

Does nitric oxide buffer arterial blood pressure variability in humans?

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Cooke, William H., Rong Zhang, Julie H. Zuckerman, Jian Cui, Thad E. Wilson, Craig G. Crandall, and Benjamin D. Levine. Does nitric oxide buffer arterial blood pressure variability in humans? *J Appl Physiol* 93: 1466–1470, 2002. First published July 12, 2002; 10.1152/jappphysiol.00287.2002.—Animal studies suggest that nitric oxide (NO) plays an important role in buffering short-term arterial pressure variability, but data from humans addressing this hypothesis are scarce. We evaluated the effects of NO synthase (NOS) inhibition on arterial blood pressure (BP) variability in eight healthy subjects in the supine position and during 60° head-up tilt (HUT). Systemic NOS was blocked by intravenous infusion of *N*^G-monomethyl-L-arginine (L-NMMA). Electrocardiogram and beat-by-beat BP in the finger (Finapres) were recorded continuously for 6 min, and brachial cuff BP was recorded before and after L-NMMA in each body position. BP and R-R variability and their transfer functions were quantified by power spectral analysis in the low-frequency (LF; 0.05–0.15 Hz) and high-frequency (HF; 0.15–0.35 Hz) ranges. L-NMMA infusion increased supine BP (systolic, 109 ± 4 vs. 122 ± 3 mmHg, *P* = 0.03; diastolic, 68 ± 2 vs. 78 ± 3 mmHg, *P* = 0.002), but it did not affect supine R-R interval or BP variability. Before L-NMMA, HUT decreased HF R-R variability (*P* = 0.03), decreased transfer function gain (LF, 12 ± 2 vs. 5 ± 1 ms/mmHg, *P* = 0.007; HF, 18 ± 3 vs. 3 ± 1 ms/mmHg, *P* = 0.002), and increased LF BP variability (*P* < 0.0001). After L-NMMA, HUT resulted in similar changes in BP and R-R variability compared with tilt without L-NMMA. Increased supine BP after L-NMMA with no effect on BP variability during HUT suggests that tonic release of NO is important for systemic vascular tone and thus steady-state arterial pressure, but NO does not buffer dynamic BP oscillations in humans.

cardiovascular control; head-up tilt; intrinsic rhythmicity

LOW-FREQUENCY ARTERIAL PRESSURE oscillations or “Mayer waves” were first described in 1877 (14), yet mechanisms underlying these low-frequency rhythms have remained elusive. Because the magnitude of systolic pressure fluctuations is directly associated with sever-

ity of end-organ damage (renal, heart, and brain) and subsequent cardiovascular complications (18), understanding mechanisms of arterial blood pressure oscillations assumes clinical importance.

In an effort to explain arterial pressure rhythms occurring at frequencies slower than respiration, at least two hypotheses have been advanced. First, because of intrinsic delays in effector responses to norepinephrine, increases of sympathetic nerve firings are manifested in cardiac and vascular responses with a time delay of ~10 s (28). With this construct, waxing and waning of arterial pressures around 0.1 Hz are likely driven by arterial baroreceptor input (9). Second, low-frequency arterial pressure rhythms have been recorded in patients with cervical spinal cord lesions (10) and in isolated mesenteric arteries (21), suggesting the possibility that the peripheral vasculature possesses intrinsic rhythmicity independent of baroreflex feedback loops. The notion that Mayer waves reflect vascular sympathetic outflow has recently been challenged (27), and therefore factors that may contribute directly to vascular rhythmicity warrant further investigation.

Increases and decreases of blood flow associated with changes in vascular shear stress activate and inhibit production of nitric oxide (NO), which directly affects peripheral vascular resistance (25). Inhibition of NO synthesis increases low-frequency arterial pressure variability in dogs (15) and rats (16), suggesting a primary role of NO in buffering changes of arterial pressure at the frequency of Mayer waves. Castellano and co-workers (3) tested the influence of NO inhibition in humans and reported reductions of low-frequency arterial pressure oscillations. However, such reductions might be expected if blockade of NO results in hypertension and subsequent sympathetic neural inhibition (25), as occurred in the study by Castellano et al. To our knowledge, the potential of NO to buffer arterial pressure oscillations in humans has not been resolved.

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Therefore, to better understand interactions between NO and arterial pressure oscillations in humans, we used passive head-up tilting to increase rather than decrease sympathetic nerve activity with systemic inhibition of NO synthase (NOS). We tested the hypothesis that inhibition of NOS with N^G -methyl-L-arginine (L-NMMA) increases arterial pressure oscillations at the low frequency on assumption of the upright posture.

