Nitric Oxide and Cardiac Autonomic Control in Humans

Saqib Chowdhary, Julian C. Vaile, Janine Fletcher, Hamish F. Ross, John H. Coote, Jonathan N. Townend

Abstract—Cardiac autonomic control is of prognostic significance in cardiac disease, yet the control mechanisms of this system remain poorly defined. Animal data suggest that nitric oxide (NO) modulates cardiac autonomic control. We investigated the influence of NO on the baroreflex control of heart rate in healthy human subjects. In 26 healthy male volunteers (mean age, 23 ± 5 years), we measured heart rate variability and baroreflex sensitivity during inhibition of endogenous NO production with N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA) (3 mg/kg per hour) and during exogenous NO donation with sodium nitroprusside (1 to 3 mg/h). Increases from baseline (Δ) in high-frequency (HF) indexes of heart rate variability were smaller with L-NMMA in comparison to hydralazine (P < 0.002); Δpercentage of successive RR interval differences >50 ms (ΔpNN50)=5 ± 15% versus 14 ± 12% (P < 0.05); and ΔHF normalized power = −2 ± 7 versus 9 ± 8 normalized units (P < 0.01), respectively. Relative preservation of these indexes was observed during unloading of the baroreflex with sodium nitroprusside compared with a matched fall in blood pressure produced by a control vasodilator, hydralazine (9 to 18 mg/h): ΔRMSSD=−8 ± 8 versus −24 ± 15 ms (P < 0.001); ΔpNN50=−6 ± 11% versus −15 ± 19% (P < 0.01); ΔHF normalized power = −7 ± 13 versus −13 ± 11 normalized units (P < 0.05), respectively. The change in cross-spectral α-index calculated as the square root of the ratio of RR interval power to systolic spectral power in the HF band (although not α-index calculated in the same way for the low-frequency bands or baroreflex sensitivity assessed by the phenylephrine bolus method) was attenuated with L-NMMA compared with phenylephrine (Δ=4 ± 8 versus 14 ± 15 ms/mm Hg, respectively; P < 0.02) and with sodium nitroprusside compared with hydralazine (Δ=−7 ± 6 and −9 ± 7 ms/mm Hg, respectively; P < 0.05). In conclusion, these data demonstrate that NO augments cardiac vagal control in humans. (Hypertension. 2000;36:264-269.)

Key Words: nitric oxide ■ heart rate ■ baroreceptors ■ autonomic nervous system ■ blood pressure

The powerful influence of autonomic control on the natural history of cardiac disease is evidenced by large trials showing that reduced heart rate variability (HRV) and baroreflex sensitivity (BRS) are independent indicators of adverse prognosis.\textsuperscript{1,2} However, the mechanisms controlling cardiovascular autonomic function remain poorly defined.

The initial suggestion that nitric oxide (NO) may be an important mediator in cardiac autonomic control came from the demonstration of discrete neuronal populations that possess NO synthase at numerous sites within known cardiac autonomic pathways.\textsuperscript{3} Animal evidence suggests that the NO synthesized at these sites is active in modulating activity within both limbs of the autonomic nervous system. NO appears to act as a sympatholytic agent, decreasing activity within sympathoexcitatory brain stem nuclei and reducing central sympathetic outflow,\textsuperscript{4,5} as well as attenuating cardiac responses to sympathetic stimulation.\textsuperscript{6} Conversely, NO increases activity in central vagal motoneurons\textsuperscript{7} and enhances the cardiac response to vagal stimulation.\textsuperscript{6,8} In addition, NO donors may increase sinoatrial nodal discharge rate directly.\textsuperscript{9} However, the results of animal experiments have not been consistent, and the influence of NO on human cardiac autonomic control remains to be determined.

Cardiac autonomic control can be assessed noninvasively from the measurement of HRV and of BRS. This study was designed to determine the effects on these indexes of the systemic administration of (1) the inhibitor of endogenous NO synthesis N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA) and (2) the exogenous NO donor sodium nitroprusside in healthy human subjects. Investigation of the autonomic effects of these agents is hampered by their influence on blood pressure and consequently on baroreceptor loading. We have therefore made comparisons with the effects of equal changes in blood pressure produced by vasoactive drugs that do not act via the NO pathway, namely, phenylephrine and hydralazine.
Subjects

Twenty-six healthy male volunteers aged 18 to 36 years (mean age, 23 ± 5 years) were studied. All subjects were normotensive (supine blood pressure measurement <140/90 mm Hg at initial screening visit), with no symptoms or signs of cardiovascular disease and on no medication. Subjects were asked to abstain from food or drink for at least 2 hours before the study and from caffeine and alcohol for 24 hours. Experimental protocols were approved by the South Birmingham local research ethics committee, and individual written consent was obtained.

