Hemodynamic and sympathto-adrenal responses to mental stress during nitric oxide synthesis inhibition

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ABSTRACT

Cardiovascular and sympatho-adrenal responses to a reproducible mental stress test were investigated in eight healthy young men, before and during intravenous infusion of the NO synthesis inhibitor L-NMMA. Before L-NMMA the stress responses included significant increases in heart rate, mean arterial pressure and cardiac output, and decreases in systemic vascular resistance and in forearm vascular resistance. Arterial plasma norepinephrine increased. At rest after 30 min of intravenous infusion of L-NMMA (0.3 mg/kg/min), mean arterial pressure increased from 98±4 to 108±3 mm Hg (p<0.001), due to an increase in systemic vascular resistance from 12.9±0.5 to 18.5±0.9 U (p<0.001). Cardiac output decreased from 7.7±0.4 to 5.9±0.3 L/min (p<0.01). Arterial plasma norepinephrine 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/min) 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 cardiac output were attenuated, but the stress-induced decreases in systemic and forearm vascular resistances were essentially unchanged. Arterial plasma norepinephrine 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 cardiac output response, and suppressed sympathetic nerve activity.

Key words: L-NMMA, nitric oxide, psychological stress, catecholamines
INTRODUCTION

Ever since the discovery by Furchgott (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, see e.g. (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 have previously found similar hemodynamic and sympatho-adrenal responses to mental stress in healthy individuals and hypertensives (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 have found NO to be of importance for vasodilatation in the forearm during mental stress (6, 10). However, the importance of NO production for the general hemodynamic stress reactions and possible influences on sympatho-adrenal responses to stress have, to our knowledge, not been studied. Since 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 sympatho-adrenal responses to stress.

The aim of the present study was therefore to study the cardiovascular and sympatho-adrenal responses to a standardized mental stress test, before and during systemic blockade of NO, to evaluate if an intact endothelial function is of importance for these reactions. Blockade of NO synthesis was achieved by i.v. infusion of L-NMMA at a dosage previously shown to elicit hemodynamic changes that were expected during NO synthesis inhibition, and to decrease the NO concentrations in nasal air by approximately 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 years (range 21-31 years), a mean body weight of 76 kg (range 65-81 kg) and a mean height of 183 cm (175-190 cm) were studied. The subjects arrived in the laboratory in the morning, after a light breakfast, and after having refrained 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 in a foot vein for infusion of L-NMMA. A 4-lumen thermo-dilution 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 mm x 700 mm, COOK, Askim, Sweden) was placed in the right brachial artery for pressure measurements and blood sampling.

Pressures were measured using Synetics transducers 14-6100 (Synetics AB, Stockholm, Sweden), and were digitised on a PC with a pressure handling program (Polygram 2.0 for Windows, Synetics AB). 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 to the heart level with the subject in the semi-recumbent 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 the pulse pressure. Pulmonary capillary wedge pressure (PCWP) was measured after inflating the tip balloon of the pulmonary catheter. Systolic and diastolic brachial arterial pressures (SAP and DAP) were measured, and mean arterial brachial pressure (MAP) was calculated as the diastolic pressure plus one-third of the pulse pressure. Heart rate (HR) was calculated from the ECG tracing (averaged over 30 second 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 cardiac output 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 occlusion plethysmograph (PE Hokansson Inc., Issaquah, WA), with simultaneous occlusion of the wrist above the arterial pressure, in order to exclude the circulation to the hand. Flow curves were recorded on a pen recorder (Houston Instruments). Each value was based on the mean of three to five curves. The arm was elevated and supported so that the forearm was slightly above the heart level.

Systemic, forearm and pulmonary vascular resistances (SVR, FVR and PVR) were calculated as MAP/CO, MAP/FBF, and (MPAP-PCWP)/CO, respectively.

Blood samples were collected simultaneously from the brachial artery and the cubital vein into ice-chilled test tubes containing EDTA (10 mM final concentration) for determinations of norepinephrine (NE) and Epi. Following centrifugation at +4°C, plasma was removed and
stored at -80°C, until analysed for catecholamines by high performance liquid chromatography (19).

L-NMMA (Clinalfa AG, Laufelingen, 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 have reached a steady state within eight to ten minutes, and have been shown to be reproducible (14).

