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Inhibition of Nitric Oxide Inhibition of Nitric Oxide Synthase Does Not Alter Dynamic Cerebral Autoregulation in Humans

Rong Zhang
UT Southwestern Medical Center

Thad E. Wilson
UT Southwestern Medical Center

Sarah Witkowski
UT Southwestern Medical Center, switkowski@smith.edu

Jian Cui
UT Southwestern Medical Center

Craig G. Crandall
UT Southwestern Medical Center

See next page for additional authors

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Inhibition of nitric oxide synthase does not alter dynamic cerebral autoregulation in humans

Rong Zhang, Thad E. Wilson, Sarah Witkowski, Jian Cui, Craig G. Crandall, and Benjamin D. Levine
Institute for Exercise and Environmental Medicine, Presbyterian Hospital of Dallas, Dallas 75231; and University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75235

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Zhang, Rong, Thad E. Wilson, Sarah Witkowski, Jian Cui, Craig G. Crandall, and Benjamin D. Levine. Inhibition of nitric oxide synthase does not alter dynamic cerebral autoregulation in humans. *Am J Physiol Heart Circ Physiol* 286: H863–H869, 2004. First published November 6, 2003; 10.1152/ajpheart.00373.2003.—The aim of this study was to determine whether inhibition of nitric oxide synthase (NOS) alters dynamic cerebral autoregulation in humans. Beat-to-beat blood pressure (BP) and cerebral blood flow (CBF) velocity (transcranial Doppler) were measured in eight healthy subjects in the supine position and during 60° head-up tilt (HUT). NOS was inhibited by intravenous N^G-monomethyl-L-arginine (L-NMMA) infusion. Dynamic cerebral autoregulation was quantified by transfer function analysis of beat-to-beat changes in BP and CBF velocity. Pressor effects of L-NMMA on cerebral hemodynamics were compared with those of phenylephrine infusion. In the supine position, L-NMMA increased mean BP from 83 ± 3 to 94 ± 3 mmHg ($P < 0.01$). However, CBF velocity remained unchanged. Consequently, cerebrovascular resistance index (CVRI) increased by 15% ($P < 0.05$). BP and CBF velocity variability and transfer function gain at the low frequencies of 0.07–0.20 Hz did not change with L-NMMA infusion. Similar changes in mean BP, CBF velocity, and CVRI were observed after phenylephrine infusion, suggesting that increase in CVRI after L-NMMA was mediated myogenically by increase in arterial pressure rather than a direct effect of cerebrovascular NOS inhibition. During baseline tilt without L-NMMA, steady-state BP increased and CBF velocity decreased. BP and CBF velocity variability at low frequencies increased in parallel by 277% and 217%, respectively ($P < 0.05$). However, transfer function gain remained unchanged. During tilt with L-NMMA, changes in steady-state hemodynamics and BP and CBF velocity variability as well as transfer gain and phase were similar to those without L-NMMA. These data suggest that inhibition of tonic production of NO does not appear to alter dynamic cerebral autoregulation in humans.

cerebral blood flow; head-up tilt; transcranial Doppler

SYSTEMIC INHIBITION of nitric oxide synthase (NOS) increases vasomotor tone and arterial pressure in humans under steady-state conditions (19). In addition, inhibition of NOS with L-arginine analogs, or in NOS gene-deficient mice, provokes large beat-to-beat blood pressure fluctuations at frequencies between 0.1 and 0.6 Hz (22, 23). These data suggest that besides the steady-state pressor effects, NO may also modulate vasomotor tone, dynamically responding to transient changes in blood pressure and/or flow, and thus may contribute to the stability of the systemic circulation (23). However, whether this function is physiologically significant in humans, and especially in the cerebral circulation, is unknown.

In humans, cerebral blood flow (CBF) velocity in the basal cerebral arteries fluctuates spontaneously, similar to arterial pressure (8, 14, 16, 36). In addition, the cerebral vasculature appears to buffer changes in CBF induced by transient changes in arterial pressure under dynamic conditions (1, 16, 36). This ability of the cerebrovascular bed has been referred to as "dynamic cerebral autoregulation." In contrast, the traditional concept of cerebral autoregulation, which emphasizes a relatively constant CBF despite large changes in arterial pressure under steady-state conditions, has been referred to as "static cerebral autoregulation" (17).

Thus, theoretically, if tonic production of NO plays an obligatory role in buffering changes in CBF induced by transient changes in pressure, dynamic cerebral autoregulation would be impaired by NOS inhibition. A recent study of dynamic cerebral autoregulation during acute hypotension induced by thigh cuff deflation suggested the presence of this regulatory mechanism (34).

