mean are considered. Thus any error in estimating CVRi in supine vs. HUT occurred in the steady state and might have influenced system static gain, whereas the calculated gain and phase relationships in
Table 4. Transfer function gain and phase for BP_{MCA-CVRi} relationship

<table>
<thead>
<tr>
<th></th>
<th>N-CO₂</th>
<th>LoCO₂</th>
<th>HiCO₂</th>
</tr>
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<tbody>
<tr>
<td><strong>Gain, CVRU/mmHg</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLF</td>
<td>0.022 ± 0.007(9)</td>
<td>0.021 ± 0.006(5)</td>
<td>0.023 ± 0.005(9)</td>
</tr>
<tr>
<td>LF</td>
<td>0.019 ± 0.006(9)</td>
<td>0.014 ± 0.006(9)</td>
<td>0.029 ± 0.012(9)</td>
</tr>
<tr>
<td>HF</td>
<td>0.014 ± 0.011(7)</td>
<td>0.008 ± 0.007(9)</td>
<td>0.025 ± 0.017(9)</td>
</tr>
<tr>
<td>Phase, degrees</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLF</td>
<td>−1.6 ± 1.2(9)</td>
<td>−9.3 ± 1.1(8)</td>
<td>1.0 ± 1.5(9)</td>
</tr>
<tr>
<td>LF</td>
<td>−9.6 ± 2.4(8)</td>
<td>−78.5 ± 3.5(9)</td>
<td>−76.4 ± 20.1(9)</td>
</tr>
<tr>
<td>HF</td>
<td>−70.6 ± 103.9(7)</td>
<td>−103.4 ± 97.8(8)</td>
<td>−141.7 ± 32.2(9)</td>
</tr>
</tbody>
</table>

Values are means ± SD of number of observations in parentheses. See Table 2 legend for definition of abbreviations. Statistical significance (P < 0.05) is as follows: supine vs. HUT for gain at LF; between arterial PCO₂ levels (N-CO₂, LoCO₂, HiCO₂) for gain at VLF and LF and phase at VLF.

the cross-spectral analysis are calculated strictly from changes about the mean value.

Transcranial Doppler ultrasound provides a continuous estimate of changes in cerebral blood flow. The major assumption of this method is that changes in cerebral blood flow are reflected primarily in MFV (measured in cm/s), while the vessel cross-sectional area (measured in cm²) remains essentially constant. Under conditions of altered BP_{MCA} and arterial PCO₂, Serrador et al. (32) were unable to detect changes in cross-sectional area of the MCA, so the assumption of constant cross-sectional area appears to be valid, although some authors present a different opinion (26). Continuous noninvasive estimates of arterial pressure by the finger cuff device have compared favorably with direct arterial measurements in several different laboratories (16, 38). For these reasons, Doppler ultrasound and continuous noninvasive blood pressure measurements have become widely accepted as a means of evaluating dynamic cerebral blood flow control.

Selection of the VLF, LF, and HF bands, consistent with Zhang et al. (37), was somewhat arbitrary, but the underlying physiology separates clearly for the VLF and HF regions, with the LF region as a transition zone. In the VLF region, there was very low coherence for BP_{MCA} → MFV but much higher coherence for BP_{MCA} → CVRI. In the HF region, the situation was reversed, inasmuch as low coherence was found for the BP_{MCA-CVRi} relationship. The low coherence for BP_{MCA} → MFV in the VLF region probably reflects the property of cerebrovascular autoregulation but could also indicate a nonlinear relationship that is not detected by coherence (24).

Normalization of gain is often employed as a means of reducing the between-subject variation and is useful in comparisons between different populations (30). The normalized gain can give an indication of the relative attenuation of the input to output (14, 19, 30). In the present experiments, we provide data for normalization only in the LoCO₂ and HiCO₂ conditions for HUT testing in Figs. 3 and 4. To obtain the normalized values, the absolute gains were divided by mean values of conductance (MFV/BP_{MCA}) or the inverse of MFV (CVRi/BP_{MCA}) for the BP_{MCA-MFV} and BP_{MCA-CVRi} relationships, respectively. In both cases, these normalization factors were greatly affected by the experimental conditions because of the alteration in steady-state vascular resistance with altered PCO₂. This effect certainly influenced normalized gain for BP_{MCA} → MFV in the LF region (Fig. 3). Our primary focus on the ability of the cerebrovascular system to maintain
relatively constant blood flow was observed from the absolute gain. Normalized gain might provide additional insight under certain conditions (14, 19, 29) but resulted in an unexpected outcome in this study (see Dynamic cerebrovascular autoregulation: BPMCA → MFV).