Procedures

All protocols were of a single-blind, random-order, crossover design. Subjects attended an initial habituation and training visit to our dedicated clinical autonomic research laboratory. At this visit they were trained to breathe to an audio signal set close to the individual’s resting respiratory frequency. All studies were performed at the same time of day and with an ambient temperature of 24 ± 1°C.

A standard 3-lead ECG signal was amplified, processed (high-frequency [HF] signal noise filter >500 Hz), and digitized at 500 Hz with the use of a National Instruments NB/MIO16XH/18 analog-to-digital converter board (National Instruments Corporation). A continuous arterial pressure signal was obtained with the Portapres device (TNO Biomedical Instrumentation) and was similarly digitized. Respiratory excursion was recorded from the amplified output of a standard strain gauge attached to an elastic strap around the subject’s chest. All signals were displayed on the screen of a personal computer running Laboratory View 5.0 software (National Instruments Corporation).

At the start of each study, a venous cannula was inserted into an antecubital vein for drug administration. Subjects were rested for 30 minutes, after which selected periods from all 3 signals were stored to disk during breathing at the predetermined frequency. Two 5-minute recordings were taken during a 30-minute normal saline infusion and an additional 2 at steady-state (<10% deviation in the mean heart rate and arterial pressure over two 60-second periods, 5 minutes apart) during the vasoactive infusion. Results were calculated as the mean of the 2 recordings. Target increments and decrements in mean arterial pressure were assessed from a continuously updated 60-second integrated mean of the Portapres signal and by intermittent arm cuff sphygmomanometry with an automated oscillometric system. All drugs were diluted in normal saline or 5% glucose immediately before the experiment.

Protocol 1: Effects of Inhibition of NO Synthesis on HRV

Fourteen subjects were randomly assigned to receive intravenous infusions of either L-NMMA (3 mg/kg per hour) or a control infusion of the pressor agent phenylephrine (12 to 42 μg/kg per hour) during the first of 2 studies. The second agent was given during a separate study visit 7 to 14 days later. At each study visit the subject rested semisupine, and data were acquired at baseline and during the drug infusion after the target rise in mean arterial pressure of ~10 mm Hg had been achieved.

Protocol 2: Effects of Exogenous NO on HRV

In a similar experimental design, 12 subjects received random-order infusions of the NO donor sodium nitroprusside (1 to 3 mg/h) and, as a control, hydralazine (9 to 18 mg/h) on separate days. Each infusion was titrated to achieve a drop in mean arterial pressure of 5 to 10 mm Hg. Initial experiments showed that in the supine position the dose of hydralazine required to achieve this target was occasionally associated with headache and nausea. Using a mild degree of orthostatic stress considerably reduced the cumulative dose required and side effects. Both experiments were therefore performed with subjects at 30° of head-up tilt during all phases of the experiment. Recordings were made, as before, at baseline and during a 60- to 90-minute infusion of each of the hypotensive agents.

To test the effect of time on this protocol, in 6 volunteers we assessed changes in mean arterial pressure and HRV during normal saline infusion (30 mL/h) at 30° head-up tilt. Data were acquired at baseline (30 minutes) and at 2 hours. No significant changes over time were observed in mean arterial pressure, RR interval, or any HRV index.

<table>
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<tr>
<th>TABLE 1. Blood Pressure, RR Interval, RR Interval Variability, and BRS (α-Index) at Baseline and During Steady-State Drug Infusion</th>
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CCV indicates coefficient of component variance.

*P<0.05, †P<0.01.
§P<0.05, ||P<0.01, ‡P<0.001, infusion vs baseline value.
Measurement of BRS

BRS was assessed by 2 methods. The α-index, describing the transfer function of variability in the systolic pressure signal to variability in the RR interval, was assessed by cross-spectral analysis in protocols 1 and 2. In addition, we used the Oxford method of measuring the RR interval response to a transient rise in blood pressure generated by an intravenous bolus injection of phenylephrine. The value of the regression line of RR interval against the preceding systolic blood pressure was accepted as baroreflex gain only if the correlation coefficient was >0.8. Quoted values represent the mean of at least 3 results.

