Original Contribution

Autonomic Blockade During Sinusoidal Baroreflex Activation Proves Sympathetic Modulation of Cerebral Blood Flow Velocity

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Background and Purpose—Pharmacological blockade showed sympathetic origin of 0.03 to 0.15 Hz blood pressure (BP) oscillations and parasympathetic origin of 0.15 to 0.5 Hz RR-interval (RRI) oscillations, but has not been used to determine origin of cerebral blood flow velocity (CBFV) oscillations at these frequencies. This study evaluated by pharmacological blockade whether 0.1 Hz CBFV oscillations are related to sympathetic and 0.2 Hz CBFV oscillations to parasympathetic modulation.

Methods—In 11 volunteers (24.6±2.3 years), we monitored RRIs, BP, and proximal middle cerebral artery CBFV, at rest, during 180 s sympathetic BP activation by 0.1 Hz sinusoidal neck suction (NS), and during 180 s parasympathetic RRI activation by 0.2 Hz NS. We repeated recordings after 25 mg carvedilol, and after 0.04 mg/kg atropine. Autoregressive analysis quantified RRI-, BP-, and CBFV-spectral powers at 0.1 Hz and 0.2 Hz. We compared parameters at rest, during 0.1 Hz, or 0.2 Hz NS, with and without carvedilol or atropine (analysis of variance, post hoc testing; significance, P<0.05).

Results—Carvedilol significantly increased RRIs and lowered BP, CBFV, and 0.1 Hz RRI-, BP-, and CBFV-powers at baseline (P=0.041 for CBFV-powers), and during 0.1 Hz NS-induced sympathetic activation (P<0.05). At baseline and during 0.2 Hz NS-induced parasympathetic activation, atropine lowered RRIs and 0.2 Hz RRI-powers, but did not change BP, CBFV, and 0.2 Hz BP- and CBFV-powers.

Conclusions—Attenuation of both 0.1 Hz CBFV and BP oscillations after carvedilol indicates a direct relation between 0.1 Hz CBFV oscillations and sympathetic modulation. Absent effects of atropine on BP, CBFV, and 0.2 Hz BP and CBFV oscillations suggest that there is no direct parasympathetic influence on 0.2 Hz BP and CBFV modulation. (Stroke. 2013;44:00-00.)

Key Words: atropine ■ carvedilol ■ cerebral blood flow ■ pharmacological autonomic blockade ■ sinusoidal neck suction ■ sympathetic nervous system

Continuous transcranial Doppler recordings of cerebral blood flow velocities (CBFV) show signal oscillations similar to 0.1 Hz Mayer1 and Traube-Hering waves,2 occurring in time series of heart rate (HR) or blood pressure (BP).

Spectral analysis of oscillations in the so-called low- (LF; 0.03–0.14 Hz) and high-frequency (HF; 0.15–0.50 Hz) ranges has become a valuable standard for estimating sympathetic and parasympathetic influences on HR and BP.3–9 Human and animal studies3–9 that assessed changes in LF and HF oscillations of HR and BP upon pharmacological sympathetic or parasympathetic blockade indicate that resting HR oscillations in the LF range are mediated by sympathetic outflow and—to an undetermined degree—also by parasympathetic activity.3–9 In contrast, LF oscillations of BP are related to sympathetic outflow only.1,9 HR oscillations in the HF range reflect parasympathetic activity,1,9 whereas BP oscillations in the HF range are primarily a mechanical consequence of respiration-induced fluctuations in venous return and cardiac output.1,9

Similarly, assessment of possible sympathetic and parasympathetic influences on CBFV modulation might be of clinical relevance and beneficial for the understanding of cerebral blood flow physiology and pathophysiology.10–14

Baroreceptor stimulation by means of sinusoidal neck suction (NS) at 6 and 12 cycles per minute, that is, at 0.1 Hz and 0.2 Hz,15 generates oscillations of HR and BP signals16 in the same frequency ranges and is suited to separately quantify sympathetic cardiac and vasomotor, as well as cardiovagal responses.16 Bernardi et al demonstrated that blockade of