To extend these previous observations, the present study examined dynamic cerebral autoregulation from spontaneous fluctuations in arterial pressure and CBF velocity. NOS activity was inhibited by intravenous infusion of N^G-monomethyl-L-arginine (L-NMMA). Dynamic cerebral autoregulation was quantified by transfer function analysis of beat-to-beat changes in arterial pressure and CBF velocity (8, 14, 16, 21, 36). We hypothesized that inhibition of tonic production of NO would alter dynamic cerebral autoregulation in humans.

METHODS

Eight healthy subjects (6 men, 2 women) with mean age of 31 ± 3 yr, mean height of 173 ± 4 cm, and mean weight of 73 ± 4 kg voluntarily participated in this study. No subject smoked or had known medical problems. Subjects were screened carefully with a medical history and a physical examination with 12-lead ECG. All subjects signed an informed consent form approved by the Institutional Review Boards of the University of Texas Southwestern Medical Center and Presbyterian Hospital of Dallas.

Beat-to-beat arterial pressure was measured with finger photoplethysmography (Finapres). The pressure transducer was positioned carefully at the heart level both in the supine position and during 60° head-up tilt and corroborated with the measurement of brachial artery pressure with a sphygmomanometer (Suntech). Beat-to-beat pressure recordings were used to quantify pressure variability. Intermittent brachial pressure recordings were used to quantify steady-state pressure and to estimate both systemic and cerebrovascular resistance.

CBF velocity was measured in the middle cerebral artery (MCA) by transcranial Doppler ultrasonography. The optimal Doppler signal was obtained according to standard techniques with the sample

Address for reprint requests and other correspondence: B. D. Levine, Institute for Exercise and Environmental Medicine, Presbyterian Hospital of Dallas, 7232 Greenville Ave., Dallas, TX 75231 (E-mail: benjaminlevine@texashealth.org).

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volume adjusted to the proximal segment of the MCA. A 2-MHz probe (Multiflow, DWL) was placed over the subject's temporal window and fixed at a constant angle with a probe holder custom made to fit each subject's facial bone structure. This approach ensured that the exact same probe angle, the same segment of the MCA, and the same instrument settings were used in all repeat studies (6).

Heart rate was monitored continuously by ECG. Respiratory excursions were monitored via a piezoelectric transducer (Pneumotrace, UFI; Morro Bay, CA). End-tidal CO_2 ($ETCO_2$) was measured with a nasal cannula interfaced with a mass spectrometer (Marquette Electronics). Cardiac output was measured by using a standard foreign gas rebreathing technique with acetylene as the soluble and helium as the insoluble gas (28). Adequate mixing of the rebreathing gas in the lung was confirmed by a constant level of helium in all cases. Stroke volume was calculated as cardiac output divided by the heart rate during rebreathing. Total peripheral vascular resistance was calculated as mean arterial pressure divided by cardiac output.

Experimental protocols. All experiments were performed in the morning at least 2 h after a light breakfast. The subjects were asked to refrain from heavy exercise and caffeinated or alcoholic beverages for at least 24 h before the tests. After at least 30 min of supine rest, cardiac output was measured, followed by a 6-min period of data collection during which the subject breathed spontaneously. The subject was then tilted passively to the 60° head-up position. After 2 min for stabilization, 6 min of data was collected, followed by a measurement of cardiac output during tilt. The subject was then returned to the supine position for a recovery period of ~45 min.

After the recovery, NOS was inhibited by intravenous infusion of L-NMMA with a loading dose of 5 mg/kg for 15 min, followed by a maintenance dose of 50 $\mu g \cdot kg^{-1} \cdot min^{-1}$ throughout the duration of the ~45-min study. Previous work demonstrated stable blood concentrations of L-NMMA and sustained inhibition of NOS with this regimen in healthy humans (15, 20). After 30 min of L-NMMA infusion, two measurements of cardiac output were obtained and averaged. A 6-min period of data collection followed in the supine position. Head-up tilt and data collection were then repeated in the presence of L-NMMA as outlined above.

On a separate day at least 1 mo from the experiments with L-NMMA, low-dose phenylephrine (0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$), an α_1-agonist with no direct cerebral vasoactive effects (4), was infused in the same group of subjects to account for the pressor effect of L-NMMA on cerebral hemodynamics in the supine position. Data collection was conducted before and after 5 min of phenylephrine infusion, when the increases in arterial pressure were stabilized at a level equivalent to those during L-NMMA infusion.