**Study protocol**

After insertion of the catheters, the subject was raised to a semi-recumbent position, and equipment for measurements of FBF was attached. The subject was left resting for 30 minutes. Thereafter, arterial and venous blood samples were taken for catecholamine analyses, immediately before pressure and heart rate measurements, followed by simultaneous measurements of CO and FBF. After this, the mental stress test was performed and measurements were repeated at three (initial phase) and ten minutes (steady-state phase) of stress, and ten minutes after stress. Then the patient was left resting for another 20 minutes, after which resting measurements without treatment were repeated. L-NMMA infusion then commenced by giving 0.3 mg/kg/min for 30 minutes, followed by measurements, and the infusion rate was lowered to 0.15 mg/kg/min to avoid accumulation of L-NMMA, as described previously (1). After 15 minutes, the stress test was repeated, with measurements at
three and ten minutes, and the L-NMMA infusion was terminated. Ten minutes after the end of the stress test measurements were repeated, and thereafter the subjects were given 5 mg isosorbidinitrate (Sorbangil®, Pharmacia & Upjohn) to counteract the effects of L-NMMA.

Statistics

Data were analysed by ANOVA, repeated measurements design with two dependent factors. The first factor is conditions, with two levels, before and after i. v. L-NMMA-infusion. The second factor is time, with four levels, time-points. Resting values before the two stress tests were compared by a post hoc test, planned comparisons. All data were analysed 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 a p-value less than 0.05 was considered as statistically significant.

RESULTS

Mental stress without treatment (CWT 1)

Mental stress induced by CWT evoked marked cardiovascular responses (Table 1, Fig. 1-3). Heart rate increased by 23 beats/minute (37±6%, p<0.001), and systolic, diastolic and mean arterial pressures increased by 16, 13 and 14 mm Hg, 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 cardiac output (p<0.001), mainly due to an increase in heart rate, but also a small increase in stroke volume (9 ml/min, 10±7%; p<0.05). Systemic vascular resistance decreased by 3.0 U
(-22±4%, p<0.01), and forearm vascular resistance by 3.1 U (-60±5%, p<0.001); forearm blood flow increased by 41 ml/L/min (208±35%, p<0.01). Mean pulmonary artery pressure increased by 1.4 mm Hg (17±6%, p<0.01), as both systolic and diastolic pulmonary arterial pressures increased. Pulmonary vascular resistance decreased slightly by 0.09 U (p<0.05). Pulmonary capillary wedge pressure increased during mental stress and reached a maximum value of 8.6 mmHg after three minutes of stress (Fig. 2), but decreased during the post-stress period (p<0.001). Right atrial pressure did not change.

Arterial plasma NE increased from 1.77±0.15 nmol L\(^{-1}\) to 2.51±0.20 nmol L\(^{-1}\) (44±9%; p<0.001). Arterial plasma Epi rose from 0.46±0.05 nmol L\(^{-1}\) to 0.68±0.06 nmol L\(^{-1}\) (53±13%, p<0.05) (Fig. 3). There were no significant changes in venous plasma NE or Epi.

**Measurements at rest during L-NMMA-infusion (Table 1, Fig. 1-3)**

At rest during L-NMMA infusion, before CWT II, there was no significant change in heart rate. Mean and diastolic arterial pressures increased by 9 mm Hg (9±1%, p<0.001) and 12 mm Hg (15±1%, p<0.001), respectively, but systolic arterial pressure was unchanged. Systemic vascular resistance increased by 5.1 U (39±6%, p<0.001), and pulmonary vascular resistance by 0.23 U (p<0.01), but there was no significant change in mean pulmonary arterial pressure, since stroke volume decreased by 23 ml/min (-17±4%, p<0.05) and cardiac output decreased by 1.6 L/min (-21±4%, p<0.01). Pulmonary capillary wedge pressure decreased by 1.7 mm Hg (-23±5%, p<0.05). There was no change in right atrial pressure. The decrease in forearm vascular resistance and the increase in forearm blood flow at rest during i.v. L-NMMA infusion were not significant.

Resting arterial plasma NE concentrations decreased from 1.77±0.15 nmol L\(^{-1}\) to 1.47±0.14 nmol L\(^{-1}\) during L-NMMA infusion (-15±5%), and venous plasma NE decreased from
2.04±0.26 nmol L⁻¹ to 1.56±0.25 nmol L⁻¹ (-24±6% ) (p<0.05 for both), whereas arterial and venous plasma Epi were unchanged.

