Role of NO in the O_2 and CO_2 responsiveness of cerebral and ocular circulation in humans

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Schmetterer, Leopold, Oliver Findl, Karin Strenn, Ursula Graselli, Johannes Kastner, Hans-Georg Eichler, and Michael Wolzt. Role of NO in the O_2 and CO_2 responsiveness of cerebral and ocular circulation in humans. *Am. J. Physiol.* 273 (*Regulatory Integrative Comp. Physiol.* 42): R2005–R2012, 1997.—It is well known that changes in P_{CO_2} or P_{O_2} strongly influence cerebral and ocular blood flow. However, the mediators of these changes have not yet been completely identified. There is evidence from animal studies that NO may play a role in hypercapnia-induced vasodilation and that NO synthase inhibition modulates the response to hyperoxia in the choroid. Hence we have studied the effect of NO synthase inhibition by N^G-monomethyl-L-arginine (L-NMMA, 3 mg/kg over 5 min as a bolus followed by a continuous infusion of 30 $\mu g \cdot kg^{-1} \cdot min^{-1}$) on the changes of cerebral and ocular hemodynamic parameters elicited by hypercapnia and hyperoxia in healthy young subjects. Mean flow velocities in the middle cerebral artery and the ophthalmic artery were measured with Doppler ultrasound, and ocular fundus pulsation amplitude, which estimates pulsatile choroidal blood flow, was measured with laser interferometry. Administration of L-NMMA reduced ocular fundus pulsations (−19%, $P < 0.005$) but only slightly reduced mean flow velocities in the larger arteries. Hypercapnia (P_{CO_2} = 48 mmHg) significantly increased mean flow velocities in the middle cerebral artery (+26%, $P < 0.01$) and fundus pulsation amplitude (+16%, $P < 0.005$) but did not change mean flow velocity in the ophthalmic artery. The response to hypercapnia in the middle cerebral artery ($P < 0.05$) and in the choroid ($P < 0.05$) was significantly blunted by L-NMMA. On the contrary, L-NMMA did not affect hyperoxia-induced (P_{O_2} = 530 mmHg) hemodynamic changes. The hemodynamic effects of L-NMMA (at baseline and during hypercapnia) were reversed by coadministration of L-arginine. The present study supports the concept that NO has a role in hypercapnia-induced vasodilation in humans.

cerebral blood flow; ocular blood flow; Doppler ultrasound; ocular fundus pulsation

IT IS WELL KNOWN THAT changes in arterial partial pressure of carbon dioxide (P_{CO_2}) and oxygen (P_{O_2}) regulate cerebral and ocular blood flow, but the mediators responsible for these changes have not yet been completely identified. One possible mediator is nitric oxide (NO), a potent vasodilating agent, which is most likely involved in the regulation of cerebral circulation (11). Despite the large number of studies on the role of NO in the regulation of the cerebral circulation and in the pathophysiology of cerebral ischemia, a considerable degree of controversy remains. Contradicting results have also been reported concerning the role of NO in cerebrovasodilation elicited by hypercapnia or hypoxia (11). The majority of studies, however, has found that NO synthase inhibitors attenuate the increase in cerebral blood flow (CBF) elicited by hypercapnia (13, 30) but not by hypoxia in rats (18, 20). The role of NO in the ocular circulation has been much less investigated. There is, however, evidence that NO also plays a major role in the control of choroidal blood flow (4, 23, 24). In contrast to the cerebral circulation, the choroid shows almost no vasoconstrictor reactivity to hyperoxia (22, 24, 28). In newborn pigs, however, the choroid shows a vasoconstrictor reactivity to hyperoxia during NO synthase inhibition (9). Hence increased NO synthesis during hyperoxia prevents a vasoconstrictor response to hyperoxia in these animals.

The aim of the present study was to characterize the effect of partial NO synthase inhibition on alterations of cerebral and ocular blood flow elicited by hypercapnia and hyperoxia in man. In contrast to animal experiments, the assessment of hemodynamic parameters in these vascular beds in humans is limited to noninvasive methods. We measured middle cerebral artery (MCA) blood flow velocity (BFV) and ophthalmic artery (OA) BFV with Doppler ultrasound (1, 7). Moreover, fundus pulsation measurements were performed, which have been shown to estimate local pulsatile ocular blood flow (26, 28).