Baseline vs. N-CO2 supine response. The baseline measurements collected during spontaneous breathing in the supine posture were very similar to those collected in the N-CO2 condition in the supine posture. There was a tendency for greater amplitude of the autospectral power at HF, but this was significant only for CVRi. The mechanism for this difference in distribution of power was related to the fixed breathing rhythm and tidal volume that concentrated spectral power at precisely 0.25 Hz (15 breaths/min). It can be concluded that the regulated breathing conditions imposed by the present experiment focused spectral power within the HF band, but they did not cause a significant change from the baseline condition.

Steady-state averaged data. The advantage of clamping PetCO2 at three different levels in the supine and HUT positions was that changes in cerebrovascular control could be identified independently as functions of posture or arterial PCO2. Consistent with the differences in arterial PCO2 were differences in steady-state MFV and CVRi that were similar to observations in previous research (1, 21, 22, 26).

With the transition from supine to 45° HUT posture, MAP remained approximately constant while BP MCA decreased. Subsequently, cerebrovascular resistance decreased to achieve approximately constant MFI in the face of reduced perfusion pressure. The finding of no significant reduction in MFV on going to HUT contrasts with several other studies (21, 29). Although part of the reason for the discrepancy with other studies might be the relatively low level of tilt in the present study, another reason for this difference could be the clamping of arterial PCO2 between the supine and HUT posture. HUT causes a reduction in arterial PCO2 in most individuals (3) so that a natural consequence of the reduced PCO2 with HUT is a relative increase in CVRi and decrease in MFV (6, 21). In the absence of change in arterial PCO2, the cerebrovascular system can adapt to maintain constant cerebral blood flow, at least in the face of small reductions in perfusion pressure.

Dynamic cerebrovascular autoregulation: BPMCA → CVRi. In this study, we evaluated cerebrovascular autoregulation from transfer function analysis of BPMCA → CVRi. In normal daily life, rapid adaptations of cerebrovascular resistance are essential for maintenance of cerebral blood flow, because BPMCA varies constantly as a result of changes in posture and spontaneous fluctuations in MAP. The spontaneous fluctuations are apparent in the autospectral power for BPMCA (Fig. 2), where VLF (~0.03 Hz), LF (~0.1 Hz), and HF (~0.25 Hz) peaks can be seen. Transfer function analysis (Fig. 3) shows that the large VLF amplitude in MFV (Fig. 2) was essentially independent of changes in BPMCA, as indicated by the very low coherence in this frequency range. In contrast, there was higher coherence for BP MCA → CVRi in the VLF-to-LF range (Fig. 4), indicating that changes in BP MCA evoked changes in CVRi that were effective to various degrees as affected by PCO2 in regulating cerebral blood flow. Furthermore, the negative phase detected for BP MCA → CVRi is consistent with the expected physiological response of changes in BP MCA causing changes in CVRi.

A change in the gain of BP MCA → CVRi suggests altered autoregulation. Our results that showed reduced gain for BP MCA → CVRi during HiCO2 and increased gain during LoCO2 are consistent with alterations in the range of the plateau region for cerebrovascular autoregulation under different levels of CO2 (see also Effects of CO2 on dynamic autoregulation). The normalization process reduced but did not eliminate the difference in gain between LoCO2 and HiCO2 in the HUT tests (Fig. 4). We also found with HUT that there was a significant reduction in LF gain for BP MCA → CVRi compared with the supine position and a similar trend in the HF region. Reduced autoregulatory response in the HUT position is consistent with the results from severe levels of lower body negative pressure (38). However, this finding contrasts with the suggestion of improved autoregulation during HUT that we would obtain from our data relating to BP MCA → MFV in the HF region as well as with data from other studies of HUT (4). A physiological interpretation of reduced BP MCA → CVRi gain with HUT might be that the decline in BP MCA toward the lower limit of the autoregulatory curve restricted the ability to respond dynamically to changes in cerebral perfusion pressure.

