Effects of Unilateral and Bilateral Carotid Baroreflex Stimulation on Cardiac and Neural Sympathetic Discharge Oscillatory Patterns

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Background—Left and right carotid baroreflex afferents participate in generating the spontaneous variability of heart rate (HR), arterial pressure (AP), and muscle sympathetic nerve activity (MSNA), but the relative contribution of each side is unclear. Pathophysiological conditions unilaterally affecting carotid baroreceptor function might result in abnormal changes of HR, AP, and MSNA variability, thus markedly affecting prognosis. We tested the hypothesis that unilateral carotid baroreceptor perturbation might differentially affect HR, AP, and MSNA variability compared with stimulation of the opposite side.

Methods and Results—In 12 healthy volunteers, 4 sinusoidal neck suction procedures (0.1 Hz, from 0 to −50 mm Hg) were applied at the right, left, and combined right and left sides of the neck, in concordance or with phase opposition. Respiration was controlled at 0.25 Hz. Power spectrum analysis assessed the changes in the 0.1-Hz oscillatory component of the R-R interval, systolic AP (SAP), and MSNA variability induced by rhythmic baroreceptor stimulation. Mean R-R interval, SAP, and MSNA were unchanged during all procedures. The increase of the 0.1-Hz component of R-R and SAP variability during right and combined right and left carotid baroreceptor stimulation was greater than the changes induced by left-sided stimulation. The increase in the 0.1-Hz oscillatory component of MSNA variability was similar during all neck suction procedures.

Conclusions—Right carotid baroreflex loading was as efficient as bilateral stimulation and more effective than left carotid suction in modulating R-R and SAP variability. There was no asymmetry in neural sympathetic discharge responses after single-sided carotid baroreceptor stimulation. (Circulation. 2003;108:717-723.)

Key Words: carotid arteries ■ nervous system, sympathetic ■ baroreceptors

In humans, neural sympathetic discharge (muscle sympathetic nerve activity, MSNA) and heart rate (HR) are modulated by arterial aortic and carotid baroreflex mechanisms to buffer changes in blood pressure.1 Baroreflex-mediated excitatory/inhibitory activity acts on a beat-by-beat basis, resulting in reciprocal modifications of sympathetic and parasympathetic efferent modulation to the cardiovascular system2 that contribute to the changes of both mean values and spontaneous variability of MSNA and HR. For instance, baroreflex loading produced by pressor drug administration3 was found to reduce MSNA and increase R-R interval and the high frequency (HF) oscillatory components of both MSNA and heart beat variability. Conversely, baroreflex unloading induced by nitroglycerin administration3 or the assumption of orthostatic posture4 leads to a reduction of R-R interval and promotes the increase of MSNA and the 0.1-Hz, low-frequency (LF) component of R-R and MSNA variability.

The carotid component of arterial baroreflex activity has been extensively studied in humans by developing a variable pressure neck chamber method for bilateral1 and unilateral stimulation.5,6 However, the use of unilateral neck stimulation to evaluate possible asymmetry in carotid-cardiac and carotid-sympathetic baroreflex modulation has provided conflicting results. Indeed, animal studies have consistently reported that right carotid perturbation induced greater effects on HR than left-sided stimulation,7,8 whereas human investigations have found the right carotid-cardiac reflex response to be either greater9 or similar to the left.10 In humans, a sustained left-sided carotid baroreceptor unloading resulted in a more pronounced MSNA activation than the contralateral
perturbation, whereas in anesthetized rabbits, the carotid baroreflex modulation of the cardiac efferent sympathetic activity did not show lateralization.

So far, no studies have been performed in humans to concomitantly assess whether unilateral carotid baroreceptor loading may elicit different effects on HR, arterial pressure, and MSNA variability compared with stimulation of the opposite side. This could be of clinical importance, since unilateral arterial carotid atherosclerosis, commonly encountered in practice, or surgical interventions to treat it, might markedly impair MSNA, heart beat, and arterial pressure variability. This might lead to a marked decrease of spontaneous HR variability or an exaggerated increase of the variability of arterial pressure, conditions that are recognized to affect the prognosis of myocardial infarction, hypertension, and carotid artery damage.

This study was designed to evaluate possible differences in postganglionic sympathetic neural discharge, R-R interval, and arterial pressure variability during perturbation of carotid baroreceptor inputs from the two sides of the neck.

Methods

We studied 12 normotensive volunteers (5 women, 7 men, 32±2 years of age) without evidence of organic disease.

