Central Vagotonic Effects of Atropine Modulate Spectral Oscillations of Sympathetic Nerve Activity

Nicola Montano, MD, PhD; Chiara Cogliati, MD; Alberto Porta, MD; Massimo Pagani, MD; Alberto Malliani, MD; Krzysztof Narkiewicz, MD, PhD; Francois M. Abboud, MD; Clay Birkett, MS; Virend K. Somers, MD, PhD

Background—Low-dose atropine causes bradycardia either by acting on the sinoatrial node or by its effects on central muscarinic receptors increasing vagal activity. Any central muscarinic effects of high-dose atropine on RR interval are masked by peripheral muscarinic blockade at the sinoatrial node, which causes tachycardia. Effects of central parasympathetic activation on sympathetic activity are not known.

Methods and Results—Using power spectral analysis of RR interval, intra-arterial blood pressure, respiration, and muscle sympathetic nerve activity (MSNA), we examined the effects of both low (2 μg/kg IV) and high (15 μg/kg IV) doses of atropine. After low-dose atropine, RR increased by 9±1% (P<0.0001), the low-frequency (LF) component (in normalized units, NU) of RR variability decreased by −32±8%, and the high-frequency (HF) component increased (+74±19%); hence, LF/HF of RR variability fell by 52±10% (all P<0.01). Although overall MSNA did not change, LFNU of MSNA decreased (−15±5%), HFNU of MSNA increased (+31±3%), and LF/HF of MSNA fell (−41±8%) (all P<0.01). After high-dose atropine, LFNU of MSNA decreased (−17±12%), HFNU of MSNA increased (+22±3%), and LF/HF of MSNA fell (−51±21%) (all P<0.02).

Conclusions—Increasing central parasympathetic activity with low-dose atropine is associated with an increase in the HF and a decrease in the LF oscillations of both RR interval and MSNA variability. High-dose atropine similarly induces an increase in the HF and a decrease in the LF components of MSNA variability. Thus, central parasympathetic activation is able to modulate the oscillatory characteristics of sympathetic nerve traffic to peripheral blood vessels. (Circulation. 1998;98:1394-1399.)

Key Words: vagus nerve ■ nervous system, autonomic ■ heart rate ■ muscles ■ nervous system, sympathetic

In humans, low-dose atropine decreases heart rate1,2 and increases respiratory sinus arrhythmia3 because of an increase in parasympathetic activity. High doses of atropine cause blockade of muscarinic receptors at the cardiac sinoatrial node and are parasympatholytic, markedly increasing heart rate and decreasing heart rate variability.4,5

Whether the vagotonic effects of low-dose atropine are due to a peripheral effect on the sinoatrial node or to a central mechanism is not clear. If it is a central effect, it is not known whether a similar vagotonic effect is also exerted by high-dose atropine, because any central effect of high-dose atropine would be masked by muscarinic blockade at the sinoatrial node. We addressed this question by exploring how the vagotonic (low-dose) or vagolytic (high-dose) actions of atropine affect the activity of a neural outflow, such as muscle sympathetic nerve activity (MSNA).

Power spectral analysis of RR interval variability has been used as an indirect marker of cardiac autonomic regulation.6–9 Recent studies in normal subjects have shown that MSNA variability includes low-frequency (LF) and high-frequency (HF) components, highly coherent with those present in RR-interval and blood-pressure variability.10 Small increases in average sympathetic activity induced by experimental hypotension are accompanied by a relative dominance in the LF components of both RR and MSNA variabilities and by an increase in their LF/HF ratios. Parasympathetic activation induced by increases in arterial pressure, conversely, is accompanied by a reduction in MSNA and by a relative dominance of the HF oscillatory components of RR and MSNA and a decrease in their LF/HF ratios.10 In the present study, we obtained simultaneous measurements of RR interval, intra-arterial blood pressure, and MSNA and evaluated the spectral oscillations of each of these variables during low- and high-dose atropine in normal human subjects. We tested the following hypotheses: (1) that the vagotonic effect of low-dose atropine occurs at a central level and therefore increases the HF components not only of the RR interval but also of MSNA and (2) that despite the elimination of the HF
component of the RR interval by peripheral muscarinic blockade, central vagotonic effects of high-dose atropine result in an increase in the HF component of MSNA.

