Hemodynamic and sympathoadrenal responses to mental stress during nitric oxide synthesis inhibition

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Lindqvist, Madeleine, Anders Melcher, and Paul Hjemdahl. Hemodynamic and sympathoadrenal responses to mental stress during nitric oxide synthesis inhibition. Am J Physiol Heart Circ Physiol 287: H2309–H2315, 2004. First published July 15, 2004; doi:10.1152/ajpheart.01216.2003.—Cardiovascular and sympathoadrenal responses to a reproducible mental stress test were investigated in eight healthy young men before and during intravenous infusion of the nitric oxide (NO) synthesis inhibitor N-monomethyl-L-arginine (L-NMMA). Before L-NMMA, stress responses included significant increases in heart rate, mean arterial pressure, and cardiac output (CO) and decreases in systemic and forearm vascular resistance. Arterial plasma norepinephrine (NE) increased. At rest after 30 min of infusion of L-NMMA (0.3 mg·kg−1·min−1 iv), mean arterial pressure increased from 98 ± 4 to 108 ± 3 mmHg (P < 0.001) because of an increase in systemic vascular resistance from 12.9 ± 0.5 to 18.5 ± 0.9 units (P < 0.001). CO decreased from 7.7 ± 0.4 to 5.9 ± 0.3 l/min (P < 0.01). Arterial plasma NE decreased from 2.08 ± 0.16 to 1.47 ± 0.14 nmol/l. Repeated mental stress during continued infusion of L-NMMA (0.15 mg·kg−1·min−1) induced qualitatively similar cardiovascular responses, but there was a marked attenuation of the increase in mean arterial blood pressure, resulting in similar “steady-state” blood pressures during mental stress without and with NO blockade. Increases in heart rate and CO were attenuated, but stress-induced decreases in systemic and forearm vascular resistance were essentially unchanged. Arterial plasma NE increased less than during the first stress test. Thus the increased arterial tone at rest during L-NMMA infusion is compensated for by attenuated increases in blood pressure during mental stress, mainly through a markedly attenuated CO response and suppressed sympathetic nerve activity.

N-monomethyl-L-arginine; psychological stress; catecholamines

Since the discovery by Furchgott and Zawadski (15) that the endothelial layer of arterial rings determined their responses to acetylcholine, a large number of studies have examined the role of nitric oxide (NO) in circulatory regulation and various disease states (11, 28, 36). For example, it has been suggested that deficient NO production may contribute to the pathogenesis of essential hypertension (30). Mental stress is also a possible contributor to cardiovascular diseases such as hypertension and ischemic heart disease (12, 32). We previously found similar hemodynamic and sympathoadrenal responses to mental stress in healthy individuals and hypertensive patients (25). The pronounced forearm vasodilator response to mental stress was partially explained by epinephrine (Epi) (26), and we found no neurogenic contribution to the sustained vasodilatation during stress (24). Others found that NO was important in vasodilation in the forearm during mental stress (6, 10).

However, the importance of NO production for the general hemodynamic stress reactions and possible influences on sympathoadrenal responses to stress have, to our knowledge, not been studied. Because mental stress may be an etiological factor and trigger acute events in different cardiovascular diseases, it is of interest to clarify the role of NO in hemodynamic and sympathoadrenal responses to stress.

The aim of the present study was therefore to study the cardiovascular and sympathoadrenal responses to a standardized mental stress test, before and during systemic blockade of NO, to evaluate whether an intact endothelial function is of importance for these reactions. Blockade of NO synthesis was achieved by intravenous infusion of N-monomethyl-L-arginine (L-NMMA) at a dose previously shown to elicit hemodynamic changes that were expected during NO synthesis inhibition and to decrease the NO concentrations in nasal air by ~65% in healthy volunteers (1).

MATERIALS AND METHODS

Procedures and assays. The study was approved by the Ethics Committee of the Karolinska Hospital and the Swedish Medical Products Agency, and informed consent was obtained from all individuals.