*Mental stress (CWT II) during L-NMMA-infusion (Table 1, Fig 1-3)*

The qualitative responses of all hemodynamic parameters to CWT were preserved, although some responses were attenuated. Heart rate increased by 13 beats/min (22±4%), which was less than during CWT I (p<0.001). The increases in systolic, diastolic and mean arterial pressures were all significantly smaller than without the NO-blockade, i.e. 8 mm Hg for all (6±1%, 9±2% and 8±1%, respectively, p<0.001 for SAP and MAP, p<0.01 for DAP, compared to CWT I). It may be noted that very similar blood pressure levels were attained during CWT without and with L-NMMA infusion.

Cardiac output increased less than during CWT I, by 2.2 L/min (38±9%, p<0.001 compared to CWT I) due to the lesser increase in heart rate, since the stroke volume increase was essentially unchanged (13 ml/min, 13±6%). The decreases in systemic and forearm vascular resistances were unchanged (-3.8 U [-19±5%] and –2.2 U [-45±7%], respectively), as was the decrease in pulmonary vascular resistance (– 0.13 U). The increase in systolic pulmonary arterial pressure was less pronounced than during the first CWT (19±4%, p<0.01 compared to the first CWT), but the increase in mean pulmonary arterial pressure during CWT II was unchanged. The increase in pulmonary capillary wedge pressure during mental stress was unchanged compared to the first CWT, 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 nmol L⁻¹ to 1.74±0.20 nmol L⁻¹ (17±4 %) was markedly smaller than during the first CWT (p<0.001). The initial increase in arterial
plasma Epi was smaller than during the first CWT (36±11 %, p<0.001), but the values after ten minutes 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 the second CWT, all hemodynamic variables except systemic vascular resistance returned to the resting values with L-NMMA infusion (prior to the second CWT) within ten minutes.

DISCUSSION

The main findings of the present study are that the hemodynamic response pattern during mental stress, i.e. the ”defence reaction”, were qualitatively preserved during NO-synthesis inhibition by intravenous infusion of L-NMMA, although the increases in arterial blood pressure, heart rate, and cardiac output were markedly attenuated. NO-synthesis inhibition resulted in decreased sympathetic nerve activity, as assessed by arterial plasma NE levels, both at rest and during mental stress. It is of interest to note that blood pressures during CWT were similar without and with L-NMMA infusion, and that the cardiac output 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 systemic vascular resistance and mean arterial pressure, mainly due to an increase in diastolic arterial pressure. Forearm vascular resistance was unchanged, contrary to the increases in systemic and pulmonary vascular resistance. These findings confirm our previous results (1) which also demonstrated regional differences in the vascular response to systemic NO synthesis inhibition by i.v. infusion of L-NMMA. The increase in pulmonary vascular resistance during infusion of L-NMMA, suggesting that NO participates in the regulation of the resting arterial tone in the pulmonary vascular bed in healthy adult humans, is also in accordance with another previous study (35).

The decreases in resting cardiac output and stroke volume during L-NMMA infusion found presently are in concert with previous findings (1, 35). The lowering of stroke volume can be attributed to several factors. First, the rise in arterial pressure and consequently left ventricular afterload may have influenced ventricular emptying. Secondly, 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, pulmonary capillary wedge pressure was lower during L-NMMA infusion, indicating either a decrease in venous return as a result of less sympathetic vasoconstrictor tone to the systemic veins, or improved ventricular emptying. Thirdly, 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 due to adaptive mechanisms.

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

**Hemodynamic and sympatho-adrenal responses to mental stress before and during i.v. L-NMMA**

Cardiovascular and sympatho-adrenal responses to the mental stress test used presently, 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, e.g. (5, 34), using other mental stress tests. When 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 ten minutes (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 (3 min) Epi response. The levels at ten minutes of stress were essentially the same as during stress before infusion, indicating that the steady state level of stress was similar during the first and the second stress test, in agreement with previous findings after placebo infusion (14). Thus, the ten-minute measurements in the present study reflect treatment effects of i.v. L-NMMA on responses to mental stress.
The cardiovascular response pattern to mental stress - a cardiac output dependent increase in blood pressure with a decrease in systemic vascular resistance - was essentially preserved during systemic NO synthesis inhibition, despite the vasoconstrictor response to L-NMMA seen at rest. The systemic vasodilator response to stress was intact, but the cardiac output response was reduced during L-NMMA infusion. The latter was mainly due to attenuation of the heart rate response, since the increase in stroke volume during steady state stress was essentially unchanged. The attenuation of the heart rate 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 systemic vascular resistance may be of importance for the preserved stroke volume response to stress.