Data Analysis

Initial Processing

The ECG series for analysis were coded so that the investigator performing the analysis was blinded to the vasoreactive agent under study. All ECG series were reviewed and if necessary edited to exclude ectopic and artifact signals. The RR intervals before and after any ectopic beats were replaced by interpolation from the previous and following sinus intervals. No signal containing >1% of ectopic beats was used for analysis. R waves were detected by an individually adjusted threshold, and the fiducial point was determined by fitting a quadratic polynomial to sequential groups of 7 data samples. HRV was analyzed off-line on data lengths of 256 RR intervals.

Time Domain Analysis

We used the standard time domain measures of standard deviation of RR interval values (SDNN), root mean square of successive RR interval differences (RMSSD), and percentage of successive RR interval differences >50 ms (pNN50). Overall variability is expressed by SDNN, whereas those indexes based on successive differences in RR intervals, ie, pNN50 and RMSSD, assess HF (“beat-to-beat”) variation associated with respiratory sinus arrhythmia mediated principally by the vagus nerve.

Frequency Domain Analysis

Stationarity of the time series was tested by calculation of the mean variance of the first and last 128 beats of each recording period to verify a difference of <10% in the values for each time series. Power spectral analysis was performed with the use of the Burg algorithm (autoregressive modeling), with a model order between 8 and 20 selected to minimize the Akaike information criterion. The power at each underlying frequency was quantified by decomposing the total variability signal according to the method of Zetterberg. Powers at low frequency (LF) (centered at ~0.1 Hz) and at HF (corresponding to the observed respiratory frequency) were thus determined. Power was expressed in absolute and normalized units (nU) (power/total power >0.04 Hz) and as the coefficient of component variance (square root of power/mean RR interval).

Baroreflex Sensitivity

In the assessment of BRS by cross-spectral analysis, the recordings of RR interval and systolic blood pressure were interpolated (with a cubic spline) and then resampled at 1 Hz to produce a uniform time base. A cross spectrum of 256 samples was then analyzed by fast Fourier transforms with the use of a Hanning window on successive overlapping records of 64 samples each. The α-index was calculated as the square root of the ratio of RR interval power to systolic spectral power in both the HF (α-HF) and LF bands (α-LF).

Statistical Analysis

Data for mean arterial pressure and RR interval were compared by a 2-tailed paired Student’s t test. Differences between groups for time and frequency domain indexes of HRV and BRS were determined with the Wilcoxon signed rank test for paired data. Statistical significance was defined by a P value <0.05. Numerical values are expressed as mean±SD.

Effects of Inhibition of Endogenous NO on HRV

The frequency of metronomic breathing was within the range of 0.17 to 0.22 Hz. Baseline values for mean arterial pressure, RR interval, and indexes of HRV were not significantly different between the groups before administration of L-NMMA or phenylephrine (Table 1). Infusion of L-NMMA and phenylephrine resulted in similar rises in mean arterial pressure (11±5 and 11±3 mm Hg, respectively). Both agents increased the mean RR interval, but this increase tended to be lower with L-NMMA (+101±55 ms) than with phenylephrine (+156±110 ms) (P=0.08).

However, measures of HF, vagally mediated HRV in both time and frequency domains were significantly lower during L-NMMA infusion than during phenylephrine (absolute values are shown in Table 1). A comparison of the magnitude of change in HRV from baseline values emphasizes the markedly discrepant responses between the 2 equipressor infusions (Figure 1). The increases in HF HRV in both domains (ie, RMSSD, pNN50, and HF power) were significantly smaller in response to L-NMMA than to an equipressor phenylephrine infusion. The fall in LF power in normalized (although
Effects of Exogenous NO on HRV

Baseline levels of mean arterial pressure and RR interval were not significantly different before administration of sodium nitroprusside or hydralazine. Indexes of HRV were comparable at baseline except for RMSSD and HF absolute power, which were lower in the sodium nitroprusside group (Table 2).