Data analysis. Steady-state arterial pressure was obtained from the average of at least three measurements of brachial pressure under each experimental condition. Heart rate, CBF velocity, and $ETCO_2$ were obtained from the average of the 6-min data segments. A "cerebrovascular resistance index (CVRI)" in the supine position was calculated by dividing mean blood pressure by mean CBF velocity to estimate relative changes in cerebrovascular resistance (1). It should be noted that although the hydrostatic component of arterial pressure at the head level must be reduced during head-up tilt relative to the supine position (8), whether intracranial and cerebral venous pressure are reduced commensurately is not completely understood (18). Given these uncertainties, changes in cerebrovascular resistance during tilt were not estimated in the present study. However, we have considered that the magnitude of beat-to-beat changes in mean arterial pressure during head-up tilt should reflect that of changes in cerebral perfusion pressure (8, 21, 35).

The magnitude of beat-to-beat changes in mean arterial pressure and CBF velocity was quantified by Fourier spectral analysis. The transfer function between these variables was calculated to assess dynamic cerebral autoregulation as described previously (36). Briefly, the estimates of transfer function gain were used to quantify the ability of the cerebrovascular bed to buffer changes in CBF velocity induced by transient changes in arterial pressure at different frequencies. The phase spectrum was estimated to reflect the temporal relationship between these variables. In addition, to account for the effects of steady-state changes in arterial pressure and CBF velocity on the transfer function gain estimation in the supine position, a normalized transfer function gain was derived as a product of the estimated transfer function gain and the CVRI under these conditions (21). Finally, the coherence function between changes in arterial pressure and CBF velocity was estimated to assess the linear correlation between these variables.

Spectral power of arterial pressure and CBF velocity were calculated in the very low (0.02–0.07 Hz), low (LF, 0.07–0.20 Hz), and high (HF, 0.20–0.35 Hz) frequency ranges. Mean values of transfer function gain and phase were calculated in the LF and HF ranges. The selection of these frequencies was based on estimates of coherence function >0.5 and the unique features of the dynamic pressure-CBF velocity relationship in these ranges as described previously (8, 36).

Statistics. The data were examined by using a two-way repeated-measures ANOVA with tilt and L-NMMA as experimental factors (SigmaStat, SPSS). When statistically significant differences were observed, a further post hoc comparison was conducted by using the Student-Newman method. Comparisons between the two baselines before L-NMMA and phenylephrine infusion were conducted by using a paired test. Because no significant change was observed between the two baseline measurements, comparisons between baseline (before L-NMMA infusion), L-NMMA infusion, and phenylephrine infusion were performed by using one-way repeated-measures ANOVA. Data are expressed as means ± SE. The significance level was set at $P < 0.05$.

RESULTS

Steady-state hemodynamics. In the supine position, infusion of L-NMMA increased mean arterial pressure significantly by 13%. However, CBF velocity remained unchanged. Consequently, CVRI increased significantly by 15% (Fig. 1, Table 1). Cardiac output and stroke volume did not change, and there was a trend toward an increase in total peripheral vascular resistance (TPR) ($P = 0.07$) (Table 1). Of note, changes in blood pressure, CBF velocity, and CVRI after L-NMMA were virtually identical to those with phenylephrine infusion (Fig. 1).

During baseline tilt without L-NMMA, mean arterial pressure increased because of an increase in diastolic pressure. CBF velocity decreased, associated with a reduction in $ETCO_2$ (Table 1). Cardiac output and stroke volume were reduced by 17% and 38%, respectively, associated with a significant increase in TPR by 42% (Table 1). Head-up tilt in the presence of L-NMMA induced changes in both systemic and cerebral hemodynamics similar to those during tilt without L-NMMA (Table 1).

Beat-to-beat data analysis. In the supine position, infusion of L-NMMA did not change the spectral power of arterial pressure and CBF velocity variability at all frequencies (Fig. 2, Table 2), nor did it change the transfer function gain at the low frequencies. However, transfer function gain at the high frequencies decreased significantly by 19% (Fig. 3, Table 2). The normalized gain remained unchanged (LF: 1.42 ± 0.16 vs. 1.22 ± 0.13, $P = 0.34$; HF: 1.37 ± 0.11 vs. 1.46 ± 0.09, $P = 0.44$). There was a trend toward decreases in phase at the low frequencies after L-NMMA ($P = 0.13$, power = 0.51; Fig. 3,