Methods
We studied 14 healthy human volunteers, all male, 29±2 years old (range, 23 to 42 years). All were nonsmokers and were receiving no medications.

We recorded the ECG, intra-arterial pressure (from a catheter inserted into the radial artery), respiration (with a pneumatic chest belt sensitive to both frequency and amplitude), and efferent MSNA measured directly by microneurography. MSNA was recorded from a sympathetic nerve fascicle to muscle blood vessels in the peroneal nerve. This technique has been extensively described in previous studies.1,2 In brief, recordings were obtained by percutaneous insertion of tungsten microelectrodes into sympathetic fascicles in the peroneal nerve. The electrodes were connected to a preamplifier, and the nerve signal was fed through a band-pass filter and routed through an amplitude discriminator to a storage oscilloscope and loudspeaker. For recording and analysis, the filtered neural signal was fed through a resistance-capacitance integrating network to obtain a mean voltage display of the neural activity. Data were stored via an FM tape recorder (TEAC).

Experimental Protocol
Informed written consent was obtained from all subjects. The study was approved by the Human Subject Review Committee of the University of Iowa. All subjects were studied in a quiet, dimly lit room at a comfortable temperature. After a period of adaptation in the supine position, data were acquired over a 10-minute period of quiet rest. Then 2 intravenous boluses (2 and 15 mg/kg) of atropine were administered in a stepwise fashion, starting with the lower dose. After each atropine bolus, 15-minute periods of recordings were obtained (Figure 1). This study was conducted in 10 subjects.

To exclude the possibility of increased blood pressure after high-dose atropine contributing to an increased HF of MSNA,10 we studied 4 additional subjects (men 26±2 years old) in whom arterial pressure after high-dose atropine was maintained at slightly below the baseline level with an intravenous infusion of nitroprusside.

Spectral Analysis
Data were analyzed off-line after analog-to-digital conversion at a rate of 600 Hz per channel with a 12-bit converter (Gould).

The methodology and the software for data acquisition and spectral analysis of cardiovascular signals have been described previously.1 Briefly, a derivative-threshold algorithm provided the continuous series of RR intervals (tachogram) from the ECG signal. From the continuous arterial pressure signal, beat-by-beat systolic (systogram) and diastolic (diastogram) values were calculated, and the signal of respiratory activity was sampled once for every cardiac cycle.

As described in a previous study,10 we used a digital algorithm to automatically perform event detection and amplitude computation of MSNA. A burst in the neural activity was recognized on the basis of a user-defined voltage and time threshold. For each individual sympathetic burst, the computer program provided the time of the occurrence and its amplitude (time×voltage area). Simulation studies showed that this permitted an accurate automatic computation of the average number of bursts in a time unit (minute) and of the average burst amplitude (expressed in arbitrary units, AU). In addition, integration of the continuous MSNA signal was performed over the time window between 2 consecutive diastolic values delimiting the ith cardiac cycle of period t(i) provided by the neurogram. Accordingly, this new series of variability measures of MSNA is synchronous with the other variability signals on a beat-by-beat basis.

All variability series were analyzed by means of autoregressive parametric spectral and cross-spectral algorithms,14 which can automatically provide the number, center frequency, and associated power of each relevant oscillatory component. The very-low-frequency component (VLF, 0.00 to 0.03 Hz) requiring specific parametric spectral and cross-spectral algorithms,14 which can automatically provide the number, center frequency, and associated power of each relevant oscillatory component. The very-low-frequency component (VLF, 0.00 to 0.03 Hz) requiring specific algorithms and longer data series15 was not addressed in this study and, accordingly, was considered to be a DC component.15

The power was expressed both in absolute and in normalized units (NU), which were obtained by dividing the power of each component by total variance from which the VLF component had been subtracted and multiplying this value by 100.15

Statistical Analysis
Data are expressed as mean±SEM. Responses to atropine were tested by means of a 1-way ANOVA for repeated measures. Linear regressions were used to determine whether any relationship existed between changes in blood pressure and changes in spectral measurements of MSNA. A value of P<0.05 was considered significant.