METHODS

Subjects. We studied eight healthy subjects (6 men and 2 women; height 173 ± 4 cm, weight 73 ± 4 kg, age 31 ± 3 yr). All subjects were nonsmokers; refrained from caffeine, alcohol, and heavy exercise 24 h before the study; and had no history of cardiovascular or autonomic dysfunction. This study was conducted in accordance with the Declaration of Helsinki (1989) of the World Medical Association and was approved by the Institutional Review Boards of the University of Texas Southwestern Medical Center and the Presbyterian Hospital of Dallas. Each subject gave written, informed consent before participating.

Instrumentation. We continuously monitored heart rate with electrocardiography, beat-by-beat arterial pressure with finger photoplethysmography (Finapres, Ohmeda, Aurora, CO), brachial cuff pressure with standard sphygmomanometry (Suntech), and respiratory excursions with piezoelectric transducers (Pneumotrace, Morro Bay, CA). We positioned the Finapres transducer at heart level to avoid spurious recordings due to changes of hydrostatic pressure gradients during changes of posture. Beat-to-beat recordings of arterial pressure were used for spectral analysis and cuff pressures were used to compare absolute arterial pressures between experimental conditions.

Protocol. We studied all subjects in a quiet, temperature-controlled (25°C) laboratory in the morning, at least 2 h postprandial. After instrumentation, subjects rested quietly in the supine position for at least 30 min. We collected 6 min of data with subjects in the supine position. The subjects were then passively tilted to a 60° head-up position. After 2 min of stabilization, we collected 6 min of data during tilt. The subjects were then returned to the supine position for a recovery of ~ 45 min. After this test, systemic NOS was blocked by intravenous infusion of L-NMMA with a loading dose of 5 mg/kg for 15 min and a maintenance dose of $5 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ through the study. Previous work has demonstrated stable blood levels and sustained NOS inhibition with use of this regimen in human subjects (13). After 30 min of L-NMMA infusion, 6 min of data were collected in the supine position, followed by a repeat of the 60° head-up-tilt protocol (2-min stabilization, 6-min data collection).

Data analysis. Electrocardiogram and arterial pressure waveforms were sampled at 1 kHz and digitized at 12 bits with an analog-to-digital converter (Das-20, Metrabyte). Respiratory excursions were sampled simultaneously with electrocardiogram and arterial pressure signals at 10 Hz. Digitized signals were stored on a laboratory computer and processed with a customized software program for R wave and systolic and diastolic pressure detection. Beat-to-beat R-R interval and systolic and diastolic pressures were linearly interpolated and resampled at 2 Hz for spectral analysis. The time series of R-R interval and systolic and diastolic pressures were first trend eliminated with third-order polynomial fitting and then subdivided into 256-point segments with 50% overlap for spectral estimation. Fast Fourier trans-

forms were then implemented with each Hanning-windowed data segment and then averaged to calculate auto-spectra, cross-spectra, coherence, and transfer functions.

Spectral powers of R-R interval and systolic and diastolic pressures were calculated in the low- (0.05–0.15 Hz) and high- (0.15–0.35 Hz) frequency ranges by integrating the corresponding auto-spectra. We calculated the transfer function by dividing the cross-spectra of R-R interval and systolic pressure by the power spectra of the input signal (systolic pressure) and used the magnitude of transfer function as an estimate of spontaneous arterial baroreflex gain.

Statistics. Mean values for dependent variables of interest were examined with a 2 (condition; drug vs. no drug) \times 2 (position; supine vs. tilt) factorial analysis of variance with repeated measures on both factors. In the event of significant interactions, we decided a priori to probe further with simple main effects analysis. We considered differences to be significant when $P \leq 0.05$.

RESULTS

Inhibition of NOS with L-NMMA infusion in the supine position significantly increased systolic ($P = 0.03$) and diastolic ($P = 0.002$) pressures but did not affect R-R interval, transfer function gain, or blood pressure variabilities.