Eight healthy men with a mean age of 25 (range 21–31) yr, a mean body weight of 76 (range 65–81) kg, and a mean height of 183 (175–190) cm were studied. The subjects arrived in the laboratory in the morning, after a light breakfast and after having abstained from caffeine-containing beverages and nicotine on the day of the investigation. With the subject in the supine position, a short venous cannula (Venflon, Boc Ohmeda, Helsingborg, Sweden) was inserted into the left arm for blood sampling, and a similar venous cannula was inserted into a foot vein for infusion of L-NMMA. A four-lumen thermodilution catheter (7F, model SP510 7H, Boc Ohmeda) was introduced through a median antecubital vein in the right arm by the Seldinger technique, and the tip was placed in the pulmonary artery under fluoroscopic guidance. A Teflon catheter (1.4 × 700 mm, COOK, Askim, Sweden) was placed in the right brachial artery for pressure measurements and blood sampling.

Pressures were measured using transducers (model 14-6100, Synetics, Stockholm, Sweden) and digitized on a personal computer with a pressure-handling program (Polygram 2.0 for Windows, Synetics). An ECG lead was registered separately on a Mingocard (Siemens Elema, Solna, Sweden). Midthoracic depth, measured at the level of the fourth parasternal intercostal space, was used as reference. This level was marked on the subject, and the pressure transducers were subsequently adjusted at heart level with the subject in the semirecumbent position. Systolic and diastolic pressures in the pulmonary artery (SPAP and DPAP) and the mean pressure in the right atrium.
were measured. Mean pulmonary arterial pressure (MPAP) was calculated as diastolic pressure plus one-third of pulse pressure. Pulmonary capillary wedge pressure (PCWP) was measured after inflation of the balloon on the tip of the pulmonary catheter. Systolic and diastolic brachial arterial pressures (SAP and DAP) were measured, and mean arterial brachial pressure (MAP) was calculated as diastolic pressure plus one-third of pulse pressure. Heart rate (HR) was calculated from the ECG trace (averaged over 30-s periods).

Cardiac output (CO) was determined by the thermodilution technique with 5-ml injections of ice-cold 5% glucose. Curve analyses were performed with a thermodilution CO computer (model 9510 A, Edwards Laboratories, Santa Ana, CA). Values were obtained as the means of triplicate, or, during the initial phase of mental stress, duplicate measurements.

Forearm blood flow (FBF) was determined in the left arm by a mercury-in-Silastic strain-gauge venous plethysmograph (PE Hokansson, Issaquah, WA), with simultaneous occlusion of the artery and the cubital vein into ice-chilled test tubes containing EDTA (NE) and Epi. After centrifugation at 4°C, plasma was removed and stored at −80°C, until analyzed for catecholamines by high-performance liquid chromatography (19).

L-NMMA (Clinalfa, Laufhelingen, Switzerland) was given intravenously via the ankle catheter with an infusion pump (Perfusor Secura FT, Braun Medical, Solna, Sweden).

Mental stress was induced by a modified version of Stroop’s color word conflict test (CWT) (13). A videotape showed color words written in incongruent colors, and simultaneously a voice gave a third conflicting color for each word shown. The words were shown rapidly, and the subject was asked to mark the color he saw on a protocol where the color words were randomly listed and to disregard the two conflicting statements. Hemodynamic responses to this mental stress test reach a steady state within 8–10 min and have been shown to be reproducible (14).

Study protocol. After insertion of the catheters, the subject was raised to a semirecumbent position, and equipment for measurement of FBF was attached. After 30 min of rest, arterial and venous blood samples were taken for catecholamine analyses immediately before pressure and HR measurements; then CO and FBF were measured simultaneously. The mental stress test was performed, and measurements were repeated at 3 min (initial phase) and 10 min (steady-state phase) of stress and 10 min after stress. After another 20 min of rest, resting measurements without treatment were repeated. L-NMMA infusion then commenced by administration of 0.3 mg·kg−1·min−1 for 30 min, followed by measurements, and the infusion rate was lowered to 0.15 mg·kg−1·min−1 to avoid accumulation of L-NMMA, as described previously (1). After 15 min, the stress test was repeated, with measurements at 3 and 10 min, and the L-NMMA infusion was terminated. At 10 min after the end of the stress test measurements were repeated, the subjects were given 5 mg of isosorbidehydrate (Sorbangil, Pharmacia & Upjohn) to counteract the effects of L-NMMA.