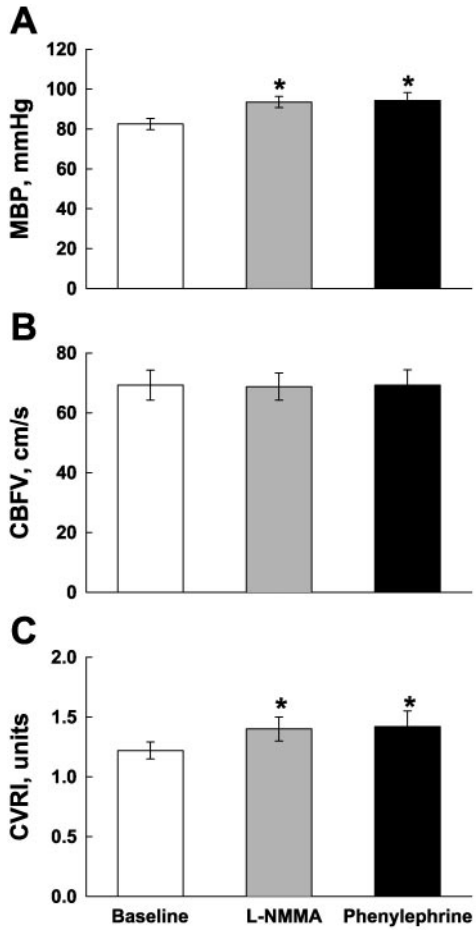

Fig. 1. Steady-state mean blood pressure (MBP; *A*), cerebral blood flow velocity (CBFV; *B*), and "cerebrovascular resistance index" (CVRI = MBP/CBFV, units = mmHg·s·cm^{-1}; *C*) obtained in the supine position at baseline, after N^G-monomethyl-L-arginine (L-NMMA) infusion, and after phenylephrine infusion. Values are means ± SE; $n = 8$. *$P < 0.05$ compared with baseline.

Table 2). Of note, no significant difference in transfer function gain, phase, or coherence was observed after phenylephrine infusion compared with those after L-NMMA (Fig. 4, Table 2).

During baseline tilt without L-NMMA, spectral power of arterial pressure and CBF velocity variability increased substantially at the low and high frequencies (Fig. 2, Table 2). Transfer function gain remained unchanged at the low frequencies, whereas it decreased significantly by 16% at the high frequencies (Fig. 3, Table 2). Similarly, there was a trend toward decreases in phase at the low frequencies during tilt associated with the significant increases in arterial pressure ($P = 0.06$, power = 0.72; Fig. 3, Table 2). Head-up tilt in the presence of L-NMMA induced changes in arterial pressure and CBF velocity variability as well as transfer function gain and phase similar to those during tilt without L-NMMA (Figs. 2 and 3, Table 2).

DISCUSSION

The primary new findings of the present study are twofold. *1*) We demonstrated for the first time that systemic inhibition of NOS does not alter CBF velocity variability even when the magnitude of these oscillations is significantly enhanced during head-up tilt. *2*) Transfer function gain between beat-to-beat changes in arterial pressure and CBF velocity at the low frequencies (<0.2 Hz) remained unchanged after L-NMMA infusion in the supine position and did not change during head-up tilt, although there was a trend toward decreases in phase at the low frequencies after L-NMMA in both positions, associated with the increases in arterial pressure. These data, in contrast to our hypothesis, suggest that inhibition of tonic production of NO does not alter dynamic cerebral autoregulation in humans.

CBF and NO. The role of NO in modulating the cerebral circulation has been studied extensively (5). Most studies in animals showed that inhibition of NOS reduces resting CBF and may attenuate CBF responses to hypercapnic stimuli (10, 30). These findings provide evidence that tonic production of NO (either endothelial or neuronal) modulates cerebral vasomotor tone. However, the data obtained in humans are rather limited and inconsistent. For example, in healthy subjects after intravenous bolus injection of L-NMMA, internal carotid CBF, measured by Doppler ultrasonography, was reduced significantly by 15% despite significant increases in arterial pressure (32). These findings were supported by a follow-up study from the same investigators using positron emission tomography to measure global CBF after L-NMMA (33). However, resting CBF after intravenous infusion of L-NMMA remained unchanged when measured by phase-contrast magnetic resonance imaging (29). Moreover, intracarotid infusion of nitroprusside (an exogenous NO donor) did not increase CBF in patients undergoing cerebral angiography, whereas infusion of L-NMMA reduced CBF by 20% (12, 13). The exact mechanism(s) underlying these discrepancies is difficult to delineate given the limitations of studies in humans regarding the temporal nature and extent of NOS inhibition in the cerebral circulation and the different methods used for CBF measurements (27).