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β-adrenoreceptors significantly reduces the NS-induced increase in 0.1 Hz oscillations in HR, confirming that this increase is mediated by efferent sympathetic nerves. Moreover, the authors showed that 0.2 Hz NS does not generate or augment 0.2 Hz HR oscillations after atropine application, indicating that 0.2 Hz HR fluctuations are mediated by parasympathetic baroreflex modulation. At the level of CBFV, slow oscillations have only been shown during baroreceptor stimulation at the lower 0.1 Hz frequency. From the simultaneous increase in 0.1 Hz CBFV fluctuations and respective HR and BP oscillations, it has been indirectly suggested that these oscillations may reflect sympathetic modulation of cerebral circulation. However, there is no direct evaluation, for example, by pharmacological blockade, of any association between 0.1 Hz CBFV oscillations and sympathetic outflow. Moreover, it has not yet been tested, whether 0.2 Hz NS generates CBFV oscillations at the same frequency that might unveil a possible parasympathetic origin of 0.2 Hz CBFV fluctuations.

We hypothesize that CBFV oscillations at 0.1 or 0.2 Hz are associated with sympathetic or parasympathetic activity and can be blocked by sympathetic, respectively, parasympathetic pharmacological blockade, similar to the pharmacological blockade of respective HR or BP oscillations described in previous human and animal studies.

In this study, we therefore evaluated whether 0.1 Hz and 0.2 Hz NS generate CBFV oscillations at the frequency of stimulation, and we tested to which extent these fluctuations may be influenced by sequential and selective pharmacological blockade of sympathetic or parasympathetic activity.

Material and Methods

Subjects

Eleven healthy men (mean age 24.6±2.3 years) participated in the study. None of the participants had any disease or was taking medication known to affect the cardiovascular or autonomic nervous system. Before testing, all participants refrained from nicotine, caffeine, or alcohol for 24 hours, and underwent a physical examination (Wasmeier G), duplex sonography of the extracranial carotid and vertebral arteries to rule out vascular pathologies, a 12-lead ECG, and an echocardiogram to rule out cardiac abnormalities, such as arrhythmias or structural heart disease. Transcranial Doppler sonography (TCD) at the temporal and suboccipital windows confirmed normal intracranial CBFVs of the vertebral, basilar, middle, posterior, and anterior cerebral arteries. The study was approved by the ethics committee of the University of Erlangen-Nuremberg. All participants have given their written informed consent after detailed information, according to the declaration of Helsinki.

Procedures

Studies were performed in a quiet room with an ambient temperature of 24°C and stable humidity. Each participant initially rested in supine position for at least 45 minutes to ensure cardiovascular stability. During this period, the monitoring devices were applied.

We continuously recorded electrocardiographic RR-intervals (RRI) using a 5-lead ECG with superficial skin electrodes. We noninvasively monitored mean arterial BP by radial artery tonometry with initial calibration at the brachial artery (Colin Pilot, San Antonio, TX). Mean CBFV of the proximal middle cerebral artery (MCA) was assessed by TCD (Multidop XL, DWL, Germany). The MCA was insonated through the temporal window, approximately 1 cm above the zygomatic arch at a depth of 35 to 55 mm using a 2 MHz Doppler probe. After optimizing the Doppler signal, the probe was attached to the skull at a fixed angle using a headband with an adjustable positioning system. Respiratory frequency was monitored by means of inductance plethysmography using 2 calibrated belts attached around the thorax and abdomen (Respirate Calibrator, Ambulatory Monitoring Inc, Airdley, NY).

Baroreceptor Stimulation

Responses of RRI, BP, and CBFV signals to baroreceptor stimulation were determined using the sinusoidal NS method previously described by Bernardi et al. The neck chamber consisted of a malleable lead collar edged with neoprene foam and was fitted to the anterior neck, over the carotid baroreceptors. Subatmospheric pressure was applied to the collar by means of a vacuum cleaner, whose power output was regulated through a control unit. Pressure within the chamber was monitored with a pressure transducer (Hugo-Sachs Elektronik, Germany) and could be set to oscillate between 0 and ~30 mm Hg at 0.1 Hz or 0.2 Hz. Breathing was paced at 15 breaths per minute (0.25 Hz) to avoid the effects of respiratory interference on cardiovascular autonomic modulation during the 0.1 Hz or 0.2 Hz NS stimulation. To familiarize study participants with the task of keeping the respiratory frequency paced at 15 cycles per minute before the study, participants were instructed to follow visual and verbal signals to inspire and expire within 4 seconds (i.e., slightly above their normal respiratory frequency).