Statistics. Data were analyzed by repeated-measures ANOVA with two dependent factors: conditions (with 2 levels, before and after intravenous L-NMMA-infusion) and time (with 4 levels, time points). Resting values before the two stress tests were compared by a post hoc test, planned comparisons. All data were analyzed with respect to raw data values, i.e., absolute values. Changes in outcome variables are also presented as relative changes, i.e., percentages. All statistical tests were two sided, and P < 0.05 was considered statistically significant.

RESULTS

Mental stress without treatment: CWT I. Mental stress induced by CWT evoked marked cardiovascular responses (Table 1, Figs. 1–3). HR increased by 23 beats/min (37 ± 6%, P < 0.001), and SAP, DAP, and MAP increased by 16, 13, and 14 mmHg, respectively (12 ± 2, 17 ± 1, and 14 ± 1%, respectively, P < 0.001 for all). There was a 3.6 l/min (50 ± 10%) increase in CO (P < 0.001), mainly due to an increase in HR, but also a small increase (9 ml/min, 10 ± 7%, P < 0.05) in stroke volume (SV). SAP decreased by 3.0 units (−22 ± 4%, P < 0.01) and FVR by 3.1 units (−60 ± 5%, P < 0.001); FBF increased by 41 ml·l−1·min−1 (208 ± 35%, P < 0.01). MAP increased by 1.4 mmHg (17 ± 6%, P < 0.01), as SAP and

<table>
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<tr>
<th>HR, beats/min</th>
<th>Rest (30 min)</th>
<th>CWT I (10 min)</th>
<th>After CWT I (+10 min)</th>
<th>L-NMMA Infusion</th>
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<td>Untreated</td>
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<tr>
<td>Rest (30 min)</td>
<td>HR, beats/min</td>
<td>63 ± 4</td>
<td>86 ± 5*</td>
<td>66 ± 5</td>
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<td>SAP, mmHg</td>
<td>142 ± 5</td>
<td>158 ± 5*</td>
<td>144 ± 4</td>
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<td>DAP, mmHg</td>
<td>77 ± 2</td>
<td>90 ± 3*</td>
<td>79 ± 3</td>
<td>108 ± 3*</td>
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<td>MAP, mmHg</td>
<td>99 ± 3</td>
<td>113 ± 3*</td>
<td>101 ± 3</td>
<td>110 ± 0.6</td>
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<td>MPAP, mmHg</td>
<td>12.5 ± 0.8</td>
<td>13.9 ± 0.6*</td>
<td>11.9 ± 0.6</td>
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<td>PCWP, mmHg</td>
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<td>6.3 ± 0.6</td>
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<td>CO, l/min</td>
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<td>11.1 ± 0.6*</td>
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<td>Sv, ml/min</td>
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<td>130 ± 5*</td>
<td>125 ± 4</td>
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<td>FBF, ml·l−1·min−1</td>
<td>18.4 ± 1.4</td>
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<td>10.4 ± 0.6*</td>
<td>12.6 ± 0.7</td>
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<td>FVR, U</td>
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<td>4.9 ± 0.4</td>
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<td>PVR, U</td>
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<td>0.60 ± 0.05*</td>
<td>0.66 ± 0.07</td>
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Values are means ± SE. N-monomethyl-L-arginine (L-NMMA) was infused intravenously. CWT, color word conflict test; HR, heart rate; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial pressure; MPAP, mean pulmonary arterial pressure; PCWP, pulmonary capillary wedge pressure; CO, cardiac output; SV, stroke volume; FBF, forearm blood flow; SVR, systemic vascular resistance; FVR, forearm vascular resistance; PVR, pulmonary vascular resistance; u, resistance units. Significant changes (based on ANOVA) during CWT I compared with rest: *, P < 0.05; **, P < 0.01; †, P < 0.001. Changes in resting values during L-NMMA infusion (30 min) compared with rest before L-NMMA: ‡, P < 0.01; ‡‡, P < 0.05. Changes between CWT II (during L-NMMA) compared with CWT I (before L-NMMA): ††, P < 0.01; †††, P < 0.001. Hemodynamic variables were also measured at 3 min of mental stress (Figs. 1–3).
DPAP increased. PVR decreased slightly by 0.09 unit ($P < 0.05$). PCWP increased during mental stress and reached a maximum of 8.6 mmHg after 3 min of stress (Fig. 2) but decreased during the poststress period ($P < 0.001$). Right atrial pressure did not change.