In the present study, we observed that CBF velocity in the MCA remained unchanged after L-NMMA infusion, associated with significant increases in arterial pressure. In addition, we

Table 1. *Steady-state hemodynamics*

	Control		L-NMMA	
	Supine	Tilt	Supine	Tilt
HR, bpm	60±4	89±4*	57±5	84±4*
SBP, mmHg	112±4	119±4	122±3†	125±4†
DBP, mmHg	68±3	82±3*	80±3†	91±3*†
PBP, mmHg	44±3	37±3*	42±2	34±2*
MBP, mmHg	83±3	95±3*	94±3†	102±3*†
CBFV, cm/s	69±5	59±5*	69±5	61±4*
CO, liter	7.27±0.52	6.05±0.33*	7.54±0.76	6.37±0.45
SV, ml	102±7	63±4*	113±8	69±6*
TPR, dyn·s·cm^5	916±43	1,300±79*	1,033±77	1,302±76
$ETCO_2$, mmHg	38±1	35±1*	37±1	35±1*

Values are means ± SE; $n = 8$ subjects. HR, heart rate; SBP, systolic pressure; DBP, diastolic pressure; PBP, pulse pressure; CBFV, cerebral blood flow velocity; CO, cardiac output; SV, stroke volume; TPR, total peripheral vascular resistance; $ETCO_2$, end-tidal CO_2. *$P < 0.05$, between supine and tilt under the same N^G-monomethyl-L-arginine (L-NMMA) conditions; †$P < 0.05$, before and after L-NMMA in the same body position.

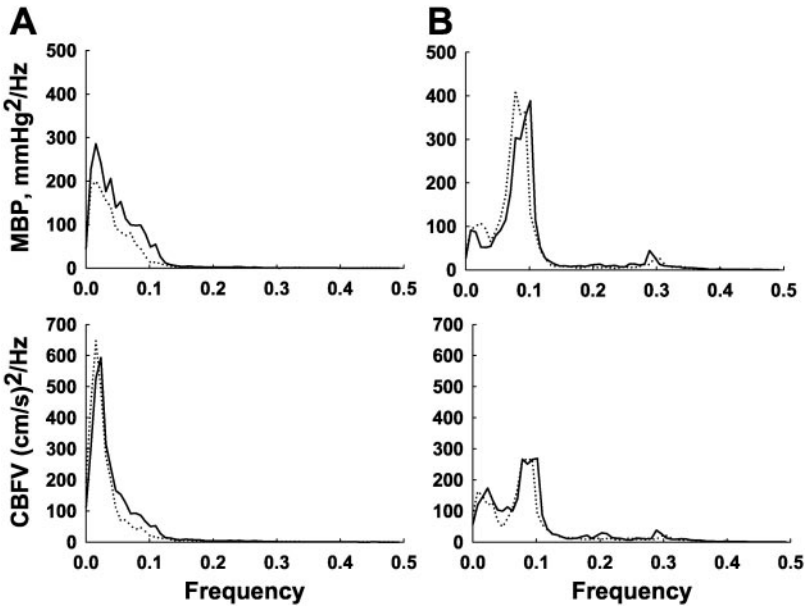

Fig. 2. Group-averaged spectra of MBP and CBFV variability in the supine position (*A*) and during tilt (*B*) before (solid line) and after (dotted line) L-NMMA.

found that with the same pressor effects of phenylephrine infusion, steady-state CBF velocity also remained unchanged and CVRI increased to the same degree as that with L-NMMA. These data suggest that the increase in CVRI after L-NMMA was mediated myogenically by an increase in arterial pressure (autoregulatory responses) rather than a direct effect of cerebrovascular NOS inhibition (17). Consequently, the absence of changes in CBF velocity observed in the present study may indicate the lack of change in resting CBF after L-NMMA (29). However, we cannot exclude the possibility that if infusion of L-NMMA induced significant vasoconstriction in the MCA as well as in the downstream resistance vessels of the cerebral circulation a true reduction of CBF may have been underestimated by the measurement of CBF velocity in the present study (32).

Cerebral autoregulation and NO. Under steady-state conditions, whether NO plays a role in static cerebral autoregulation is uncertain (11, 24, 25, 31). Studies in rats using laser Doppler flowmetry to measure CBF suggest that NO is an important mediator of cerebral vasodilation during hemorrhagic hypotension and that NOS inhibition shifts the lower limit of the autoregulatory curve to higher pressures (11, 26). However, measurement of CBF with either autoradiography (24) or the ^{133}Xe clearance method failed to support this conclusion (31). In addition, static cerebral autoregulation was not altered by intracarotid infusion of L-NMMA in primates (25).