In every subject, we recorded the ECG (Gould), noninvasive blood pressure (Finapres, Ohmeda 2300) and respiratory activity by using a thoracic bellows connected to a pressure transducer. MSNA was obtained from the right peroneal nerve by the microneurography technique. Briefly, a unipolar tungsten electrode was placed in the right peroneal nerve near the fibular head for multunit postganglionic sympathetic nerve recording. The raw neural signal was amplified (1000-fold amplification), fed to a band-pass filter (bandwidth between 700 and 2000 Hz), rectified, and integrated (time constant, 0.1 second) by a nerve traffic analyzer system (Bioengineering Department, University of Iowa).

Integrated MSNA, ECG, arterial pressure, respiratory activity, and neck suction signals were digitized at 300 samples/s by an analog-to-digital board (AT-MIO 16E2, National Instruments) and stored on the hard disk of a personal computer for off-line analysis.

Protocol

Neck suction was applied by 2 separate lead neck cuffs connected to a pressure transducer for negative pressure value monitoring and to a computer-controlled vacuum engine for suction (Figure 1). A valve provided a sinusoidal suction profile for pressure ranging from 0 up to −50 mm Hg. Cuffs were placed on both sides of the neck just under the jaw angle and gently fastened with rubber strings.

Baseline data acquisition was initiated 30 minutes after the instrumentation was in place. Thereafter, the subjects’ respiratory frequency was controlled at 0.25 Hz (ie, 15 breaths per minute) by asking the subject to follow the rhythm of a programmed metronome without changing the depth of breath. Subsequently, a sinusoidal neck suction oscillating from 0 to −50 mm Hg at a frequency of 0.1 Hz was randomly placed at the right side, left side, and combined right and left sides in concordance and in phase opposition for 3 minutes each. A full recovery was allowed after each suction procedure.

The experimental protocol was approved by both the Hospital Sacco and the Vanderbilt University Institutional Review Boards. All subjects provided written informed consent.

Data Analysis

The principles of autoregressive spectrum and cross-spectrum analysis of R-R interval, SAP, and MSNA variability have been described in detail elsewhere. The power of the 0.1-Hz (LF) and 0.25-Hz (HF) components of the power spectra is provided in absolute units. Absolute values of each component were computed as the integral of the single oscillatory component LF and HF.

Microneurography was considered to reflect MSNA according to criteria previously established. Different numbers of recruited sympathetic fibers and different amplification conditions (noise, needle position) resulted in different recorded burst amplitude among subjects. Therefore, the changes in the spectral components of MSNA variability during the neck suction are presented as percent changes from controlled respiration condition.

Data are expressed as mean±SEM. One-way repeated-measures ANOVA and Dunnett’s post-test were used to evaluate differences between each side of neck suction and the controlled respiration alone. The Student’s t test for paired observations was used to evaluate the changes induced by controlled respiration compared with baseline. Differences were considered significant at values of P<0.05.

Results

Changes in the hemodynamics and right peroneal neural sympathetic discharge induced by the sinusoidal suction procedures during controlled respiration at 15 breaths per minute (0.25 Hz) are presented in Table 1. Mean values of HR, arterial blood pressure, and MSNA were unaffected by all suction procedures compared with controlled breathing. This latter, in turn, did not induce major changes in the hemodynamics and neural sympathetic discharge compared with spontaneous breathing values.

Traces in Figures 2 and 3 show the effects of carotid baroreflex stimulation on MSNA and HR. A 0.1-Hz discharge
activity, synchronous with 0.1-Hz neck suction, was evident in MSNA during all procedures, whereas a 0.1-Hz oscillation in HR was induced only by right and combined right- and left-sided stimulations.

**Carotid Sympathetic Reflex Modulation**

Right, left, and combined right- and left-sided sinusoidal 0.1-Hz neck suctions (Table 2) increased the 0.1-Hz oscillatory component of MSNA variability in comparison with controlled respiration. However, no side preference could be observed. Indeed, as shown in the power spectra of Figures 4 and 5, the 0.1-Hz components of MSNA were increased to the same extent during all suction procedures.

The summation of changes induced in the 0.1-Hz component of MSNA by separate right- and left-side baroreceptor stimulation was higher than the power of the same oscillatory component obtained by combined right- and left-side neck suction procedures (Figure 6, upper bar graphs).

**Carotid Cardiac Reflex Modulation**

The 0.1-Hz oscillatory component of R-R variability was significantly increased by the right side alone and combined right- and left-side neck suctions in phase (Table 2 and Figures 4 and 5), whereas the stimulation of both sides in phase opposition had only minor effects ($P<0.6$). Left-sided stimulation did not modify the LF component of R-R variability.

Summation of the 0.1-Hz component of R-R variability achieved by individual right- and left-side neck suctions was similar to the amplitude of the 0.1-Hz component during concomitant right and left carotid baroreceptor stimulation in concordance of phase (Figure 6, lower bar graph). However, when the neck stimulation was performed bilaterally but in phase opposition, the 0.1-Hz, LF oscillation was lower than the sum of the LF components obtained from individual right and left neck suction (Figure 6, lower bar graphs).