Recording of Signals at Baseline, During 0.1 Hz and 0.2 Hz NS, Without and With Sympathetic or Parasympathetic Blockade

An initial 5-minute baseline recording was made during 0.25 Hz breathing without NS. The 6 cycles per minute, that is, 0.1 Hz NS, and the 12 cycles per minute, that is, 0.2 Hz NS, stimulation were then performed for 3 minutes each, with breathing paced at 0.25 Hz throughout.

Measurements Before and After Partial α- and β-Adrenoceptor Blockade and Parasympathetic Blockade

The study comprised visits on 3 consecutive days. During the first visit, measurements before and during 0.1 Hz and 0.2 Hz NS were performed without any premedication. During the second (or third) visit, recordings were repeated immediately after administration of 0.04 mg/kg of the muscarinic receptor antagonist atropine. During the third (or second) visit, participants received 25 mg of the partial α- and β-adrenoceptor antagonist carvedilol orally 2 hours before testing. As a safety precaution, a cardiologist (Wasmeier G) with experience in emergency medicine was present in the examination room during the entire tests.

Data Acquisition and Analysis

All data were digitized by a custom-made analogue-to-digital converter at a sampling rate of 300 Hz and fed to a Macintosh PowerBook computer (Apple Inc), manually cleaned from artifacts by linear interpolation and stored for offline analysis. A C-language program identified all QRS complexes in each sequence, located the peak of each R-wave, and calculated and interpolated linearly between adjacent values to construct a corresponding continuous time series.

From a 60-second interval at baseline and during 0.1 Hz or 0.2 Hz NS, we calculated mean values and standard deviation of all biosignals. Fluctuations in RRI, BP, CBFV, respiration, and pressure within the neck chamber were characterized by applying power spectrum analysis to these signals using an autoregressive algorithm with a linear detrrending option and model order estimation according to Akaike information criteria. Spectral power refers to the amount of variability of a signal (eg, BP or CBFV) at a specific frequency. It is

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assessed as the magnitude of the oscillations at a given frequency and is expressed as units.2,22

Autoregressive spectral analysis allows not only for quantitative evaluation of cardiovascular regulation in humans by assessing spontaneous oscillations in various biosignals, it also provides information about the transmission of NS-induced 0.1 Hz or 0.2 Hz oscillations on various biosignals.21,23 NS applied at 0.1 Hz induces oscillations at 0.1 Hz to the level of RRI, BP, and CBFV as previously shown, for example, by Bernardi et al and Lagi et al.25 The response to 0.1 Hz NS at the level of BP is considered an index of sympathetic baroreceptor activation, whereas the response to 0.1 Hz NS at the RRI level reflects sympathetic and also vagal effects of baroreceptor stimulation on the heart.4 As shown by Bernardi et al, faster 0.2 Hz oscillations in neck pressure are transmitted only to the level of the HR or RRI by modulation of parasympathetic activity.4 The 0.2 Hz frequency was chosen to provide responses close to, but distinct from, the frequency of respiration (0.25 Hz).3,19

We assessed the responses to 0.1 Hz or 0.2 Hz NS as the changes in spectral powers at 0.1 Hz or 0.2 Hz in the RRI, BP, and CBFV compared with the baseline values of spectral powers.4

Additionally, we calculated 2 parameters reflecting the integrity of cerebral autoregulation (CA). Intact CA assures constant cerebral blood flow in the face of BP changes and depends on mechanisms such as the myogenic component, that is, the capacity of vascular smooth muscles to dampen BP fluctuations, on intact endothelial function, and on neurogenic, primarily sympathetic influences.8,25 The dynamics of autoregulation can be compared with a high-pass filter.26 Rapid BP perturbations are transferred to CBFV, whereas slow BP changes are damped.26-27 As CA is a frequency-dependent phenomenon,27 its function can be evaluated by comparing BP and CBFV oscillations in the frequency domain.26-27 Transfer function analysis with BP as the input signal and CBFV as the output signal27 can be used to determine the gain between both variables.5,27 If autoregulation is functioning effectively, spontaneously occurring changes in BP should only minimally influence CBFV, that is, there should be little coherence between both variables or the transfer function between BP and CBFV oscillations should have only a small gain.2,27 Increasing BP activates autoregulation that assures counter-regulation of the vascular resistance bed with vasodilatation.25 The counter-regulation causes CBFV to be at its maximal value before the causative BP oscillation has reached its maximum value.2,26 Therefore, the relation between BP and CBFV oscillations can be described by calculating the phase shift between the leading CBFV and the lagging BP signal.26 The coherence between BP and CBFV signal oscillations might span from 0 (ie, no association) to 1 (ie, maximal association).25 If coherence is >0.5, the 2 signals are considered to have a stable phase relation for a given frequency of oscillation.2,27 Before and after pharmacological blockade, we assessed NS-induced changes in transfer function gain and phase shift between BP oscillations and CBFV oscillations at 0.1 Hz, that is, at the frequency that is considered to reflect oscillations mediated by sympathetic outflow.28