The direct effects of L-NMMA on vascular responses to stress cannot be separately evaluated in the present study, since 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 one minute) during mental stress, is attenuated when L-NMMA is given intraarterially (6, 10). It has also been shown by microdialysis, that skeletal muscle blood flow (in the leg) was reduced during local infusion of L-NMMA, both at rest and during dynamic exercise (18), and that the vasodilator response to contralateral handgrip is reduced by intraarterial L-NMMA (33). In the present study, however, i.v. L-NMMA had no effect on either the resting forearm vascular resistance 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 a lesser NO dependency of the sustained, compared to 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).

It is interesting to note that 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 i.v. 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). Previous studies in the present mental stress model have shown that inhibition of the cardiac output response to stress by beta-adrenoceptor blockade is 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 cardiac output (beta-adrenoceptor blockade; (14) ) is acutely altered by treatments.

**Sympathetic nerve activity during i.v. 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 systemic vascular resistance and decreased venous plasma NE levels (7). The sympathetic
(and hemodynamic) responses to NO blockade by i.v. L-NMMA injections are dose dependent, and at high doses, the sympatho-inhibitory effects were comparable to those evoked by the alfa-adrenergic vasoconstrictor drug phenylephrine (23). Others have found that infusion of L-NMMA at a low dose that decreased heart rate 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 still have suggested that there is in fact no evidence for nitric oxide being involved 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 (9)).

Another possible explanation for the decreases of NE in plasma during L-NMMA infusion might be a prejunctional effect of NO on the release of NE. To our knowledge there exists only inconsistent in vitro and animal studies addressing this issue (8, 21, 27, 37), and 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 i.v. L-NMMA infusion is compensated for by smaller increases in blood pressure during mental stress, mainly through a markedly attenuated cardiac output response, and suppressed sympathetic nerve activity.
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REFERENCES


FIGURE LEGENDS

Fig. 1
Line graphs show resting values and responses to mental stress (color word conflict test; CWT) for hemodynamic variables, untreated (filled symbols, solid lines), and during intravenous infusion of L-NMMA (open symbols, dotted lines). "Effects" relate to the effects of L-NMMA on resting values and on the stress reaction. N.s = not significant. Values are means.

Fig. 2
Line graphs show resting values and responses to mental stress (color word conflict test; CWT) for hemodynamic variables, untreated (filled symbols, solid lines), and during intravenous infusion of L-NMMA (open symbols, dotted lines). "Effects" relate to the effects of L-NMMA on resting values and on the stress reaction. N.s = not significant. Values are means.

Fig. 3
Line graphs show resting values and responses to mental stress (color word conflict test; CWT) for forearm blood flow, forearm vascular resistance, arterial norepinephrine and arterial epinephrine, untreated (filled symbols, solid lines), and during intravenous infusion of L-NMMA (open symbols, dotted lines). "Effects" relate to the effects of L-NMMA on resting values and on the stress reaction. N.s = not significant. Values are means.
Fig. 1

Mean arterial pressure, mm Hg

Rest 3min 10min Rest 10 min

CWT

Untreated

During L-NMMA

Effect at rest p<0.001
Effect on response p<0.001

Cardiac output, L/min

Rest 3min 10min Rest 10 min

CWT

Untreated

During L-NMMA

Effect at rest p<0.001
Effect on response p<0.001

Heart rate, beats/min

Rest 3 min 10 min Rest 10 min

CWT

Untreated

During L-NMMA

Effect at rest n.s.
Effect on response p<0.001

Systemic vascular resistance, U

Rest 3 min 10 min Rest 10 min

CWT

Untreated

During L-NMMA

Effect at rest p<0.001
Effect on response n.s.
Fig. 2

- **Stroke volume, L**
  - Untreated: Rest 3min 10min Rest 10min
  - During L-NMMA: Rest 3min 10min Rest 10min
  - Effect at rest p<0.05
  - Effect on response n.s.