### Table 1. Hemodynamic, Respiratory Frequency, and MSNA in Baseline Condition, During Controlled Respiration, and 0.1-Hz Sinusoidal Suction Procedures Applied to Different Sides of the Neck

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Controlled Respiration $(0.25 \text{ Hz})$</th>
<th>Right</th>
<th>Left</th>
<th>Right+Left</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, bpm</td>
<td>63±3</td>
<td>66±3</td>
<td>64±3</td>
<td>65±3</td>
<td>63±2</td>
</tr>
<tr>
<td>R-R, ms</td>
<td>970±42</td>
<td>925±38</td>
<td>955±37</td>
<td>946±48</td>
<td>969±40</td>
</tr>
<tr>
<td>SAP, mm Hg</td>
<td>116±3</td>
<td>116±3</td>
<td>117±3</td>
<td>114±3</td>
<td>116±4</td>
</tr>
<tr>
<td>DAP, mm Hg</td>
<td>73±2</td>
<td>74±2</td>
<td>75±2</td>
<td>71±3</td>
<td>72±3</td>
</tr>
<tr>
<td>Resp, cycles/min</td>
<td>14±1</td>
<td>15±0</td>
<td>15±0</td>
<td>15±0</td>
<td>15±0</td>
</tr>
<tr>
<td>MSNA, bursts/min</td>
<td>16±2</td>
<td>16±2</td>
<td>18±1</td>
<td>17±2</td>
<td>18±2</td>
</tr>
</tbody>
</table>

HR indicates heart rate; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; Resp, respiratory frequency; MSNA, muscle sympathetic nerve activity at the right peroneal nerve; and $\Phi$, phase delay between right and left sinusoidal suction procedures.
Effects on Systolic Arterial Pressure Variability
The effects on systolic arterial pressure (SAP) 0.1-Hz oscillation induced by the side-specific baroreceptor perturbation were similar to those observed for R-R interval variability (Table 2), in that there was a significant increase of the 0.1-Hz fluctuation during right-sided and combined right- and left-sided neck suction in phase.

**Discussion**
In the present study, monolateral and bilateral carotid baroreflex sinusoidal loading at 0.1 Hz did not induce major changes in hemodynamics and MSNA compared with the controlled respiration condition. However, the power of 0.1-Hz fluctuations of MSNA, R-R interval, and SAP variability increased.

In humans, baroreflex responsiveness oscillates concomitantly with spontaneous respiration; thus, to minimize the potential confounding effect of changes in respiratory activity on baroreflex sensitivity, we controlled breathing frequency at 0.25 Hz and kept the depth of breathing constant during all suction procedures.

The results of the present study suggest that: (1) The carotid cardiac and the carotid sympathetic baroreflex modulation in response to unilateral neck suction is not uniform in humans under conditions of controlled respiratory rate. Unilateral carotid baroreflex stimulation did not elicit any functional asymmetry in the sympathetic nerve discharge variability. Conversely, right- and combined right- and left-side in-phase neck suction was more effective in modulating HR variability at 0.1 Hz than the stimulation of the left side of the neck. (2) The different response of MSNA and R-R variability to concomitant bilateral baroreflex loading indicates that right and left carotid baroreceptors act with an inhibitory interaction in modulating the pattern of neural sympathetic discharge and with a simple summation interaction in modulating R-R variability.

**Unilateral Carotid Baroreflex Modulation of MSNA, R-R Interval, and SAP Variability**
The contribution of arterial baroreflex modulation to MSNA, HR, and blood pressure variability has been extensively addressed in both animal and human studies. For instance, arterial baroreflex loading achieved by phenylephrine administration, by enhancing parasympathetic and reducing sympathetic efferent activities, was found to increase the HF component of both R-R interval and MSNA variability.\(^3\) Stimuli eliciting an increase of sympathetic efferent activity through arterial baroreflex unloading, such as the administration of nitroprusside or the assumption of the upright posture,\(^4\) were found to induce a more significant 0.1-Hz oscillatory pattern in sympathetic neural discharge, R-R, and SAP variability. These observations suggest that baroreflex activity may concur in modulating the amplitude of different oscillatory components constituting the variability of MSNA, R-R, and arterial pressure by shifting and synchronizing most of their spontaneous variability into LF (0.1 Hz) or HF (≈0.25 Hz) oscillatory bands, according to the prevailing reflex sympathetic or vagal activation.

In this context, in a subject with carotid baroreflex failure\(^{21}\) caused by bilateral neck irradiation, we found a disruption in the normal 0.1-Hz and respiratory oscillatory patterns of MSNA,
R-R interval, and SAP variability, suggestive of a major role played by the carotid component of overall arterial baroreceptor regulatory activity in modulating the spontaneous variability of those parameters.