Statistical Analysis
We used the Kolmogorov-Smirnov test to test for normal distribution of data. Normally distributed data are presented as mean±standard deviation. Differences in cardiovascular parameters between measurements performed without and with pharmacological blockade were evaluated by analysis of variance for repeated measurements (ANOVA, general linear model), with “neck suction” (before and during NS) as first within subject factor and “blockade” (with and without pharmacological blockade) as second within subject factor. Suitability of the ANOVA model was assessed by Mauchly Test of Sphericity. In case of violation of the sphericity assumption, the Greenhouse Geisser correction was used. In case of significant ANOVA results, post hoc single comparisons were performed using t test for paired groups of normally distributed data, or the Wilcoxon test, in case of not normally distributed data. A commercially available statistics program (SPSS, SPSS Inc, Chicago, IL) was used for data analysis. Significance was set at P<0.05.

Results

Responses to 0.1 Hz NS With and Without Carvedilol

Effect of Carvedilol on BP, RRI, and CBFV at Rest and During 0.1 Hz NS

With carvedilol medication, BP and CBFV values were significantly lower and RRI values were significantly higher than corresponding values without adrenergic blockade, both at baseline and during 0.1 Hz NS. The 0.1 Hz NS without carvedilol medication induced a significant increase in BP (82.7±5.0 mm Hg versus 86.4±8.8 mm Hg) and CBFV (45.7±18.8 cm/s versus 50.0±22.0 cm/s) from baseline values, whereas RRI values during NS were not significantly lower than RRIIs without NS (946.0±101.6 ms versus 912.5±65.1 ms; P=0.067; Table 1). The 0.1 Hz NS with carvedilol medication induced only slight and no longer significant increase in BP and CBFV from baseline values, and RRI remained unchanged.

Effect of Carvedilol on Spectral Powers of 0.1 Hz RRI, BP, and CBFV Oscillations, and on 0.1 Hz Transfer Function Gain and Phase Shift Between BP and CBFV at Rest and During 0.1 Hz NS

Carvedilol medication also resulted in significantly lower spectral powers of 0.1 Hz RRI and BP oscillations—at baseline and during 0.1 Hz NS—than corresponding powers without adrenergic blockade (Table 1).

For CBFV, adrenergic blockade lowered 0.1 Hz CBFV-powers significantly at baseline, that is, without NS (P=0.041) and during NS (P=0.029) from corresponding powers without blockade. The 0.1 Hz NS without adrenergic blockade significantly increased 0.1 Hz RRI-, BP-, and CBFV-powers from powers at baseline (Table 1). The 0.1 Hz NS with blockade still increased 0.1 Hz RRI- and BP-powers (P<0.05); however, powers were smaller with than without adrenergic blockade (P<0.05). For CBFV, NS with blockade no longer increased 0.1 Hz CBFV-powers significantly, and 0.1 Hz CBFV-powers during NS were significantly lower with than without carvedilol (Table 1).

Carvedilol had no effect on the 2 parameters of cerebral autoregulation, transfer function gain and phase shift between 0.1 Hz oscillations of BP and CBFV, calculated at baseline and during 0.1 Hz NS. Moreover, 0.1 Hz NS did not change the transfer function gain and phase shift between 0.1 Hz oscillations of BP and CBFV from baseline values (Table 1).