Results
Baseline Conditions
In control conditions, average MSNA burst frequency and amplitude were 25±7 bursts per minute (39±9 bursts/100 beats) and 650±150 AU, respectively; RR interval was 1028±39 ms; and mean systolic and diastolic arterial pressures were 115±2 and 84±3 mm Hg, respectively (Table 1).

Spectral analysis of MSNA variability revealed 2 major oscillatory components, LF (0.10±0.004 Hz) and HF (0.24±0.01 Hz). A similar spectral profile was also observed in RR interval and systolic arterial pressure (SAP) variabilities (Figure 2; Table 2).

Effects of Low-Dose Atropine
Bolus infusion of the low dose of atropine (2 µg/kg) did not alter MSNA (either in burst frequency or amplitude). The RR
interval increased significantly (+9±1%; \(P<0.0001\)), whereas SAP was unchanged (Table 1; Figure 1).

Despite the absence of changes in absolute measurements of MSNA, low-dose atropine had significant effects on spectral oscillatory components of MSNA (Figures 2 and 3). Normalized LF_{MSNA} power was reduced (−15±5%; \(P<0.008\)) and normalized HF_{MSNA} power increased (+31±3%; \(P<0.007\)). The LF/HF_{MSNA} ratio decreased (−41±8%; \(P<0.002\)). These changes in MSNA spectral profile were mirrored by similar changes in RR interval variability. Normalized LF_{RR} decreased (−32±8%; \(P<0.004\)) and normalized HF_{RR} increased (+74±19%; \(P<0.003\)). LF/ HF_{RR} was also reduced (−52±10%; \(P<0.001\)). SAP variability measures did not reveal significant changes (Table 2). The center frequency of respiration and its variance were not significantly modified (from 0.27±0.01 to 0.25±0.02 Hz).

**Effects of High-Dose Atropine**

Compared with control conditions, administration of high-dose atropine (15 μg/kg) produced a substantial decrease in MSNA burst frequency (−25±9%; \(P<0.01\)) as well as in RR interval (−41±2%; \(P<0.001\)), whereas SAP increased slightly but significantly (+7±3%; \(P<0.02\)) (Table 1; Figure 1).

Normalized LF_{MSNA} decreased (−17±12%; \(P<0.02\)), as did the LF/HF_{MSNA} (−51±21%; \(P<0.01\)), whereas normalized HF_{MSNA} increased (+22±3%; \(P<0.02\)). RR interval variance was drastically reduced (−99±0.2%; \(P<0.0001\)), accompanied by a reduction of LF_{RR} and HF_{RR} in absolute values (−96±0.1% and −99±0.1%, respectively; \(P<0.0001\)). However, normalized LF_{RR} was markedly increased (+59±25%; \(P<0.001\)), whereas normalized HF_{RR} was reduced (−63±12%; \(P<0.001\)). LF/HF_{RR} increased markedly (1293±540%; \(P<0.0001\)) (Figures 2 and 3). SAP variance as well as variance and center frequency of respiration did not change significantly (Table 2). By use of linear regressions, no significant correlations were found between changes in SAP and alterations in normalized LF_{MSNA}, HF_{MSNA}, and LF/HF_{MSNA}.