Under dynamic conditions, the magnitude of oscillations in CBF and CBF velocity in rats was enhanced substantially by intravenous infusion of the NOS inhibitor N^{ω}-nitro-L-arginine methyl ester (3). These data suggest that tonic production of NO may attenuate oscillations in CBF induced by spontaneous changes in arterial pressure. Moreover, dynamic cerebral autoregulation in humans during acute hypotension induced by thigh cuff deflation was impaired after intravenous bolus injection of L-NMMA (34). These data, similar to the observations in peripheral vascular beds (23), indicate that NO may modulate cerebral vasomotor tone dynamically in response to transient changes in blood pressure and/or flow.

Table 2. *Spectral analysis of blood pressure and CBFV variability*

	Control		Phenylephrine	L-NMMA	
	Supine	Tilt	Supine	Supine	Tilt
MBP_{VLF}, $mmHg^2$	6.63±2.07	3.85±0.81	4.39±1.73	4.90±1.38	5.53±0.94
MBP_{LF}, $mmHg^2$	3.47±1.30	13.09±3.89*	1.55±0.57	1.61±0.43	11.44±3.03*
MBP_{HF}, $mmHg^2$	0.14±0.04	1.91±0.71*	0.25±0.09	0.17±0.05	1.33±0.39
$CBFV_{VLF}$, $(cm/s)^2$	8.75±2.22	4.99±1.34	5.46±1.69	6.72±2.44	4.16±0.88
$CBFV_{LF}$, $(cm/s)^2$	3.44±0.89	10.90±3.01*	2.13±0.85	1.81±0.37	8.76±2.58*
$CBFV_{HF}$, $(cm/s)^2$	0.31±0.05	2.10±0.59*	0.51±0.22	0.24±0.05	1.46±0.29*
$Gain_{LF}$, $cm \cdot s \cdot mmHg^{-1}$	1.00±0.09	0.96±0.05	0.98±0.06	1.00±0.08	0.91±0.07
$Gain_{HF}$, $cm \cdot s \cdot mmHg^{-1}$	1.22±0.09	1.03±0.07*	1.14±0.09	0.99±0.08†	0.95±0.07
$Phase_{LF}$, radians	0.76±0.07	0.55±0.04	0.68±0.06	0.59±0.08	0.49±0.06
$Phase_{HF}$, radians	0.22±0.09	0.23±0.04	0.22±0.16	0.21±0.10	0.21±0.05
$Coherence_{LF}$, units	0.65±0.07	0.77±0.03	0.56±0.07	0.54±0.07	0.70±0.06*
$Coherence_{HF}$, units	0.60±0.04	0.76±0.02*	0.60±0.05	0.56±0.05	0.68±0.06

Values are means ± SE; $n = 8$ subjects. VLF, very low frequency; LF, low frequency; HF, high frequency. *$P < 0.05$, between supine and tilt under the same L-NMMA conditions; †$P < 0.05$, before and after L-NMMA in the same body position.