Arterial plasma NE increased from $1.77 \pm 0.15$ to $2.51 \pm 0.20$ nmol/l ($44 \pm 9\%$, $P < 0.001$). Arterial plasma Epi rose from $0.46 \pm 0.05$ to $0.68 \pm 0.06$ nmol/l ($53 \pm 13\%$, $P < 0.05$; Fig. 3). There were no significant changes in venous plasma NE or Epi.

Measurements at rest during l-NMMA infusion. At rest during l-NMMA infusion, before CWT II, there was no significant change in HR (Table 1, Figs. 1–3). MAP and DAP increased by 9 mmHg (9 ± 1\%, $P < 0.001$) and 12 mmHg (15 ± 1\%, $P < 0.001$), respectively, but SAP was unchanged. SVR increased by 5.1 units (39 ± 6\%, $P < 0.001$) and PVR by 0.23 units ($P < 0.01$), but there was no significant change in MPAP, because SV decreased by 23 ml/min (−17 ± 4\%, $P < 0.05$) and CO decreased by 1.6 l/min (−21 ± 4\%, $P < 0.01$). PCWP decreased by 1.7 mmHg (−23 ± 5\%, $P < 0.05$). There was no change in right atrial pressure. The decrease in FVR and the increase in FBF at rest during intravenous l-NMMA infusion were not significant.

Resting arterial plasma NE concentrations decreased from $1.77 \pm 0.15$ to $1.47 \pm 0.14$ nmol/l during L-NMMA infusion (−15 ± 5\%), and venous plasma NE decreased from $2.04 \pm 0.26$ to $1.56 \pm 0.25$ nmol/l (−24 ± 6\%, $P < 0.05$ for both), whereas arterial and venous plasma Epi were unchanged.

**Mental stress (CWT II) during l-NMMA-infusion.** The qualitative responses of all hemodynamic parameters to CWT were preserved, although some responses were attenuated (Table 1, Figs. 1–3). HR increased by 13 beats/min (22 ± 4\%), which was less than during CWT I ($P < 0.001$). The increases in SAP, DAP, and MAP were significantly smaller than without the NO blockade, i.e., 8 mmHg for all (6 ± 1, 9 ± 2, and 8 ± 1\%, respectively, $P < 0.001$ for SAP and MAP, $P < 0.01$ for DAP, compared with CWT I). Very similar blood pressure levels were attained during CWT without and with l-NMMA infusion.

CO increased less than during CWT I, by 2.2 l/min (38 ± 9\%, $P < 0.001$ compared with CWT I), as a result of the smaller increase in HR, because the SV increase was essentially unchanged (13 ml/min, 13 ± 6\%). The decreases in SVR and FVR were unchanged [−3.8 units (−19 ± 5\%) and −2.2 units (−45 ± 7\%), respectively], as was the decrease in PVR (−0.13 unit). The increase in SPAP was less pronounced than...
during CWT I (19 ± 4%, P < 0.01 vs. CWT I), but the increase in PCWP during mental stress was unchanged compared with CWT I, but the PCWP values were lower during L-NMMA infusion both before and during stress.

The increase in arterial plasma NE from 1.47 ± 0.14 to 1.74 ± 0.20 nmol/l (17 ± 4%) was markedly smaller than during CWT I (P < 0.001). The initial increase in arterial plasma Epi was smaller than during CWT I (36 ± 11%, P < 0.001), but the values after 10 min of stress (previously shown to be steady-state values during this test) were essentially the same during the two stress tests; thus the stress levels were similar during the two stress tests.

After CWT II, all hemodynamic variables except SVR returned to the resting values with L-NMMA infusion (before CWT II) within 10 min.