**Effects of High-Dose Atropine and Intravenous Nitroprusside**

In 4 subjects, we sought to exclude any possible effects of the increased blood pressure after high-dose atropine contributing to an increase in HF of MSNA. In 3 of these 4 subjects, infusion of intravenous nitroprusside was used to maintain blood pressure slightly below baseline. In the fourth subject, blood pressure after high-dose atropine was lower than the baseline even without the use of nitroprusside. For all subjects, blood pressure at baseline was 116±5 mm Hg, and after high-dose atropine (and intravenous nitroprusside in 3 subjects) it was 110±5 mm Hg. MSNA was significantly decreased when expressed as bursts per 100 beats (−35±7%; \(P<0.04\)) but not when expressed as bursts per minute (from 19±6 to 22±5). Normalized LF_{MSNA} decreased (−48±10%; \(P<0.03\)). HF_{MSNA} increased, but not significantly, from 36±8 to 45±6 NU (\(P=0.3\)). A significant decrease in LF/HF_{MSNA} (−61±13%; \(P<0.05\)) was also evident in these 4 subjects.
The mechanism by which low-dose atropine slows heart rate is not clear. It has been suggested that the bradycardia is due to the stimulation of pacemaker muscarinic M1 receptors or to a direct effect on pacemaker cells, independent of muscarinic receptors; however, such a mechanism would be unlikely to enhance the HF rhythmicity of RR-interval variability.

The present study demonstrates that the decrease in heart rate after low-dose atropine is accompanied by an increase in the HF oscillatory components of both RR interval and MSNA variability. These simultaneous changes suggest that low-dose atropine acts centrally and that the parasympathetic effect of low-dose atropine manifests as a rhythmic pattern consisting of an increase in the HF and a decrease in the LF oscillations.

Similar findings have been reported by Tougas et al. who used a mechanical rather than a pharmacological intervention. In their study, the vagal response to esophageal distension was accompanied by an increase of HF and a decrease of LF in the power spectrum of heart rate variability.

Our data support earlier studies by Gilbey et al. who observed an increase in single cardiac vagal motor neuron firing activity in the nucleus ambiguus after iontophoretic application of atropine. In studies in humans, Raczkowska et al. reported an increase in the magnitude of respiratory sinus arrhythmia after low-dose atropine.

Previous reports have examined the effect of scopolamine, a muscarinic blocker, on heart period and its variability. In studies in healthy subjects, in patients after myocardial infarction, and in patients with congestive heart failure, scopolamine was found to increase indices of parasympathetic activity. In patients after cardiac transplantation and presumed cardiac denervation, Epstein et al. showed that low-dose atropine did not increase RR interval, suggesting that the decrease in heart rate is not due to direct effects of atropine on the pacemaker cells.

### Discussion

This study shows, first, that the vagotonic effect of low-dose atropine (slowing of heart rate) is accompanied by a relative increase in the HF components of both RR interval and MSNA variabilities. Changes in the oscillatory properties of MSNA occur in the absence of any clear changes in burst frequency of MSNA.

Second, we have shown that even with high-dose atropine, there is an indication of a central parasympathetic effect that is revealed only by examination of the HF oscillation of MSNA. This conclusion could not have been drawn from measurements of oscillations in RR interval variability alone because of peripheral muscarinic blockade of the sinoatrial node with high-dose atropine. Both low and high doses of atropine are accompanied by a relative increase in central parasympathetic activity, reflected by a decreased LF/HF ratio of both RR and MSNA during low-dose atropine and a decreased LF/HF ratio of MSNA during high-dose atropine.

### Effects of Low-Dose Atropine

This study demonstrates that the decrease in heart rate is not due to direct effects of atropine on the pacemaker cells, independent of muscarinic receptors; however, such a mechanism would be unlikely to enhance the HF rhythmicity of RR-interval variability.

The present study demonstrates that the decrease in heart rate after low-dose atropine is accompanied by an increase in the HF oscillatory components of both RR interval and MSNA variability. These simultaneous changes suggest that low-dose atropine acts centrally and that the parasympathetic effect of low-dose atropine manifests as a rhythmic pattern consisting of an increase in the HF and a decrease in the LF oscillations.

Similar findings have been reported by Tougas et al. who used a mechanical rather than a pharmacological intervention. In their study, the vagal response to esophageal distension was accompanied by an increase of HF and a decrease of LF in the power spectrum of heart rate variability.