Responses to 0.2 Hz NS With and Without Atropine

Effect of Atropine on BP, RRI, and CBFV at Rest and During 0.2 Hz NS

Atropinization resulted in significantly lower RRIIs, that is, higher HRs, both at baseline and during 0.2 Hz NS than corresponding values without parasympathetic blockade. In contrast, atropine did not change BP and CBFV values at baseline and during 0.2 Hz NS from corresponding values without atropinization (Table 2).
Regardless of atropinization, 0.2 Hz NS did not significantly change RRI, BP, and CBFV values from baseline values.

**Effect of Atropine on Spectral Powers of 0.2 Hz RRI, BP, and CBFV Oscillations, and on 0.1 Hz Transfer Function Gain and Phase Shift Between BP and CBFV at Rest and During 0.2 Hz NS**

Atropinization significantly lowered 0.2 Hz RRI-powers, both at baseline and during 0.2 Hz NS, compared with powers without blockade (Table 2). In contrast, atropine did not change 0.2 Hz BP- and CBFV-powers at baseline and during NS from powers without blockade (Table 2).

Without atropinization, 0.2 Hz NS significantly increased 0.2 Hz RRI-powers from powers at baseline, but did not change 0.2 Hz BP- and CBFV-powers.

After atropinization, 0.2 Hz NS still increased 0.2 Hz RRI-powers from powers at baseline, but again had no effect on 0.2 Hz BP- and CBFV-powers.

Atropine had no effect on transfer function gain and phase shift between 0.2 Hz oscillations of BP and CBFV, calculated at baseline and during 0.2 Hz NS. Moreover, 0.2 Hz NS did not change the transfer function gain and phase shift between 0.2 Hz oscillations of BP and CBFV from baseline values (Table 2).

**Discussion**

Our study confirms that 0.1 Hz NS increases sympathetic tone as evidenced by the increases in BP and in the—exclusively sympathetically mediated—0.1 Hz spectral powers of BP modulation.

Moreover, the significantly lower BP values and lower sympathetically mediated 0.1 Hz BP-powers after than before carvedilol confirm that 25 mg of the α- and β-receptor blocker are sufficient to reduce sympathetic outflow at rest, as well as during 0.1 Hz NS-induced sympathetic activation.

Effects of carvedilol on CBFV and 0.1 Hz CBFV oscillations were similar to effects on BP and sympathetic 0.1 Hz BP oscillations: the sympathetic blockade lowered CBFV values at baseline and during 0.1 Hz NS-induced sympathetic activation, and also reduced 0.1 Hz CBFV spectral powers at baseline and during sympathetic activation (P<0.05).

The results reconfirm that 0.1 Hz NS is suited to activate sympathetic outflow to vasomotor nerves, and show that 25 mg of the α- and β-adrenoreceptor blocker was sufficient to significantly reduce sympathetic BP modulation.

Still, the question arises whether (1) the 0.1 Hz NS-induced increases and carvedilol-induced decreases in CBFV and in 0.1 Hz CBFV modulation also reflect an increase, respectively decrease, in sympathetic outflow onto the cerebral vasculature; or whether (2) these changes in CBFV and 0.1 Hz CBFV powers are secondary to, and passively driven by the increases, respectively decreases, in BP and in sympathetic 0.1 Hz BP modulation.

Two findings support the conclusion that changes in CBFV and 0.1 Hz CBFV-powers reflect direct sympathetic effects and not passive changes secondary to sympathetic changes in BP modulation:

First, the CBFV changes—that occurred in parallel with the BP changes—were recorded from TCD insonation at the proximal MCA segments. The CBFV increase seen during BP increase, that is, during sympathetic activation, likely not only reflected passive CBFV acceleration because of increased BP, but also resulted from increased sympathetic outflow onto the insonated MCA segment and a subsequent vasoconstriction of this insonated segment. At proximal segments of cerebral arteries, sympathetic innervation is much denser and far more efficient than at small, distal arteries downstream of the insonated segment.