METHODS

Subjects

The study protocols were approved by the Ethics Committee of Vienna University School of Medicine. Ten healthy male volunteers participated in *protocol 1* (age range 23–33 yr, mean ± SD 26.6 ± 3.1 yr), and eight other healthy male volunteers participated in *protocol 2* (age range 21–29 yr, mean ± SD 24.3 ± 2.7). Written informed consent to participate was obtained. Each subject passed a screening examination, including physical examination and medical history; hematological status; clinical chemistry; hepatitis A, B, C, and human immunodeficiency virus serology; and urine analysis, to determine health status. Subjects were excluded if any abnormality was found as part of the pretreatment screening and examinations unless the investigator considered an abnormality to be clinically irrelevant. Furthermore, an ophthalmic examination including slit-lamp biomicroscopy, indirect funduscopy, tonometry, and determination of refraction and visual acuity was performed. Inclusion criteria were normal ophthalmic findings and ametropy <3 diopters.

Study Protocols

Protocol 1. We performed a double-blind, randomized, placebo-controlled, two-way crossover study. A study schedule is given in Fig. 1. After an overnight fast, all subjects rested for at least 20 min in a sitting position to establish a stable baseline. At baseline, measurements of fundus pulsation

Scheduled time (min)	-30	-10	0	5	15	20	30	35	45	50	60
resting time	x										
L-NMMA or placebo			----------								
5 % CO_2 + 95 % air breathing					----------						
100 % O_2 breathing									----------		
fundus pulsation measurement		x		x		x		x		x	
sonography of the OA		x		x		x		x		x	
sonography of the MCA		x		x		x		x		x	
blood pressure and pulse rate		x		x		x		x		x	
measurement of exhaled NO		x		x		x		x		x	
blood gas analysis				x		x		x		x	

Fig. 1. Time schedule of study *protocol 1*. L-NMMA, N^G-monomethyl-L-arginine; OA, ophthalmic artery; MCA, middle cerebral artery.

amplitude (FPA), mean BFV (MFV) in the OA and the MCA, blood pressure (BP), pulse rate (PR), and exhaled NO were performed. Thereafter, N^G-monomethyl-L-arginine (L-NMMA) or placebo was administered intravenously in a randomized sequence on different study days. The dose of L-NMMA was 3 mg/kg over 5 min as a bolus followed by a continuous infusion of 30 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 55 min. Two identical saline syringes were prepared for the placebo study day to obtain double-blind conditions. Measurements of hemodynamic parameters and of exhaled NO were performed in a predetermined sequence (BFV in the MCA, FPA, BFV in the OA, BP, PR, and exhaled NO). Fifteen minutes after the start of the drug infusion, a 15-min breathing period of 5% CO_2 with 95% air was started. A further resting period of 15 min was followed by a 15-min breathing period of 100% O_2. Measurement of hemodynamic parameters and exhaled NO as well as blood gas analysis from arterialized blood of the earlobe were performed between *minutes 5* and *15*, *20* and *30*, *35* and *45*, and *50* and *60* after the start of drug administration. The washout period between the two study days was at least 7 days.

Protocol 2. This study was performed to investigate whether L-NMMA-induced hemodynamic effects can be reversed by L-arginine. A study schedule is given in Fig. 2. After an overnight fast, all subjects rested for at least 20 min in a sitting position to establish a stable baseline. At baseline, the same measurements as in *protocol 1* and blood gas analysis were performed. Thereafter, a 15-min breathing period of 5% CO_2 with 95% air was started. After a 15-min resting period, an L-NMMA infusion was started. The dose of L-NMMA was 3 mg/kg over 5 min as a bolus followed by a continuous infusion of 30 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 70 min. Fifteen minutes after the start of infusion, another 15-min breathing period of 5% CO_2 with 95% air with a subsequent 15-min resting period was scheduled. Thereafter, 100 mg/kg L-arginine was coadministered over 30 min. Fifteen minutes after the start of L-arginine infusion, a third 15-min breathing period of 5% CO_2 with 95% air was started. Measurements of hemodynamic parameters and exhaled NO were performed between *minutes 5* and *15*, *35* and *45*, *50* and *60*, *80* and *90*, and *95* and *105*. Additionally, blood gas analyses were done at baseline and during the three breathing periods.

Rationale for L-NMMA doses. Previous experiments have shown that 3 mg/kg L-NMMA infused over 5 min did not cause any adverse clinical event, alter electrocardiogram, or affect clinical chemistry or hematology tests (13). However, the dose of 3 mg/kg is appropriate to induce a small increase in blood pressure (13, 23, 24) as well as a decrease in ocular blood flow (23, 24) in healthy subjects. The continuous L-NMMA dose was chosen because it has been shown to decrease renal blood flow and exhaled NO to a constant level (32).