Our data support earlier studies by Gilbey et al. who observed an increase in single cardiac vagal motor neuron firing activity in the nucleus ambiguus after iontophoretic application of atropine. In studies in humans, Raczkowska et al. reported an increase in the magnitude of respiratory sinus arrhythmia after low-dose atropine.

Previous reports have examined the effect of scopolamine, a muscarinic blocker, on heart period and its variability. In studies in healthy subjects, in patients after myocardial infarction, and in patients with congestive heart failure, scopolamine was found to increase indices of parasympathetic activity. In patients after cardiac transplantation and presumed cardiac denervation, Epstein et al. showed that low-dose atropine did not increase RR interval, suggesting that the decrease in heart rate is not due to direct effects of atropine on the pacemaker cells.

### Table 2: Spectral Measurements of RR Interval, MSNA, and SAP Variability During Control and After Infusion of a Low and a High Dose of Atropine (n=10)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Atropine, 2 µg/kg</th>
<th>Atropine, 15 µg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variance, ms$^2$</td>
<td>5391±2237</td>
<td>5060±1407</td>
<td>33±13$^\dagger$</td>
</tr>
<tr>
<td>LF, ms$^2$</td>
<td>1623±530</td>
<td>1615±577</td>
<td>23±8$^\dagger$</td>
</tr>
<tr>
<td>LF, nu</td>
<td>61±5</td>
<td>41±7$^*$</td>
<td>87±4$^\dagger$</td>
</tr>
<tr>
<td>LF, Hz</td>
<td>0.10±0.01</td>
<td>0.09±0.01</td>
<td>0.06±0.004$^\dagger$</td>
</tr>
<tr>
<td>HF, ms$^2$</td>
<td>1417±887</td>
<td>2092±865$^*$</td>
<td>1.45±0.4$^\dagger$</td>
</tr>
<tr>
<td>HF, nu</td>
<td>32±5</td>
<td>53±7$^*$</td>
<td>10±3$^\dagger$</td>
</tr>
<tr>
<td>HF, Hz</td>
<td>0.26±0.01</td>
<td>0.24±0.02</td>
<td>0.26±0.01</td>
</tr>
<tr>
<td>LF/HF</td>
<td>2.34±0.41</td>
<td>1.12±0.38$^*$</td>
<td>19.31±5.2$^\dagger$</td>
</tr>
<tr>
<td>MSNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LF, nu</td>
<td>46±5</td>
<td>39±5$^*$</td>
<td>35±4$^*$</td>
</tr>
<tr>
<td>LF, Hz</td>
<td>0.105±0.004</td>
<td>0.089±0.006</td>
<td>0.098±0.007</td>
</tr>
<tr>
<td>HF, nu</td>
<td>31±3</td>
<td>42±5$^*$</td>
<td>39±3$^*$</td>
</tr>
<tr>
<td>HF, Hz</td>
<td>0.24±0.01</td>
<td>0.24±0.01</td>
<td>0.26±0.01</td>
</tr>
<tr>
<td>LF/HF</td>
<td>1.65±0.30</td>
<td>0.87±0.12$^*$</td>
<td>1.14±0.02$^*$</td>
</tr>
<tr>
<td>SAP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variance, mm Hg$^2$</td>
<td>10±2.5</td>
<td>7.2±1.8</td>
<td>8.9±3.7</td>
</tr>
<tr>
<td>LF, mm Hg$^2$</td>
<td>4.7±1.8</td>
<td>4.8±1.8</td>
<td>3.0±1.2</td>
</tr>
<tr>
<td>LF, nu</td>
<td>73±4</td>
<td>63±7</td>
<td>59±7</td>
</tr>
<tr>
<td>LF, Hz</td>
<td>0.078±0.008</td>
<td>0.075±0.008</td>
<td>0.056±0.007</td>
</tr>
<tr>
<td>HF, mm Hg$^2$</td>
<td>0.9±0.17</td>
<td>1.44±0.41</td>
<td>0.9±0.24</td>
</tr>
<tr>
<td>HF, nu</td>
<td>23±4</td>
<td>30±6</td>
<td>26±5</td>
</tr>
<tr>
<td>HF, Hz</td>
<td>0.27±0.01</td>
<td>0.25±0.01</td>
<td>0.26±0.01</td>
</tr>
</tbody>
</table>