In the present study, right and left unilateral carotid baroreflex sinusoidal loading at 0.1 Hz resulted in a similar increase of the LF component of MSNA variability. Our results are in keeping with the finding observed in vagotomized, aortic denervated rabbits of similar dynamic transfer characteristics in right and left carotid sinus baroreflexes but differ from data obtained in humans by Williamson et al., who found that a 5-second duration of pressure applied at the left side of the neck had a greater influence on MSNA than the same procedure applied to the right side. Differences in the modality of carotid baroreflex stimulation may account for the conflicting findings.

A functional asymmetry in HR response to unilateral carotid sinus reflex stimulation, that is, maximal changes during right-side baroreceptor activation/deactivation similar to the results of the present study, has been described by Bernardi and colleagues and others in humans, although not consistently, and has been related to differences in the right-left-side innervation of the heart. In fact, in animals, right cardiac efferent fibers, both sympathetic and vagal, elicited greater chronotropic effects than the left-side fibers, and a right-side vagotomy induced a more pronounced increase of HR than left vagal transection.

In our study, changes in the 0.1-Hz oscillatory component of SAP variability during unilateral right baroreflex stimulation were greater than the modification in LF induced by left-side stimulation, thus diverging from the changes observed in the MSNA oscillatory pattern but paralleling those in R-R variability. This finding might reflect the direct LF modulation exerted by the 0.1-Hz oscillatory component of HR variability on cardiac output.

**Bilateral Carotid Baroreflex Modulation of MSNA and R-R Interval Variability**

In our study, the simple sum of the 0.1-Hz changes in R-R variability after unilateral right and left carotid stimulation was similar to the effects achieved by combined bilateral carotid baroreflex loading. This observation diverges from other findings obtained by using different methodologies in anesthetized dogs, healthy subjects, and athletes.

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**Figure 4.** Example of effects induced by a 0.1-Hz sinusoidal neck suction procedure, placed on the right (R) and left (L) sides separately, on R-R interval and MSNA variability. Notice that individual R and L suction increased the 0.1-Hz component of the spectra of MSNA variability by the same extent compared with controlled respiration. As to R-R interval variability, only right-side neck suction markedly enhanced the 0.1-Hz oscillation. Resp indicates respiration; MSNA, muscle sympathetic nerve activity; PSD, power spectrum density; a.u., arbitrary units; and Hz, Hertz.
those studies, right and left carotid-cardiac reflexes exhibited an inhibitory summation. Interestingly enough, such a pattern was evident in our data when considering the bilateral carotid-sympathetic baroreflex modulation. Indeed, the sum of the individual right-plus-left stimulations was greater than the effects obtained by concomitant bilateral neck suction on MSNA variability. Therefore, right and left carotid baroreflex modulatory activity seems to work after different modalities of interaction on R-R interval as compared with MSNA variability, possibly reflecting a different inhibitory function exerted by carotid afferents projecting into the contralateral medulla.29

The neck suction technique is characterized by several limitations. Indeed, intense carotid baroreceptor loading was found to also affect cardiac vagal modulation, as assessed by a decrease of sinus arrhythmia. In addition, aortic baroreceptors, which are unaffected by the suction procedure, tend to counterbalance the effects of carotid baroreflex stimulation. This tonic stabilizing effect might dampen some of the changes induced by neck suction, mostly on MSNA, which was hypothesized to be particularly sensitive to aortic baroreceptor modulation.30 This mechanism might account for the different effects of carotid suction on MSNA variability as compared with those induced on HR and blood pressure variability observed in the present study.

Finally, we could not directly exclude that the responses induced by unilateral stimulations might have been blunted by the opposition of the contralateral carotid baroreceptor afferents. To overcome this limitation, at least partially, we stimulated carotid baroreceptors concomitantly in phase opposition. That methodology clarified that the effects on R-R variability of bilateral neck stimulation in phase opposition were significantly reduced compared with the sum of the single effects of right and left neck suctions. In contrast, such an effect was not present on MSNA variability. Thus, contralateral carotid afferents seem to play an inhibitory role in modulating only heart period variability.
Clinical Implications

Human baroreflex failure has a variety of clinical presentations. The finding in the present study that changes in HR and blood pressure variability, during right-side carotid baroreceptor rhythmic loading, were of the same magnitude of those obtained by bilateral neck stimulation and greater than the effects induced by left-side suction, suggests that even mild abnormalities in right carotid baroreflex function might markedly affect R-R and SAP variability. Since these variability measurements have been found to be independent risk factors for several pathological conditions including myocardial infarction, hypertension and carotid atherosclerosis, the presence of abnormalities in right carotid baroreflex function might prove useful as another independent factor in risk stratification models.

References

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