Phase relationships. Interpreting the relative phase lead of MFV preceding BP MCA has created confusion in terms of anticipated cardiovascular physiology (7, 8). Cencetti et al. (7) concluded that the phase lead of MFV before BP MCA indicated sympathetic neural control of the cerebrovascular system, rather than autoregulation, although this conclusion has been criticized (15, 23). Here we show that the phase lead of MFV before BP MCA is simply a mathematical consequence of natural phase lag of CVRi responding to changes in BP MCA by the mechanisms of autoregulation. The data for MFV, BP MCA, and CVRi have been reconstructed in Fig. 5 to illustrate the gain and phase relationships at one specific frequency in the LF region (selected to be 0.1 Hz). It can be appreciated that as BP MCA starts to rise (vertical reference line in Fig. 5), CVRi continued to decrease, and there was a lag of ~2.1 to 2.3 s (76.4° to 82.5°) before CVRi increased. During this time, because MFV = BP MCA/CVRi, it will be relatively high and, indeed, will appear to increase before the increase in BP MCA. The phase relationships displayed in Fig. 4 are consistent with a pure time delay of ~2 s for frequencies up to ~0.15 Hz. Above this frequency, the high-pass nature of cerebrovascular autoregulation is reflected by a phase approaching 0° for BP MCA → MFV and BP MCA → CVRi. The greater the phase lag for CVRi behind BP MCA, the smaller will be the phase lead.
Effects of CO₂ on dynamic autoregulation. Figures 3–5 have been used to highlight the differences in gain and phase under LoCO₂ and HiCO₂. There was a small elevation in BP_MCA in the HiCO₂ test compared with LoCO₂, and the amplitude of the oscillation in BP_MCA was slightly less in LoCO₂, but these differences would not be sufficient to affect the cerebrovascular response by themselves. The primary effect of CO₂ was on cerebrovascular resistance (1, 20, 21, 33). Even with the relatively small range of only 10-mmHg difference in PETCO₂ between the LoCO₂ and HiCO₂ trials, there were clear differences in cerebrovascular responses. The overall vasodilation in response to increased PCO₂ was responsible for the reduction in CVR_i and, consequently, the elevated mean level of MFV. The increased PCO₂ was also directly responsible for the smaller oscillations in CVR_i in response to changes in BP_MCA.

In the VLF range, there was a main effect of PCO₂ on the phase relationship for CVR_i after BP_MCA. Because MFV is dependent on the changes in CVR_i relative to those of BP_MCA, the phase relationship between BP_MCA and MFV must also change. With elevated PCO₂, there was a significant reduction of the phase lead of MFV before BP_MCA, consistent with previous research (2, 12, 22). Examination of the phase relationships between the three variables in Fig. 5 provides an explanation for this result. Impaired autoregulation really means that the oscillations in BP_MCA are less effectively damped, so that MFV more closely tracks changes in pressure.

Dynamic cerebrovascular autoregulation: BP_MCA → MFV. Giller (13) first described the frequency-dependent nature of autoregulation. Since that time, many authors have explored dynamic cerebrovascular autoregulation from the BP_MCA-MFV relationship (4, 7, 8, 38). It has become common to characterize impaired autoregulation by observing greater variations in MFV, greater transfer gain between BP_MCA and MFV, greater coherence, and a decreased phase relationship between BP_MCA and MFV (2, 12, 37). In general, however, these relationships exist in the higher frequencies, while there is no coherence in the lower frequencies (4, 37). That is, autoregulation operates to reduce or eliminate the BP_MCA-MFV relationship (13).

In the present study, transfer function analysis of the absolute gain between BP_MCA and MFV failed to detect any effect of PCO₂ (Table 3). Previous research has established that dynamic cerebrovascular autoregulation is a function of arterial PCO₂ (1, 2, 12, 22, 37). However, in some studies the sample size was limited and the differences in gain induced by elevated CO₂ were small (37) or detected only at the lowest frequency (22).

Contrary to expectations where HiCO₂ was hypothesized to impair autoregulation, the normalized gain for the LoCO₂ condition was greater than that for the HiCO₂ condition in the LF region (Fig. 3). This finding was quite unexpected, inasmuch as it indicates reduced autoregulatory efficiency in LoCO₂ compared with HiCO₂. The normalized gain is greatly influenced by the normalizing factor, which in this case was the mean value of conductance. Thus the normalized gain was reduced more for HiCO₂, because conductance was markedly increased. Alternatively, the relatively stiffer vessels in LoCO₂ were less able to dampen the effects of variations in BP_MCA at these frequencies. This latter observation has a parallel in studies of renal vascular autoregulation, where inhibition of nitric oxide synthesis caused a relative increase in resistance and increased transfer function gain (18). In this case, the dynamic autoregulation might be considered...
to be impaired, even though the static gain is improved by LoCO₂ (1, 20, 33).