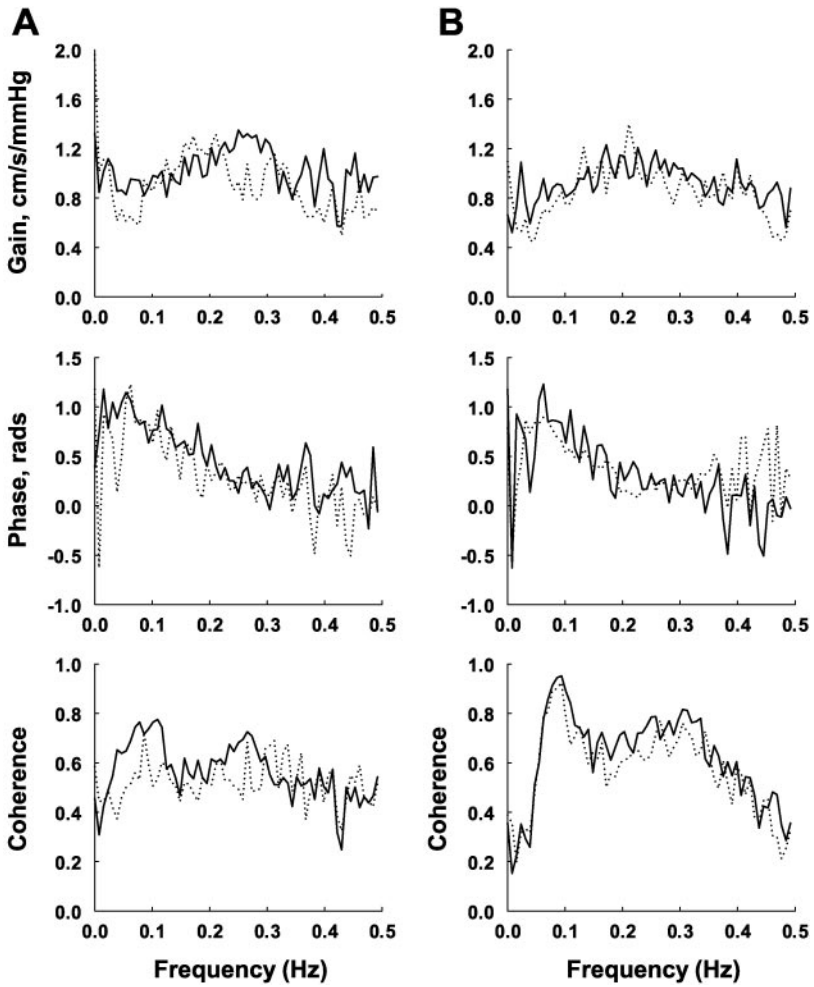

Fig. 3. Group-averaged transfer function gain, phase, and coherence function in the supine position (*A*) and during tilt (*B*) before (solid line) and after (dotted line) L-NMMA.

However, in the present study, we found that both arterial pressure and CBF velocity variability did not change after L-NMMA even when the magnitude of these oscillations was enhanced substantially during head-up tilt. In addition, transfer function gain between these variables at low frequencies (where autoregulation is likely to be most effective) remained unchanged after L-NMMA and did not change during head-up tilt. These data, in contrast to previous observations after bolus injection of L-NMMA (34), suggest that inhibition of tonic production of NO does not alter dynamic cerebral autoregulation in humans.

We contend that these discrepancies are not likely to be an artifact of differences in the pressure stimuli used in the present and previous studies. First, under steady-state conditions, the magnitudes of increases in mean arterial pressure in the supine position after L-NMMA infusion are similar in the present and previous studies (34). Second, the magnitude of hypotensive stimuli induced by the thigh cuff release maneuver in previous studies was only ~10 mmHg, which is well within the range of spontaneous changes in arterial pressure observed in the present study and reported by others (8, 14, 16, 21). Importantly, we demonstrated previously (36) that changes in CBF velocity during transient hypotension induced by the thigh cuff release maneuver could be predicted by the estimates of transfer function between the spontaneous changes in arterial pressure and CBF velocity. However, direct comparisons between the present and previous studies are difficult to make because of the different regimens used for L-NMMA infusion and the methods used for the quantification of dynamic cerebral autoregulation.

To reach the conclusions of the present study, several important issues regarding the transfer function gain and phase estimates must be addressed. First, we have considered that transfer function gain at the higher frequencies (>0.20 Hz) reflects mainly the impedance properties of the cerebrovascular bed. In contrast, transfer function gain at the lower frequencies likely reflects primarily the properties of dynamic cerebral autoregulation (36). Thus the reduction of transfer function gain at the high frequencies during tilt and after L-NMMA observed in the present study likely reflect increased cerebrovascular impedance under these conditions.

Second, in the present study, normalized transfer function gain during tilt was not calculated because of the uncertainty of changes in intracranial pressure, thereby compromising the estimates of cerebrovascular resistance under these conditions. However, because the magnitudes of increases in steady-state arterial pressure and changes in CBF velocity during tilt were similar before and after L-NMMA, any confounding effect of

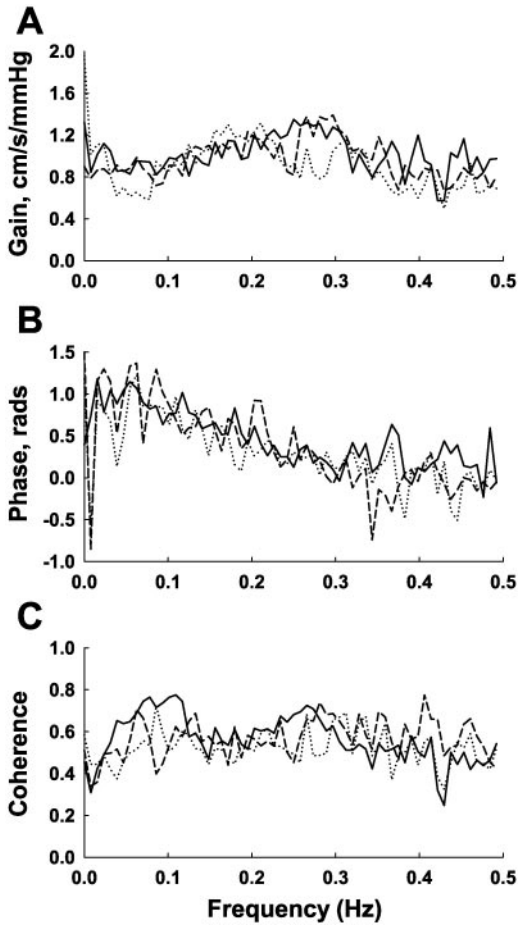

Fig. 4. Group-averaged transfer function gain (*A*), phase (*B*), and coherence function (*C*) in the supine position at baseline (solid line), after L-NMMA infusion (dotted line), and after phenylephrine infusion (dashed line).