Passive 60° head-up tilting significantly decreased R-R intervals ($P = 0.0001$), R-R interval variability at the high frequency ($P = 0.03$), and transfer function gain between systolic pressure and R-R interval at both the low ($P = 0.007$) and high ($P = 0.002$) frequencies. Tilting significantly increased systolic and diastolic pressure variabilities at both low and high frequencies (systolic: low-frequency power, $P = 0.0001$ and high-frequency power, $P = 0.049$; diastolic: low-frequency power, $P = 0.003$ and high-frequency power, $P = 0.02$) but did not affect mean cuff pressures or the coherence between systolic pressure and R-R interval. Passive 60° head-up tilting after inhibition of NOS had no additional effect on any of the variables we measured compared with the responses to head-up tilt without L-NMMA. Specifically, systolic arterial pressure oscillations at the frequency of Mayer waves increased similarly with head-up tilt both before and after L-NMMA infusion ($P = 0.43$).

Figure 1 shows representative arterial pressure oscillations with tilt before and after L-NMMA infusion. Numerical results for all dependent variables of interest across all measurement conditions are shown in Table 1.

DISCUSSION

We tilted subjects to a 60° head-up position before and after systemic inhibition of NOS to test the hypothesis that NO contributes importantly to the dynamic regulation of arterial pressure rhythms. Our two primary findings are 1) L-NMMA infusion increases arterial pressure in the supine position, suggesting that either central or direct modulation of vascular tone by NO contributes to steady-state arterial pressure control, and 2) L-NMMA infusion has no effect on arterial pressure oscillations either supine or during tilt, suggesting that NO contributes minimally, if at

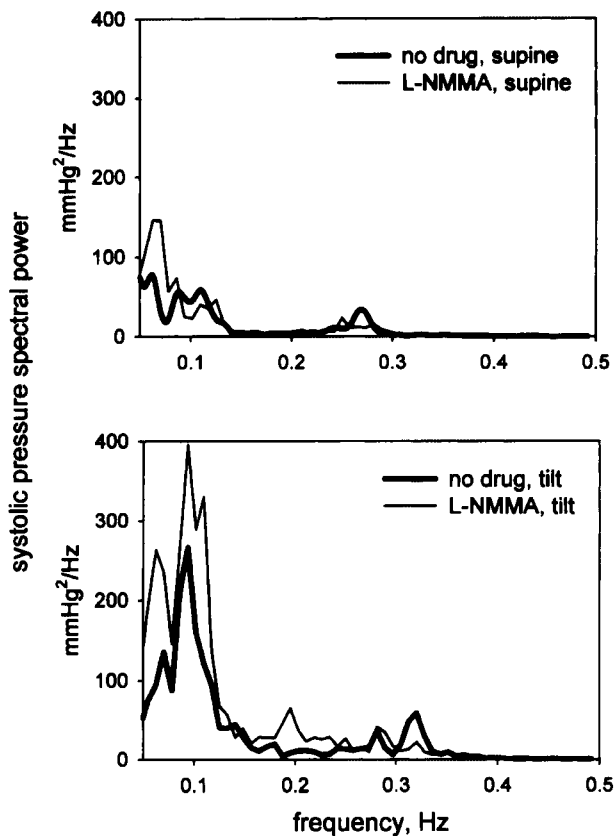


Fig. 1. Systolic pressure oscillations for 1 representative subject during supine (*top*) and head-up-tilt (*bottom*) measurements. L-NMMA, *N*^G-monomethyl-L-arginine.

all, to the modulation of low-frequency blood pressure rhythms.

Effects of NO on supine, resting humans. Afferent and efferent signals controlling arterial pressure derive, initially, from prevailing cardiac output and peripheral vascular resistance. Because humans exist in

a “closed-loop” environment, it is not possible to know with certainty which signal precedes which, but it is clear that numerous mechanisms controlling either cardiac output and/or peripheral resistance generate and control steady-state arterial pressure and arterial pressure oscillations. In addition to reflex autonomic neural control, vascular tone is determined importantly by local factors, including tonic release of endothelium NO, which is a powerful vasodilator (6). NO is produced by the enzyme NOS, whose precursor is L-arginine, and can be blocked by analogs of L-arginine, including L-NMMA. In the present study, inhibition of NOS with L-NMMA significantly increased supine arterial pressures, confirming a role for NO in the control of steady-state pressure. Others have reported similar results (11, 12). Of note, significant changes of arterial pressures after L-NMMA infusion in the present study did not translate into changes of vagal-cardiac control or arterial baroreflex sensitivity, as estimated by R-R interval oscillations at the high frequency and transfer function analysis between systolic pressures and R-R intervals. The coherence function, which reflects the strength of the linear association between input and output signals at specific frequencies, was significant [by convention ≥ 0.50 (8)] before and after L-NMMA infusion (Table 1), suggesting that inhibition of NO does not uncouple the usual relation between systolic pressure R-R interval responses. R-R intervals were unchanged despite the significant increases of steady-state arterial pressure after L-NMMA, which may reflect a central influence of NO (29) resulting in rapid baroreflex resetting. The notion of rapid resetting of arterial baroreflexes is not without precedent. For example, carotid-cardiac baroreflex gain is unchanged during exercise despite increases of carotid sinus pressure (20).

