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

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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, LF NU of MSNA decreased (−15±5%), HF NU of MSNA increased (+31±3%), and LF/HF of MSNA fell (−41±8%) (all P<0.01). After high-dose atropine, LF NU of MSNA decreased (−17±12%), HF NU 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 pneumotach 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. Briefly, 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 to the peroneal nerve. The electrodes were connected to a preamplifier, and the nerve signal was fed through an amplitude discriminator to a storage oscilloscope and, accordingly, was considered to be a DC component.

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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. Briefly, a derivative-threshold algorithm provided the continuous series of RR intervals (tachogram) from the ECG signal. 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, 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, 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 series was not addressed in this study and, accordingly, was considered to be a DC component. 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.

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

### Table 1. Mean RR Interval, MSNA Measures, and SAP at Baseline 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, ms</td>
<td>1028±39</td>
<td>1122±38*</td>
<td>599±16†</td>
</tr>
<tr>
<td>MSNA, bursts/min</td>
<td>29±9</td>
<td>25±8</td>
<td>12±7*</td>
</tr>
<tr>
<td>MSNA, bursts/100 beats</td>
<td>39±9</td>
<td>39±6</td>
<td>13±5†</td>
</tr>
<tr>
<td>MSNA, AU</td>
<td>650±150</td>
<td>456±102</td>
<td>223±78†</td>
</tr>
<tr>
<td>SAP, mm Hg</td>
<td>115±2</td>
<td>116±2</td>
<td>123±3*</td>
</tr>
</tbody>
</table>

*P<0.05 vs control; †P<0.05 low vs high dose.
Effects of Atropine on MSNA Variability

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<0.0001), whereas SAP increased slightly but significantly (7%±3%; P<0.02) (Table 1; Figure 1).

Normalized LF<sub>MSNA</sub> decreased (−17%±12%; P<0.02), as did the LF/HF<sub>MSNA</sub> (−51%±21%; P<0.01), whereas normalized HF<sub>MSNA</sub> increased (+22%±3%; P<0.02). RR interval variance was drastically reduced (−99%±2%; P<0.0001), accompanied by a reduction of LF<sub>RR</sub> and HF<sub>RR</sub> in absolute values (−96%±0.1% and −99%±0.1%, respectively; P<0.0001). However, normalized LF<sub>RR</sub> was markedly increased (+59%±25%; P<0.0001), whereas normalized HF<sub>RR</sub> was reduced (−63%±12%; P<0.0001). LF/HF<sub>RR</sub> 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<sub>MSNA</sub>, HF<sub>MSNA</sub>, and LF/HF<sub>MSNA</sub>.

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.

Intravenous Nitroprusside

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Effects of Low-Dose Atropine

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.

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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

Acknowledgments

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References


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