$^*P<0.05$ vs control; $^\dagger P<0.05$ low vs high dose.
Effects of High-Dose Atropine

The increased normalized HF component, decreased normalized LF component, and decreased LF/HF ratio of MSNA oscillations after low-dose atropine are also evident after high-dose atropine. However, these vagotonic effects of high-dose atropine are apparent only by examination of the alteration in MSNA oscillations. These findings are consistent, in part, with data from Katona et al., who showed that atropine increased cardiac vagal efferent activity in anesthetized dogs in a dose-response fashion. We were unable to demonstrate, however, that high-dose atropine reduced the LF/HF ratio of MSNA beyond the level achieved with low-dose atropine.

It is unlikely that the increase in arterial pressure after high-dose atropine might have caused a reduction in the LF component of MSNA. Indeed, even when blood pressure after high-dose atropine was lowered to slightly below baseline levels (nitroprusside substudy), a significant reduction in LF_{NI} and LF/HF of MSNA was evident in all 4 subjects.

Our findings also have implications for understanding the genesis of the LF oscillatory components in cardiovascular variability. Previous studies of RR variability noted that muscarinic blockade at the sinus node induced tachycardia, a drastic reduction of RR variance, and reduced absolute values of both LF and HF components of RR variability. Accordingly, reduction in the absolute value of the LF component of RR variability after atropine has been interpreted as evidence that this component is generated mainly by vagal mechanisms at rest. Absolute measures of oscillatory power are exquisitely dependent on variance of the signal. Hence, it is likely that the increased RR variance due to unopposed sympathetic activity after vagal blockade with high-dose atropine results in a reduction of the absolute LF component of RR variability. As shown in the 15-fold magnified spectral window in the top right panel of Figure 3, after high-dose atropine all remaining power in the RR interval variability is condensed into the LF component, leading to high values of normalized LF power (Table 2). These findings are similar to those noted by Bernardi and colleagues after atropine administration in normal subjects and by Rimoldi et al. in studies in conscious dogs. Thus, an alternative conclusion is that the relative sympathetic predominance in RR modulation after vagal blockade with high-dose atropine is evident from the marked increase in the normalized LF of RR variability and in the LF/HF ratio of RR variability.

Conclusions

Central parasympathetic activation induced by blockade of inhibitory muscarinic receptors of central vagal nuclei causes bradycardia and induces a relative predominance of the HF oscillatory components not only of the RR interval but also of MSNA. Although high-dose atropine blocks vagal activity at the cardiac level, causing tachycardia, a central muscarinic receptor blockade causing vagal activation is suggested by a relative increase in the HF component of MSNA. Thus, central parasympathetic activation may also modulate the oscillatory components of sympathetic traffic directed to peripheral blood vessels.

Although both LF and HF components are present in sympathetic discharge variability, sympathetic excitation appears to be related to a relative increase in the LF oscillation of sympathetic neural outflow and RR interval variability. Similarly, LF and HF components are also present in vagal discharge variability, but vagal excitation appears to be linked to a relative increase in the HF component of sympathetic outflow and RR variability. These rhythms may reflect a central pattern organization in which excitation (or sympathetic activation) is associated with increased LF rhythmicity and inhibition (or vagal activation) with increased HF rhythmicity.


Central Vagotonic Effects of Atropine Modulate Spectral Oscillations of Sympathetic Nerve Activity
Nicola Montano, Chiara Cogliati, Alberto Porta, Massimo Pagani, Alberto Malliani, Krzysztof Narkiewicz, Francois M. Abboud, Clay Birkett and Virend K. Somers

_Circulation_. 1998;98:1394-1399
doi: 10.1161/01.CIR.98.14.1394

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/98/14/1394