**Table 1. Cardiovascular Responses to 0.1 Hz NS Without and With 25 mg Carvedilol (n=11)**

<table>
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<tr>
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<th>Before NS</th>
<th>During NS</th>
<th>T Test Before and During NS</th>
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<tr>
<td>RR-interval, ms</td>
<td></td>
<td></td>
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<tr>
<td>Without carvedilol</td>
<td>946.0±101.6*</td>
<td>912.5±65.1*</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>With carvedilol</td>
<td>1087.3±150.4</td>
<td>1034.7±11.0</td>
<td>&gt;0.05</td>
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<tr>
<td>0.1-power of RR-interval, ms²</td>
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<tr>
<td>Without carvedilol</td>
<td>304.3±403.9*</td>
<td>796.6±460.6*</td>
<td>&lt;0.05</td>
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<tr>
<td>With carvedilol</td>
<td>209.4±150.1</td>
<td>393.4±291.7</td>
<td>&lt;0.05</td>
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<tr>
<td>BP, mm Hg</td>
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<tr>
<td>Without carvedilol</td>
<td>82.7±5.0*</td>
<td>86.4±8.8*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>With carvedilol</td>
<td>74.1±9.0</td>
<td>76.5±9.1</td>
<td>&gt;0.05</td>
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<tr>
<td>0.1-power of BP, mm Hg²</td>
<td></td>
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<tr>
<td>Without carvedilol</td>
<td>2.1±3.4*</td>
<td>3.5±2.9*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>With carvedilol</td>
<td>0.8±0.9</td>
<td>1.4±1.8</td>
<td>&lt;0.05</td>
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<tr>
<td>CBFV, cm s⁻¹</td>
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<tr>
<td>Without carvedilol</td>
<td>45.7±18.8*</td>
<td>50.0±22.0*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>With carvedilol</td>
<td>38.1±16.2</td>
<td>42.0±21.6</td>
<td>&gt;0.05</td>
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<tr>
<td>0.1-power of CBFV, cm² s⁻²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without carvedilol</td>
<td>2.0±0.9*</td>
<td>3.7±2.0*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>With carvedilol</td>
<td>1.2±0.9</td>
<td>1.9±2.1</td>
<td>&gt;0.05</td>
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<tr>
<td>0.1 Hz gain, cm s⁻¹ mm Hg⁻¹</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Without carvedilol</td>
<td>1.35±0.69</td>
<td>1.21±0.61</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>With carvedilol</td>
<td>1.68±0.89</td>
<td>1.71±2.00</td>
<td>&gt;0.05</td>
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<tr>
<td>0.1 Hz phase shift, rad</td>
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<tr>
<td>Without carvedilol</td>
<td>−1.10±0.31</td>
<td>−1.32±0.51</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>With carvedilol</td>
<td>−1.00±0.43</td>
<td>−1.20±0.82</td>
<td>&gt;0.05</td>
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BP indicates blood pressure; CBFV, cerebral blood flow velocity; and NS, neck suction.

*Significant differences between parameters with and without carvedilol.
In principle, the recorded CBFV acceleration could result from vasoconstriction at the insonated vessel segment or from vasodilatation of peripheral resistance vessels downstream of the insonated segment. Yet, downstream vasodilatation is not a physiological response in healthy persons. Their intact CA increases cerebrovascular resistance in response to BP increases and causes vasoconstriction—not vasodilatation—of small resistance vessels. Thus, the CBFV acceleration accompanying BP increase, that is, sympathetic activation, likely reflects increased sympathetic outflow onto the proximal, insonated MCA segments.

Similarly, the decreases in CBFV and 0.1 Hz CBFV modulation recorded upon sympathetic blockade in parallel with the decreases in BP and in sympathetic 0.1 Hz BP modulation, are likely because of a decrease in sympathetic outflow onto the proximal, insonated MCA segments, which, in turn, caused vasodilatation of the proximal, insonated MCA, and thus slowed CBFV.

Second, the transfer function gain and phase shift between 0.1 Hz oscillations of BP and CBFV remained constant throughout all recordings, that is, at baseline, during 0.1 Hz NS-induced sympathetic activation, before and after sympathetic blockade. If changes in CBFV or 0.1 Hz CBFV oscillations were only passively driven by BP changes, one should expect changes in the phase angle between the 0.1 Hz oscillations of BP and CBFV, which explains why sinusoidal oscillations of CBFV are leading, whereas the BP oscillations are lagging, resulting in negative phase angles (Table 1).