Our results confirm that NO contributes importantly to maintenance of supine, steady-state arterial pres-

Table 1. Cardiovascular responses to L-NMMA infusion in the supine and 60° head-up positions

Variable	Supine		Tilt	
	No drug	L-NMMA	No drug	L-NMMA
R-R interval, ms	1,045.5 ± 65.3	1,086.5 ± 63.6	689.5 ± 83.0†	734.1 ± 35.0†
SP, mmHg	109.3 ± 4.0	121.6 ± 3.0*	124.4 ± 6.7	126.9 ± 5.1
DP, mmHg	67.7 ± 2.3	78.3 ± 3.0*	62.7 ± 2.9	88.1 ± 3.2*
R-RLFP, ms ²	1,122.4 ± 306.4	851.3 ± 201.3	640.8 ± 105.7	1,044.0 ± 158.3
R-RHFP, ms ²	638.3 ± 220.1	541.8 ± 171.7	95.7 ± 47.1†	129.0 ± 43.1†
SPLFP, mmHg ²	8.6 ± 3.3	5.3 ± 1.3	19.2 ± 4.1†	18.7 ± 3.2†
SPHFP, mmHg ²	1.0 ± 0.2	0.8 ± 0.2	5.8 ± 2.1†	3.6 ± 1.0†
DPLFP, mmHg ²	4.6 ± 1.5	2.8 ± 0.6	13.5 ± 3.4†	13.6 ± 2.9†
DPHFP, mmHg ²	0.3 ± 0.1	0.3 ± 0.1	1.1 ± 0.3†	0.8 ± 0.2†
CohLF	0.6 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
CohHF	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
TFLF, ms/mmHg	11.7 ± 1.7	13.7 ± 2.7	5.1 ± 0.4†	7.0 ± 0.8†
TFHF, ms/mmHg	18.0 ± 2.9	20.0 ± 3.3	3.2 ± 0.5†	4.9 ± 0.8†

Values are means ± SE. L-NMMA, *N*^G-monomethyl-L-arginine; SP, systolic pressure; DP, diastolic pressure; R-RLFP and R-RHFP, R-R interval spectral power at the low and high frequency; SPLFP and SPHFP, systolic pressure spectral power at the low and high frequency; DPLFP and DPHFP, diastolic pressure spectral power at the low and high frequency; CohLF and CohHF, systolic pressure-to-R-R interval coherence at the low and high frequency; TFLF and TFHF, transfer function gain between systolic pressure and R-R interval at the low and high frequency. *Significantly different from corresponding no-drug value, $P \leq 0.05$. †Significantly different from corresponding supine value, $P \leq 0.05$. No significant tilt × drug interactions were detected.

sure at rest but provide evidence against a significant regulatory effect on arterial pressure rhythms. As suggested by others (25), the pressor effect of NO inhibition likely decreases sympathetic nerve traffic, which in turn is associated with blunted arterial pressure oscillations (17). To confirm whether NO contributes to low-frequency arterial pressure rhythms, it is necessary to maintain high levels of sympathetic neural activation. To accomplish this, we tilted our subjects to a 60° head-up position.

Effects of NO during 60° head-up tilt. Moving from the supine to standing position causes a large portion of the circulating blood volume to move below the level of the heart, and this volume must be pumped against a hydrostatic gradient to maintain arterial pressure and cerebral perfusion. Accordingly, compensatory mechanisms have evolved such as venous backflow valves, skeletal and respiratory muscle pumps, and autonomic reflexes that respond to reductions in ventricular stroke volumes (and thereby changes in arterial pulse amplitude) by increasing heart rate and peripheral vascular resistance secondary to vagal withdrawal and sympathetic stimulation. We found, as others have before (7, 26), that passive head-up tilting decreases high-frequency R-R interval spectral power and transfer function gain at low and high frequencies. These data are consistent with a general withdrawal of vagal neural control during tilt.