Methods of Evaluation

Laser interferometric measurement of fundus pulsations. Pulse-synchronous pulsations of the ocular fundus were assessed by laser interferometry on the subject's right eye. The method is described in detail by Schmetterer et al. (26). Briefly, the eye is illuminated by the beam of a single-mode laser diode with a wavelength (λ) of 783 nm. The light is reflected at both the front side of the cornea and the retina. The two reemitted waves produce interference fringes from which the distance changes between cornea and retina during a cardiac cycle can be calculated. Distance changes between cornea and retina lead to a corresponding variation of the interference order [$\Delta N(t)$]. This change in interference order can be evaluated by counting the fringes moving inward and outward during the cardiac cycle. Changes in optical distance [$\Delta L(t)$], corresponding to the cornea-retina distance changes, can then be calculated by $\Delta L(t) = \Delta N(t) \cdot \lambda/2$. The maximum distance change is called FPA and estimates the local pulsa-

Scheduled time (min)	-30	-10	0	5	15	30	35	45	50	60	75	80	90	95	105
resting time	x														
L-NMMA or placebo						----------									
L-arginine											----------				
5 % CO_2 + 95 % air breathing			----------					----------					----------		
fundus pulsation measurement		x		x			x		x			x		x	
sonography of the OA		x		x			x		x			x		x	
sonography of the MCA		x		x			x		x			x		x	
blood pressure and pulse rate		x		x			x		x			x		x	
measurement of exhaled NO		x		x			x		x			x		x	
blood gas analysis		x		x					x					x	

Fig. 2. Time schedule of study *protocol 2*.

tile blood flow (28). The short-term and day-to-day variability of the method is small, which allows the detection of even small changes in local pulsatile blood flow following pharmacological stimulation (28). To obtain information on the choroidal blood flow, the macula, where the retina lacks vasculature, was chosen for measurements (25, 27).

Doppler sonography. In the OA and the MCA, the MFV was measured as the time mean of the spectral outline. BFV in the MCA was assessed with transcranial Doppler using a 2-MHz probe (1). BFV in the OA was assessed with Duplex imaging using a 7.5-MHz Doppler probe (7).

Measurement of exhaled NO. Exhaled NO was measured with a chemoluminescence detector (nitrogen oxides analyzer, model 8840, Monitor Labs) connected to a strip-chart recorder. Calibration of the instrument was done with certified gases (300 parts per billion NO in N_2; AGA, Vienna, Austria), diluted in nitrogen by precision flow meters. A baseline signal was obtained with pure nitrogen. One thousand milliliters per minute of the exhaled air was allowed to enter the inlet port. Subjects were instructed to fully inflate their lungs, hold their breath for 10 s, and exhale for 10 s into a Teflon tube. Three consecutive readings were made at each measurement point under nasal occlusion. The end-expiratory values from the strip recorder readings were used for analysis. This assures that inspired NO from the ambient air does not distort the results (14). This method of quantifying the degree of endogenous NO synthesis has already been used previously (15).

Noninvasive measurement of systemic hemodynamics. Systolic and diastolic BP (SBP, DBP) were measured on the upper arm by an automated oscillometric device. Pulse-pressure amplitude was calculated as SBP − DBP; mean arterial pressure (MAP) was calculated as 1/3 SBP + 2/3 DBP. PR was automatically recorded from a finger pulse-oxymetric device; electrocardiogram was taken from a standard device (HP-CMS patient monitor; Hewlett Packard, Palo Alto, CA).

Blood gas analysis. Blood gas values were determined from capillary blood samples of the earlobe. After the earlobe was spread with nicotinate plus nonylvanillamid paste (Finalgon; Thomae, Biberach, Germany) to induce capillary vasodilation, a lancet incision was made. The arterialized blood was drawn into a thin glass capillary tube. Arterial pH, P_{CO_2}, and P_{O_2} were determined with an automatic blood gas analysis system (AVL 995-Hb, Graz, Austria).