Only active mechanisms of CA buffer the transmission of BP fluctuations onto CBFV, and result in the negative phase shift between the oscillations of the CA-input signal BP and the CA-output signal CBFV. If CBFV oscillations were not actively modulated, but resulted passively from BP oscillations, phase angles between 0.1 Hz oscillations of CBFV and BP would change with changes in BP, for example, during 0.1 Hz NS-induced sympathetic activation or upon carvedilol-induced sympathetic blockade. Such passive phase angle changes have been reported in patients with impaired CA. In these patients, intracranial arterial stenoses, autonomic neuropathy, or intracerebral microangiopathy restrict CA and account for the passively driven, BP-dependent changes in CBFV and in phase angles between CBFV and BP oscillations.

In healthy persons, Cencetti et al also found a constant relation between LF (0.03–0.15 Hz) oscillations of CBFV and LF oscillations in RRI and in BP. The authors also wondered whether CBFV oscillations are secondary to passive transmission, for example, from the aorta, or originate independently in the periphery. From their finding of negative phase angles between LF CBFV and BP oscillations, with CBFV preceding BP fluctuations, the authors concluded that passive transmission of BP oscillations onto CBFV is unlikely, and that LF CBFV oscillations most likely originate from sympathetic modulation in the cerebral circulation itself.

In summary, the 0.1 Hz oscillations of CBFV most likely do not represent secondary, passive signal fluctuations, but very likely result from direct sympathetic vasomotor modulation, similar to the 0.1 Hz oscillations of BP.

### Table 2. Cardiovascular Responses to 0.2 Hz NS Without and With 0.04 mg/kg Atropine (n=11)

<table>
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<tr>
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<th>Before NS</th>
<th>During NS</th>
<th>T Test Before and During NS</th>
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<tbody>
<tr>
<td>RR-interval, ms</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Without atropine</td>
<td>935.5±146.4*</td>
<td>899.7±145.4*</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>With atropine</td>
<td>590.2±45.3</td>
<td>594.3±48.8</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>0.2-power of RR-interval, ms²</td>
<td></td>
<td></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Without atropine</td>
<td>141.5±194.7*</td>
<td>378.5±773.4*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>With atropine</td>
<td>2.7±2.8</td>
<td>4.9±5.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>BP, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without atropine</td>
<td>81.6±11.1</td>
<td>83.2±10.8</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>With atropine</td>
<td>86.6±6.0</td>
<td>88.0±9.1</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>0.2-power of BP, mm Hg²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without atropine</td>
<td>0.6±0.5</td>
<td>0.5±0.5</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>With atropine</td>
<td>0.7±0.5</td>
<td>0.3±0.5</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>CBFV, cm s⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without atropine</td>
<td>40.6±15.3</td>
<td>39.9±14.8</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>With atropine</td>
<td>45.7±15.9</td>
<td>41.3±12.5</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>0.2-power of CBFV, cm² s⁻²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without atropine</td>
<td>0.5±0.3</td>
<td>0.9±0.8</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>With atropine</td>
<td>0.8±0.8</td>
<td>0.7±0.7</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>0.2 Hz gain, cm s⁻¹ mm Hg⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without atropine</td>
<td>1.09±0.44</td>
<td>1.32±0.85</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>With atropine</td>
<td>1.34±0.58</td>
<td>2.37±1.60</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>0.2 Hz phase shift, rad</td>
<td>−1.09±0.35</td>
<td>−1.26±0.40</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>−1.29±0.76</td>
<td>−0.57±1.48</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

BP, blood pressure; CBFV, cerebral blood flow velocity; NS, neck suction.

*Significant differences between parameters with and without atropine.
Atropinization Effects Show That 0.2 Hz Oscillations of CBFV Are Not Directly Related to Parasympathetic Modulation

Although carvedilol buffered sympathetic activity, and thus reduced HR, BP, CBFV, and sympathetic 0.1 Hz oscillations of BP and CBFV, 0.04 mg/kg atropine only reduced parasympathetic outflow to the heart, and thus induced a decrease in RRI and in parasympathetic 0.2 Hz RRI modulation at baseline and during 0.2 Hz NS. However, atropine had no effects on BP and CBFV or on 0.2 Hz oscillations of BP and CBFV.