The hypotensive responses to infusions of sodium nitroprusside and hydralazine were well matched (change in mean arterial pressure $-8.2 \pm 2$ and $-7.3 \pm 3$ mm Hg, respectively) but produced discrepant effects on heart rate, with less acceleration during sodium nitroprusside than during hydralazine infusion (change in mean RR interval $-98.58 \pm 58$ versus $-212.81 \pm 81$ ms, respectively; $P<0.0001$). The HRV responses were also significantly different. HF, vagally mediated indexes of HRV in the time domain (ie, RMSSD and pNN50) were significantly greater during sodium nitroprusside infusion than during hydralazine infusion (Table 2). It can be seen that despite the fall in blood pressure, there was relative preservation of HF HRV with sodium nitroprusside. In the frequency domain, the pattern is less clear because of a large reduction in total power with hydralazine. In absolute units, both HF and LF powers appeared to be higher with sodium nitroprusside than hydralazine, although there were no significant differences when the change in total power was corrected for by the use of normalized units. However, when changes from baseline are examined, it can be seen that the effects of the 2 agents on HF variability in both domains were discrepant with smaller changes during sodium nitroprusside than during hydralazine administration (Figure 2).

Effects of NO on BRS

Cross-spectral analysis of RR interval and systolic blood pressure variability revealed that the $\alpha$-LF index was not calculable because of noncoherence for 25% of data in protocol 1 and 14% of data in protocol 2, whereas $\alpha$-HF was calculable for all data. Baseline values of $\alpha$-HF and $\alpha$-LF were not significantly different for either protocol. $\alpha$-HF (although not $\alpha$-LF) was both significantly lower and increased less during infusion of L-NMMA than during infusion of phenylephrine (Table 1 and Figure 1). Sodium nitroprusside resulted in significantly less attenuation of $\alpha$-HF and $\alpha$-LF than phenylephrine (Figure 2).

Assessment of BRS by the phenylephrine bolus method revealed similar baseline values before L-NMMA and phenylephrine infusion (17.4 $\pm 6.1$ ms/mm Hg, respectively). When L-NMMA and phenylephrine infusion were compared, values were not significantly different (18.6 $\pm 4.7$ and 18.4 $\pm 6.5$ ms/mm Hg, respectively; $P=0.9$). Compared with baseline, BRS did not change significantly in response to L-NMMA, whereas there was a significant (18%) rise in BRS with phenylephrine ($P=0.02$).

Discussion

Direct recording of cardiac vagal and sympathetic activity is not possible in humans, but there is good evidence that HF indexes of HRV in both time and frequency domains reflect vagal modulation of heart rate in the conscious human. This includes the demonstration that HF variability is proportional to efferent vagal activity in anesthetized dogs and in humans is strongly correlated with heart rate acceleration in
response to atropine.\textsuperscript{13,16} Furthermore, HF variability is virtually abolished by atropine.\textsuperscript{16,17} We have shown that despite a rise in blood pressure equal to that obtained with phenylephrine, systemic inhibition of NO synthase by L-NMMA was associated with less bradycardia and lower measures of HF HRV. We suggest that this is a result of removal of a tonic excitatory effect of NO on baroreflex-mediated cardiac vagal activity. In support of this, we found that infusion of the exogenous NO donor sodium nitroprusside was associated with less tachycardia and relative preservation of cardiac vagal control (as measured by HF HRV) compared with a control infusion of hydralazine. These data constitute the first controlled evidence of an important role for NO in cardiac vagal control in human subjects.

Although we have shown that NO appears to modulate cardiac vagal control of heart rate, we cannot exclude the possibility of a sympathetic nervous influence on our results. LF power has been used as an index of sympathetic activity (especially when expressed in normalized units),\textsuperscript{18} but there is undoubtedly a large vagal component to this oscillation.\textsuperscript{17} Similarly, use of the LF/HF ratio as a measure of “sympathovagal balance”\textsuperscript{12} has been the subject of recent debate.\textsuperscript{19} Thus, we are reluctant to draw any firm conclusions on the relative activity of the sympathetic nervous system during these drug infusions.