We did not observe detectable alterations of baroreflex function, and cardiac rate seemed to increase appropriately with head-up tilt after L-NMMA infusion. These results conflict with those of Spieker et al. (23), who documented a lack of compensatory tachycardia during orthostatic stress after infusion of L-NMMA in healthy young subjects. The apparent vagal activation during orthostatic stress in the study by Spieker et al. was not observed in our subjects: R-R intervals and R-R interval spectral power at the high frequency decreased similarly during head-up tilt with and without L-NMMA (Table 1). However, Spieker et al. induced an orthostatic challenge with lower body negative pressure of only -30 mmHg. Fluid shifts and hemodynamic changes similar to those seen with 60° head-up tilt are achieved with negative pressures above approximately -50 mmHg (19), and therefore estimates of vagal control from data in the present study and the study by Spieker et al. may not be comparable.

Withdrawal of vagal and increases of sympathetic outflow during standing are associated with increases of arterial pressure oscillations at frequencies around 0.1 Hz (7), and therefore head-up tilting lends insight into mechanisms that generate and control autonomic rhythms at the frequency of Mayer waves. Although the genesis and control of Mayer waves are not understood completely, evidence suggests the presence of at least two redundant systems that may function either independently or in concert. First, it seems clear that low-frequency arterial pressure rhythms are generated, in part, by baroreceptor activation and inhibition of sympathetic nerve activity (17, 28) and mediated through changes of peripheral vascular resistance (5)

[although sympathetic nerve activity does not necessarily predict arterial pressure fluctuations (27)]. Second, some, but not all, quadriplegic patients studied by Guzzetti et al. (10) displayed low-frequency arterial pressure rhythms. Rizzoni et al. (21) demonstrated spontaneous low-frequency vasomotor oscillations in isolated mesenteric arteries, and Zhang et al. (30) documented the presence of low-frequency arterial pressure oscillations after complete ganglion blockade with trimethaphan. These data suggest that intrinsic peripheral vascular rhythmicity may contribute to the genesis of Mayer waves. Because NO has a half-life of ~6 s (25), it is possible that vascular rhythmicity at the low frequency is mediated by production and inhibition of NO.

It could be argued that head-up tilting maximizes arterial pressure oscillations such that further increases with NO inhibition are not physiologically possible. To ensure that further increases of arterial pressure oscillations during head-up tilt are theoretically possible (and therefore that potential modulating effects of NO are detectable), we only tilted our subjects to a 60° head-up position. Arterial pressure spectral power increases linearly with progressive head-up tilt and is not maximized at 60° (7). In the present study, low-frequency arterial pressure oscillations were statistically identical during tilt with and without NO inhibition. If NO contributes significantly to the regulation of Mayer waves via control of peripheral vascular tone, we should have observed detectable alterations of low-frequency arterial pressure rhythms after L-NMMA during tilt. Although we cannot say whether potential contributions by NO are too small to be detected with our analyses, or whether other mechanisms, including the prevailing autonomic balance between vagal and sympathetic activities override or obscure direct vascular effects of NO, our data do not support the hypothesis that NO plays a critical role in buffering dynamic changes of arterial pressure in humans.

We could not distinguish central vs. peripheral effects of L-NMMA or directly confirm NOS inhibition in this study. However, others have confirmed sustained inhibition of NOS with identical infusion protocols (13). Spieker et al. (23) and Sander et al. (22) documented significant increases of supine blood pressures, and Stamler et al. (24) showed reductions of serum NO levels of 65% with L-NMMA concentrations lower than those used in the present study. The pressor response we observed in our subjects in the supine position indicates the efficacy of our infusion protocol. However, lack of specific confirmation of NOS inhibition with direct measurements limits interpretation of our results. We cannot attribute the lack of alteration of Mayer waves after L-NMMA infusion specifically to endothelial mechanisms. Although NO clearly regulates peripheral resistance vessel tone directly at the endothelium (24), NO also directly affects cardiac contractility (2, 23) and is localized at specific neuronal sites throughout the central nervous system (1, 22),

including postganglionic parasympathetic and preganglionic sympathetic neurons (4).

Summary. Inhibition of NOS increases arterial pressure oscillations at low frequencies in rats (16) and dogs (15) and increases steady-state arterial pressures in humans (11, 12). On the basis of this evidence and evidence that the peripheral vasculature seems to possess intrinsic rhythmicity (10, 21, 30), we tested the hypothesis that NO contributes importantly to the control of arterial pressure rhythms in humans. Our data confirm a primary role for NO in maintaining stable arterial pressures at rest but provide evidence against the notion that dynamic oscillations of pressure, including naturally occurring rhythms at the frequency of Mayer waves, depend critically on tonic NO activity.

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