the cerebrovascular resistance on the estimation of transfer function gain during tilt, if present, should be equivalent and thus should have minimal effect on the conclusions of this study.

Finally, we caution that with the small number of subjects relative to the large variability of phase estimates, the possibility of a phase change at the low frequencies after L-NMMA infusion cannot be excluded in the present study (Table 2). However, we suggest that the following observations argue against the possibility that L-NMMA has direct effects on the phase relationship between the spontaneous changes in arterial pressure and CBF velocity. First, the characteristics of spectral distribution of phase estimates at coherence function >0.5 were similar before and after L-NMMA both in the supine position and during head-up tilt (Fig. 3). Second, although there was a trend toward decreases in phase at the low frequencies after L-NMMA infusion, these changes were associated with significant increases in arterial pressure and similar to those observed after phenylephrine infusion (Table 2). In addition, the reduction in phase during tilt associated with hypertensive stimuli did not change in the presence of L-NMMA. Thus these data suggest that changes in phase with L-NMMA, if they did occur, are more likely mediated by the increases in arterial pressure rather than direct effects of L-NMMA on the phase estimates in the present study.

Study limitations. First, as with other studies in humans, the major limitation of this study is the uncertainty of the degree and the specificity of L-NMMA inhibition of NOS activity in the cerebral circulation (5, 9). The loading dose and maintenance dose of L-NMMA used were chosen carefully and were similar to the infusion regimen used by others, which documented sustained NOS inhibition in healthy humans (15, 20). The significant pressor effects of L-NMMA observed in the present study demonstrate the efficacy of NOS inhibition in the systemic circulation. We therefore suggest that tonic endothelium NO production in the cerebral circulation was inhibited similarly by systemic infusion of L-NMMA.

However, given the relatively small dose of L-NMMA used in our human subjects, we are not certain that neuronal NO released from the brain parenchyma and/or from the autonomic nerves innervating the cerebral blood vessels was also inhibited by systemic infusion of L-NMMA (5, 7, 27). At present, no data are available to demonstrate that L-NMMA passes the blood-brain barrier efficiently in humans. Thus the interpretation of the findings of the present study may be confounded by the presence of neuronal NO as well as other vasoactive factors interacting with the inhibition of endothelium NO in the cerebral circulation (5). However, recent studies in animals have demonstrated elegantly that it is the endothelium rather than neuronal NO that plays an important role in the control of resting CBF (2). Moreover, it has been shown that it is the endothelium rather than neuronal NO that likely responds to the biophysical signal of beat-to-beat changes in arterial pressure and/or flow and thus modulates vasomotor tone dynamically to maintain the stability of the systemic circulation (22, 23).

Second, because of uncertainties regarding changes in intracranial pressure during head-up tilt, we did not use phenylephrine infusion to account for the hypertensive effects of L-NMMA infusion during tilt. However, it should be emphasized that the primary purpose of head-up tilt in the present study was to enhance arterial pressure variability and to determine whether dynamic cerebral autoregulation would be altered by L-NMMA infusion under these conditions. In addition, although arterial pressure measured at the heart level increased during tilt, cerebral perfusion pressure may remain unchanged or even be reduced associated with the reduction in hydrostatic pressure during tilt (8, 21). Thus hypertensive effects of L-NMMA infusion during tilt on the estimates of transfer function, if they did occur, should be similar to those observed in the supine position with infusion of L-NMMA and phenylephrine.

In summary, with systemic infusion of L-NMMA sufficient to increase arterial pressure significantly, we observed that beat-to-beat arterial pressure and CBF velocity variability remained unchanged even when the magnitude of these oscillations was enhanced substantially during head-up tilt. Moreover, transfer function gain between the changes in arterial pressure and CBF velocity at low frequencies persisted after L-NMMA and did not change during tilt. These data provide evidence that inhibition of tonic production of NO does not appear to alter dynamic cerebral autoregulation in humans.

GRANTS

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