The observed effect of NO on vagal activity during a sustained rise or fall in blood pressure could have been mediated by effects on either the sensitivity or “gain” of the baroreflex or a change in the reflex set point. When BRS was measured by cross-spectral analysis, α-HF was higher during phenylephrine than L-NMMA infusion and fell less during sodium nitroprusside than during hydralazine administration, suggesting an enhancement of baroreflex gain by NO. Changes in α-LF were directionally similar but were not statistically significant (possibly because of the loss of analyzable data through noncoherence). There may also be differences in the information held within each α-index. Since α-HF is determined at a frequency at which sympathetic influence is not effective, this index represents gain in the vagal limb of the baroreflex,\textsuperscript{20} while the α-LF index is open to influence by both limits of the autonomic nervous system.\textsuperscript{20} The Oxford method of measuring BRS, which is historically the “gold standard,” failed to show a clear influence of NO. Thus, our results do not allow firm conclusions to be drawn on the possible effects of NO on human baroreflex gain.

Previous information on the effects of NO on autonomic activity in humans is limited. Studies comparing the response of muscle sympathetic nerve activity to an infusion of L-NMMA with appropriate baroreflex controls indicated an inhibitory action of endogenous NO on sympathetic nerve activity in humans.\textsuperscript{21} Castellano et al\textsuperscript{22} studied the effects of L-NMMA on HRV in 7 healthy volunteers. No controls for alterations in blood pressure were studied, making the results difficult to interpret, but in agreement with our results, there were no significant changes in absolute values of HF or LF RR interval powers or in cross-spectral BRS despite a clear rise in blood pressure.

Our data appear to indicate that NO exerts a tonic enhancing effect on human cardiac vagal control. The site of action cannot be determined from our results, but animal data suggest that this may be due to actions of NO synthesized within neurons at a number of sites. NO has been shown to increase neuronal activity within the nucleus tractus solitarius (the primary relay site for baroreceptor afferent fibers), as well as enhancing the activity of medullary vagal motoneurons.\textsuperscript{7} In the efferent limb of the reflex, NO has been shown to enhance the bradycardic effects of efferent vagal and muscarinic stimulation.\textsuperscript{6} “Indirect” vagal activity (the ability of the efferent vagus to antagonize sympathetic cardiac responses) is also enhanced by NO.\textsuperscript{23}

In vitro studies have shown that NO may also have direct nonneurally mediated effects on the sinus node, increasing heart rate by stimulating the I\textsubscript{f} current.\textsuperscript{8} In our study the NO donor (sodium nitroprusside) caused less tachycardia than the non–NO-dependent agent hydralazine, while inhibition of NO synthesis caused less bradycardia than the α\textsubscript{1}-adrenergic agonist phenylephrine. Thus, any direct effects of NO on the sinus node in humans would appear to have been overwhelmed by opposing effects of NO on cardiac autonomic control.

There are a number of limitations to our study. It is not possible for us to be certain that the stimulus to the baroreceptor was identical between our study drugs and their
controls. We chose to use mean arterial pressure to control for baroreceptor stimulus because there is good evidence that this value (rather than pulse pressure or rate of pressure change) is the main determinant of baroreceptor discharge. Furthermore, we did not measure central aortic pressure directly but assessed blood pressure in the upper limb. However, the avoidance of invasive procedures permitted a more accurate estimation of resting cardiac autonomic tone.

It is also possible that the vasoactive drugs we used may exert different mechanical effects on the arterial baroreceptors. In dogs phenylephrine was found to constrict carotid arteries, resulting in augmentation of the baroreceptor response. In dogs phenylephrine was found to constrict carotid arteries, resulting in augmentation of the baroreceptor response. Furthermore, there was no significant difference in baroreceptor firing whether intraluminal pressure was raised by phenylephrine, angiotensin II, or an aortic balloon, and Hirooka et al showed that the baroreceptor response was independent of the effects of drugs on arterial diameter. We therefore believe that it is unlikely that any mechanical effects on arterial baroreceptors could explain our results.

Clinical Implications
Our study provides the first demonstration that both endogenous and exogenous NO modulate baroreflex-mediated cardiac vagal control in humans, as indicated by measures of HRV and BRS. The clinical relevance of this finding is emphasized by the clear demonstration that both of these measures of cardiac autonomic control are of prognostic importance in patients with cardiac disease. Further work is required to assess whether these prognostic markers can be favorably influenced by manipulation of the NO pathway in patients with cardiac disease. Finally, until recently it was common to assess BRS in humans with the use of nitrovasodilators to unload the reflex. Our data raise doubts about the results obtained by this method because of the possibility of a direct autonomic effect of the NO donor itself.

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