- **Mean pulmonary arterial pressure, mm Hg**
  - Untreated: Rest 3min 10min Rest 10min
  - During L-NMMA: Rest 3min 10min Rest 10min
  - Effect at rest n.s.
  - Effect on response n.s.

- **Pulmonary capillary wedge pressure, mm Hg**
  - Untreated: Rest 3min 10min Rest 10min
  - During L-NMMA: Rest 3min 10min Rest 10min
  - Effect at rest p<0.05
  - Effect on response n.s.

- **Pulmonary vascular resistance, U**
  - Untreated: Rest 3min 10min Rest 10min
  - During L-NMMA: Rest 3min 10min Rest 10min
  - Effect at rest p<0.01
  - Effect on response n.s.
Fig. 3

Arterial norepinephrine, nmol*L⁻¹

- Rest 3 min 10 min
- Effect at rest p<0.05
- Effect on response p<0.001

Untreated

During L-NMMA

Forearm blood flow, mL*L⁻¹*min⁻¹

- Rest 3 min 10 min
- Effect at rest n.s.
- Effect on response n.s.

Untreated

During L-NMMA

Arterial epinephrine nmol*L⁻¹

- Rest 3 min 10 min
- Effect at rest n.s.
- Effect on response p<0.001

Untreated

During L-NMMA

Forearm vascular resistance, U

- Rest 3 min 10 min
- Effect at rest n.s.
- Effect on response n.s.

Untreated

During L-NMMA

Fig. 3
Table 1.
Hemodynamic responses to mental stress (color word conflict test=CWT), before and during intravenous infusion of L-NMMA. Significant changes during CWT I compared to rest are denoted \(*\ast\ast\ast=p<0.001\), \(*\ast\ast=p<0.01\) and \(*p<0.05\); changes in resting values during L-NMMA infusion (30 min) compared to rest before L-NMMA are denoted \(\dagger\dagger\dagger=p<0.001\), \(\dagger\dagger=p<0.01\) and \(\dagger=p<0.05\); changes between CWT II (during L-NMMA) compared to CWT I (before L-NMMA) are denoted \(\ddagger\ddagger\ddagger=p<0.001\), \(\ddagger\ddagger=p<0.01\) and \(\ddagger=p<0.05\). Hemodynamic variables were also measured at three minutes of mental stress (these data are indicated in the figures).

Values are mean ±SEM. Significances of responses to CWT are based on ANOVAs, including all values. Abbreviations: 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.
TABLE 1. Hemodynamic responses to mental stress (color word conflict test; CWT) before and during i.v. infusion of L-NMMA.

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<td>After CWT I +10 min</td>
<td>Rest 30 min</td>
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<td><strong>HR, beats/min</strong></td>
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<td><strong>MAP, mmHg</strong></td>
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<td>101±3</td>
<td>108±3†††</td>
<td>116±3‡‡‡</td>
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<tr>
<td><strong>MPAP, mmHg</strong></td>
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<td><strong>PCWP, mmHg</strong></td>
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<td>8.2±0.5</td>
<td>5.9±0.3††</td>
<td>8.1±0.5†††</td>
</tr>
<tr>
<td><strong>SV, ml/min</strong></td>
<td>121±10</td>
<td>130±5*</td>
<td>125±4</td>
<td>98±3†</td>
<td>111±7</td>
</tr>
<tr>
<td><strong>FFB, ml/L/min</strong></td>
<td>18.4±1.4</td>
<td>59.0±10.4**</td>
<td>21.5±2.3</td>
<td>27.3±4.8</td>
<td>56.6±8.5</td>
</tr>
<tr>
<td><strong>SVR, U</strong></td>
<td>13.4±0.9</td>
<td>10.4±0.6**</td>
<td>12.6±0.7</td>
<td>18.5±0.9†††</td>
<td>14.7±0.8</td>
</tr>
<tr>
<td><strong>FVR, U</strong></td>
<td>5.5±0.5</td>
<td>2.4±0.5***</td>
<td>4.9±0.4</td>
<td>4.6±0.7</td>
<td>2.4±0.4</td>
</tr>
<tr>
<td><strong>PVR, U</strong></td>
<td>0.69±0.09</td>
<td>0.60±0.05*</td>
<td>0.69±0.06</td>
<td>0.92±0.09†††</td>
<td>0.79±0.08</td>
</tr>
</tbody>
</table>