DISCUSSION

The main findings of the present study are that the hemodynamic response pattern during mental stress, i.e., the "defense reaction," was qualitatively preserved during NO synthesis inhibition by intravenous infusion of L-NMMA, although the increases in arterial blood pressure, HR, and CO were markedly attenuated. NO synthesis inhibition resulted in decreased sympathetic nerve activity, as assessed by arterial plasma NE levels at rest and during mental stress. Blood pressures during CWT were similar without and with L-NMMA infusion, and the CO and plasma NE responses to stress during L-NMMA infusion may be compensatorily reduced to allow the individual to reach a similar blood pressure level during stress, despite the relative lack of endogenous NO. The possibility that blood pressure may be the primarily regulated variable during mental stress is therefore discussed.

Effects of L-NMMA infusion at rest. At rest, intravenous L-NMMA infusion increased SVR and MAP, mainly as a result of an increase in DAP. FVR was unchanged, contrary to the increases in SVR and PVR. These findings confirm our previous results (1), which also demonstrated regional differences in the vascular response to systemic NO synthesis inhibition by intravenous infusion of L-NMMA. The increase in PVR during infusion of L-NMMA, suggesting that NO participates in regulation of the resting arterial tone in the pulmonary vascular bed in healthy adult humans, is also in accordance with results of another previous study (35).

The decreases in resting CO and SV during L-NMMA infusion are consistent with previous findings (1, 35). The lowering of SV can be attributed to several factors. First, the rise in arterial pressure and, consequently, left ventricular afterload may have influenced ventricular emptying. Second, reflexogenic withdrawal of sympathetic nerve activity to the heart, as suggested by lowered arterial levels of plasma NE (see below), may have decreased the inotropic drive to the heart. In both situations, a rise in filling pressure of the left ventricle would have been expected. However, PCWP was
lower during L-NMMA infusion, indicating a decrease in venous return as a result of less sympathetic vasoconstrictor tone to the systemic veins or improved ventricular emptying. Third, there is the possibility of a direct effect of NO on the myocyte. In vitro studies have mostly demonstrated depressant effects (2, 3, 22), but later in vivo findings indicate a small positive inotropic effect in the human heart by endogenous NO and a biphasic dose dependency of myocardial contractility on NO, which may explain discrepant results (31). Taken together, our findings of preserved or lowered ventricular filling pressure during NO blockade, despite sympathetic withdrawal and raised afterload, support the idea that endogenous NO may increase myocardial contractility in resting healthy humans. However, direct effects of NO on the myocyte are difficult to evaluate in the intact organism as a result of adaptive mechanisms.

The degree of NO synthesis inhibition was not evaluated in the present study, but we previously showed a pronounced decrease in NO production, as assessed by nasal measurements with a chemiluminescence method, with the dose regimen used (1). Thus the nasal NO concentration reached a minimum after 45 min of L-NMMA infusion and remained at this low level until ≥30 min after the end of the infusion. On the basis of these observations, NO production was probably decreased by ~65% compared with baseline when the second stress test was performed in the present study.

Hemodynamic and sympathoadrenal responses to mental stress before and during intravenous L-NMMA. Cardiovascular and sympathoadrenal responses to the mental stress test used in this study, a modified Stroop CWT, have been described in detail previously (14, 25). The response pattern created by this mental stress test is similar to that found by others (5, 34) using other mental stress tests. When the test is repeated, responses to the present version of CWT show an attenuation of the initial peak response due to familiarization with the test procedures, but reproducible steady-state responses are reached within 10 min (14). Arterial plasma Epi levels were not altered at rest during L-NMMA infusion, but there was significant attenuation of the stress response due to a reduction of the initial (at 3 min) Epi response. The levels at 10 min of stress were essentially the same as those during stress before infusion, indicating that the steady-state level of stress was similar during the first and the second stress tests, in agreement with previous findings after placebo infusion (14). Thus the 10-min measurements in the present study reflect treatment effects of intravenous L-NMMA on responses to mental stress.