According to the finding of smaller gain values for BP₉MCA → MFV in the HF region, HUT appeared to enhance autoregulation. Although this was consistent with another study that used tilt as the orthostatic stress (4), it contrasted with findings from a study that employed high levels of lower body negative pressure (−50 mmHg) (38) as well as with our data from BP₉MCA → CVRi. The reason for the discrepancy where HUT appears to enhance autoregulation while lower body negative pressure impairs it needs to be resolved. Given the ability of the BP₉MCA-CVRi relationships to accurately describe the effects of PCO₂ on autoregulation, the present study provides reason to believe that HUT causes impaired, not enhanced, autoregulation.

Comparison of cerebral with renal vascular responses. Frequency domain analysis has been used extensively to study autoregulation in the renal circulation (14, 19). Several parallels and some differences can be observed compared with the cerebral circulation. The renal studies have been performed on anesthetized or conscious animals in which renal blood pressure spontaneously varied or was manipulated (14, 18, 19). On the basis of different response times, two distinct mechanisms have been revealed: a myogenic response and a feedback mechanism based on tubuloglomerular filtration (14, 19). In the cerebral circulation, there appears to be one primary autoregulatory mechanism, although it might be found to have different mechanistic components, as in the mesenteric circulation (9). The rapidity of the cerebrovascular response observed in the present study is consistent with a myogenic mechanism. In the animal studies, inhibition of the myogenic component of renal autoregulation by the calcium channel blocker nifedipine caused increased normalized gain and reduced phase shift for the pressure-flow relationship (19). Directionally similar changes in normalized gain and phase were observed after inhibition of nitric oxide synthesis (18), even though nifedipine caused a slight (−18%) increase in renal vascular conductance while nitric oxide synthesis inhibition reduced conductance (−53%). Previous research suggested that cerebrovascular autoregulation is impaired in HiCO₂ compared with LoCO₂ (1, 20, 33). Consistent with this study and with the studies of renal circulation, the phase relationship for BP₉MCA → MFV was reduced in HiCO₂. However, increased normalized gain was found in the LoCO₂, rather than in the HiCO₂, tests. The mechanism responsible for the disparity between renal and cerebral circulation based on the pressure-flow relationship is not obvious, although the effect of absolute vascular conductance on normalized gain appears to be an important factor.

The transfer function between blood pressure and renal vascular resistance has been investigated (14, 19), and the outcome has been consistent with transfer function analysis to the pressure-flow relationship. In our study, the hypothesized effect of HiCO₂ on the cerebrovascular response was evident in the BP₉MCA → CVRi response and not BP₉MCA → MFV, so that the former appears superior in detecting changes in autoregulation.

Perspectives

Autoregulation is an important property in the cerebral (1, 25) and other vascular beds (14, 19). In most cases, autoregulation has been investigated by the blood flow response to changes in perfusion pressure. This study determined the effect of the input variable (blood pressure) on the manipulated variable (vascular resistance), which in turn acts to minimize changes in the regulated variable (blood flow) (15). In our study, transfer function analysis of the input-output relationship for BP₉MCA → CVRi was capable of detecting changes in gain and phase relationships as a result of altered arterial PCO₂ and HUT within the VLF-to-LF (0.03–0.2 Hz) range. This is critical, inasmuch as it is in this region that autoregulation is operative. In contrast, the previous methods that computed transfer function relationships between BP₉MCA and MFV relied on a lack of signal within this frequency range as an index of efficient autoregulation. Furthermore, normalized gain for BP₉MCA → MFV yielded an unexpected finding that was contrary to concepts of autoregulation during altered arterial PCO₂ and HUT on the basis of previous studies of static system gain (1, 20, 33). The BP₉MCA → CVRi transfer function relies on the inherent physical link between the two measured variables BP₉MCA and MFV to calculate CVRi. We believe that transfer function analysis of BP₉MCA → CVRi provides an intuitively attractive means of assessing cerebrovascular autoregulation, inasmuch as improved autoregulation is denoted by increased gain and reduced phase lag, as opposed to reduced gain and phase lead, if one uses the BP₉MCA-MFV relationship. The BP₉MCA → CVRi transfer function can be widely applied in research and clinical settings to investigate physiological and pathological factors that alter cerebrovascular autoregulation.

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