The cardioval changes seen with atropinization confirm previous findings that 0.2 Hz oscillations of RRI reflect parasympathetic modulation,\(^4\) whereas absent atropine effects on 0.2 Hz BP oscillations—\(\sim\) as well as absent effects of 0.2 Hz NS on the 0.2 Hz BP oscillations—confirm the conclusion of previous studies\(^8,30\) that BP oscillations in the frequency range from 0.15 to 0.5 Hz are not directly mediated by changes in parasympathetic outflow, but result from secondary mechanical oscillations because of respiration-induced fluctuations in venous return and cardiac output.\(^4,5\)

Similarly, the absent effects of atropine on CBFV and on 0.2 Hz CBFV oscillations confirm that changes in parasympathetic outflow have no significant influence on CBFV modulation at this frequency range.\(^5,30\) Instead, 0.2 Hz CBFV oscillations most likely arise from mechanical oscillations, secondary to BP oscillations and to respiration-induced changes in venous cardiac return and cardiac output.\(^6,41,42\) As mentioned above, CA functions as a high-pass filter\(^26,27,43\) that transfers BP oscillations at frequencies >0.1 Hz onto CBFV,\(^4\) yielding secondary, mechanically induced CBFV oscillations at frequencies identical to the BP oscillations in the HF range.\(^6,41,42\) As atropine had no effects on BP, CBFV, or on 0.2 Hz modulation of either signal, gain and phase shift between 0.2 Hz oscillations of BP and CBFV also remained unchanged.

To summarize, our findings of carvedilol dampening HR, BP, CBFV, and 0.1 Hz oscillations of BP as well as CBFV indicate that not only 0.1 Hz oscillations of BP, but also 0.1 Hz oscillations of CBFV are because of sympathetic influence on both signals.

In contrast, 0.2 Hz NS had no effects on BP and CBFV, nor on the 0.2 Hz modulation of either signal, whereas it confirmed its known activation of parasympathetic outflow to the heart.\(^30\) Similarly, atropinization did not change BP and CBFV or 0.2 Hz powers of BP and CBFV oscillations. Thus, our data confirmed that 0.2 Hz oscillations of BP are mechanically mediated oscillations, secondary to changes in respiration and cardiac output.\(^6,41,42\) Moreover, our results showed that 0.2 Hz oscillations of CBFV also do not result from direct parasympathetic modulation, but are secondary to BP oscillations and their causation.

In conclusion, our study demonstrates that 0.1 Hz oscillations of CBFV are influenced or mediated by sympathetic activity, whereas 0.2 Hz oscillations of CBFV are not mediated by parasympathetic modulation but seem to result from mechanical oscillations, as do the 0.2 Hz oscillations of BP.

Clinical Implications

Our findings confirm the contribution of sympathetic activity to cerebral blood flow modulation, and show that sympathetic cerebral blood flow modulation can be estimated by noninvasive TCD monitoring and spectral analysis of CBFV. Diseases associated with changes in sympathetic activity may alter cerebral perfusion and compromise cerebral autoregulation.\(^12,25,33\) Disorders of reduced sympathetic modulation, such as diabetes mellitus with autonomic neuropathy, compromise cerebral autoregulation, and thus contribute to impaired brain perfusion and cerebrovascular risk.\(^24\) Similarly, disorders associated with increased sympathetic tone, such as arterial hypertension, acute stroke,\(^44\) or poststroke status\(^45\) may also be associated with impaired cerebral blood flow because of direct effects of sympathetic hyperactivity on cerebral perfusion. Sympathetic hyperactivity occurs in many conditions, for example, in patients with a history of traumatic brain injury,\(^46\) and might alter cerebral perfusion. Impaired CBFV modulation might, in turn, contribute to the pathophysiology of unexplained mortalities in this large group of traumatic brain injury patients.\(^46,49\)

In acute subarachnoid hemorrhages, increased sympathetic tone likely contributes to vasospasms,\(^50,54\) and can be identified by routine TCD recordings with additional spectral analysis. Monitoring of sympathetic CBFV modulation might support therapeutic decisions. Although catecholamines might be considered in situations of critically reduced sympathetic modulation, sympatholytic treatment, for example, \(\beta\)-blockers, might be introduced in cases of critically increased sympathetic modulation, for example, during acute subarachnoid hemorrhage with increased risk of sympathetically triggered vasospasms.\(^41,44\)

In summary, monitoring of the sympathetic cerebrovascular modulation may improve clinical assessment and therapeutic interventions in patients with cerebrovascular disorders. Our results show that CBFV oscillations with a peak frequency at 0.1 Hz can be used as marker of the sympathetic cerebrovascular modulation.

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Disclosures

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