The cardiovascular response pattern to mental stress, a CO-dependent increase in blood pressure with a decrease in SVR, was essentially preserved during systemic NO synthesis inhibition, despite the vasoconstrictor response to L-NMMA at rest. The systemic vasodilator response to stress was intact, but the CO response was reduced during L-NMMA infusion. The
latter was mainly due to attenuation of the HR response, because the increase in SV during steady-state stress was essentially unchanged. The attenuation of the HR response could be related to reflex inhibition of the autonomic nervous system response to stress during L-NMMA, as mirrored by lower resting levels and markedly attenuated increases in arterial NE during NO blockade. The similar levels of afterload (arterial pressure) and the intact decrease of SVR may be of importance for the preserved SV response to stress.

The direct effects of L-NMMA on vascular responses to stress cannot be separately evaluated in the present study, because L-NMMA was given intravenously, and interference with cardiovascular reflexes is likely. It has previously been shown that the marked forearm vasodilatation that is normally seen early (within 1 min) during mental stress is attenuated when L-NMMA is given intra-arterially (6, 10). It has also been shown by microdialysis that skeletal muscle (leg) blood flow was reduced during local infusion of L-NMMA at rest and during dynamic exercise (18) and that the vasodilator response to contralateral handgrip is reduced by intra-arterial L-NMMA (33). In the present study, however, intravenous L-NMMA had no effect on the resting FVR or the marked forearm vasodilator response to sustained mental stress. This discrepancy is most likely due to reflexogenic counterregulation during systemic NO blockade and, possibly, to less NO dependency of the sustained, compared with the initial, vasodilatation. Arterial Epi levels were unaffected by NO blockade at rest and during stress, and this may, probably in combination with a reflexogenic decrease in sympathetic vasoconstrictor nerve activity, explain the intact forearm vasodilator response to stress (16, 26).

The elevated resting vascular tone during systemic NO synthesis inhibition did not lead to amplification of the pressor response to mental stress. Furthermore, there is no evidence that intravenous L-NMMA exaggerates pressor responses to other types of stress, such as static exercise (4). The different influences of local (6, 10) and systemic (present results) NO synthesis inhibition on forearm vascular responses to mental stress underscore the importance of reflexogenic regulation of skeletal muscle blood flow in the control of arterial blood pressure during stress (20). In previous studies in which the present mental stress model was used, inhibition of the CO response to stress by β-adrenoceptor blockade was associated with an altered vascular response and similar blood pressure levels during mental stress after treatment with propranolol and placebo (14). Taken together, these findings support the hypothesis that blood pressure may be the primarily regulated variable during stress and that compensatory mechanisms come into play when vascular tone (NO synthesis inhibition; present results) or CO [β-adrenoceptor blockade (14)] is acutely altered by treatments.

**Sympathetic nerve activity during intravenous L-NMMA.** The decrease in sympathetic nerve activity during L-NMMA infusion, as assessed by arterial and venous plasma NE levels, is in accordance with our previous findings and suggests that the increase in arterial pressure causes withdrawal of sympathetic vasoconstrictor nerve activity (1). Our results are also in accordance with a previous study showing an increase in SVR and decreased venous plasma NE levels (7). The sympathetic (and hemodynamic) responses to NO blockade by intravenous L-NMMA injections are dose dependent, and at high doses the sympathoinhibitory effects were comparable to those evoked by the α-adrenergic vasoconstrictor drug phenylephrine (23). Others found that infusion of L-NMMA at a low dose that decreased HR and increased arterial pressure did not affect sympathetic nerve activity, as measured with a microneurographic method in the leg; however, when arterial pressure was elevated to the same degree by infusion of phenylephrine, muscle sympathetic nerve activity decreased (29). Others suggested that there is no evidence for involvement of NO in the tonic restraint of central sympathetic outflow (17). The effects of NO on the baroreflex control of sympathetic activity are thus complex (see also Ref. 9).

Another possible explanation for the decreases in NE in plasma during L-NMMA infusion might be a prejunctional effect of NO on the release of NE. To our knowledge, only inconsistent in vitro and animal studies have addressed this issue (8, 21, 27, 37), and there are no data supporting an influence of NO on human peripheral adrenergic neurotransmission in vivo.

In conclusion, arterial blood pressure seems to be the primarily regulated variable during mental stress. The increased arterial tone at rest during intravenous L-NMMA infusion is compensated for by smaller increases in blood pressure during mental stress, mainly through a markedly attenuated CO response, and suppressed sympathetic nerve activity.

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