Data Analysis

Exponential curves give the best fit between CBF and P_{CO_2}. Hence the reactivity of CBF to changes in P_{CO_2} has been calculated as $\Delta\ln CBF/\Delta P_{CO_2} \times 100$ (31). In our study neither MFV as measured in the MCA or the OA nor FPA is a direct measure of total blood flow. Despite this limitation, we calculated the reactivity to changes in P_{CO_2} as $\Delta\ln MFV/\Delta P_{CO_2} \times 100$ and $\Delta\ln FPA/\Delta P_{CO_2} \times 100$ for each subject after L-NMMA or placebo infusion, respectively. In the same way, the reactivity to changes in O_2 was calculated.

Statistical analysis was done with the CSS Statistica software package (StatSoft, Tulsa, OK). Data are presented as percent of baseline. Standard deviations and standard errors of the mean were calculated. For *protocol 1*, changes in hemodynamic parameters were analyzed with two-way repeated-measures analysis of variance (ANOVA) using the absolute values of L-NMMA and placebo study days, respectively. The effect of the gas-breathing periods was expressed as percent change of the preceding values. The reactivity to changes in P_{O_2} and P_{CO_2} was compared during L-NMMA and placebo, respectively, using paired *t*-tests. For *protocol 2*, the effect of L-NMMA and L-arginine on the parameters under study was analyzed by repeated-measures ANOVA using the absolute values. The effect of the gas-breathing periods was expressed as percent change of the preceding values. The reactivity to changes in P_{CO_2} was compared at baseline, during L-NMMA, and during L-NMMA and coadministration of L-arginine, using paired *t*-tests with Bonferroni correction for multiple comparisons. A P value of <0.05 was considered the level of significance. For data description, values are given as means ± SE.

Table 1. *Baseline values of hemodynamic parameters of the 2 study days (protocol 1)*

	Day 1	*Day 2*
Fundus pulsation amplitude, μm	3.6 ± 0.4	3.5 ± 0.4
Mean flow velocity in OA, cm/s	16.1 ± 2.1	16.3 ± 1.5
Mean flow velocity in MCA, cm/s	49.5 ± 3.2	51.5 ± 4.0
Exhaled NO, ppb	73.4 ± 14.1	75.9 ± 14.6
Systolic blood pressure, mmHg	113.6 ± 2.9	110.3 ± 3.4
Diastolic blood pressure, mmHg	58.3 ± 3.2	57.9 ± 3.0
Pulse-pressure amplitude, mmHg	55.5 ± 3.1	53.0 ± 3.1
Mean arterial pressure, mmHg	78.0 ± 1.0	75.9 ± 0.8
Pulse rate, min^{-1}	60.6 ± 4.8	58.7 ± 3.9

Values are means ± SE; n = 10. Washout period between the 2 study days was at least 7 days. OA, ophthalmic artery; MCA, middle cerebral artery; ppb, parts per billion.

RESULTS

Effects of L-NMMA and L-Arginine

Baseline values of the measured parameters for the two different trial cohorts are given in Tables 1 and 2, respectively. There were no significant differences between the two study days at baseline.

FPA was reduced by −19% during administration of the NO synthase inhibitor ($P < 0.005$ vs. placebo and vs. baseline, Fig. 3). This decrease in FPA (Fig. 4) was almost abolished by coadministration of L-arginine (−3% vs. baseline, $P < 0.001$). In contrast, the effect of L-NMMA on MFV in the OA was small: whereas MFV was not affected in one trial cohort (Fig. 3), a small decrease in MFV in the OA was observed during L-NMMA administration (−9%) in the other cohort, which was again antagonized by L-arginine (+4% vs. baseline, $P < 0.05$). MFV in the MCA was not affected by L-NMMA or L-arginine. As expected, exhaled NO was reduced by −41% during L-NMMA administration ($P < 0.005$ vs. baseline and placebo, Fig. 3). Coadministration of L-arginine increased exhaled NO to 82% of baseline ($P < 0.001$, Fig. 4). Administration of L-NMMA

Table 2. *Baseline values of hemodynamic parameters (protocol 2)*

Fundus pulsation amplitude, μm	4.1 ± 0.7
Mean flow velocity in OA, cm/s	18.1 ± 2.6
Mean flow velocity in MCA, cm/s	53.3 ± 4.2
Exhaled NO, ppb	51.6 ± 9.1
Systolic blood pressure, mmHg	122.5 ± 3.6
Diastolic blood pressure, mmHg	64.2 ± 3.5
Pulse-pressure amplitude, mmHg	56.3 ± 3.5
Mean arterial pressure, mmHg	81.3 ± 2.2
Pulse rate, min^{-1}	58.2 ± 5.3

Values are means ± SE; n = 8.

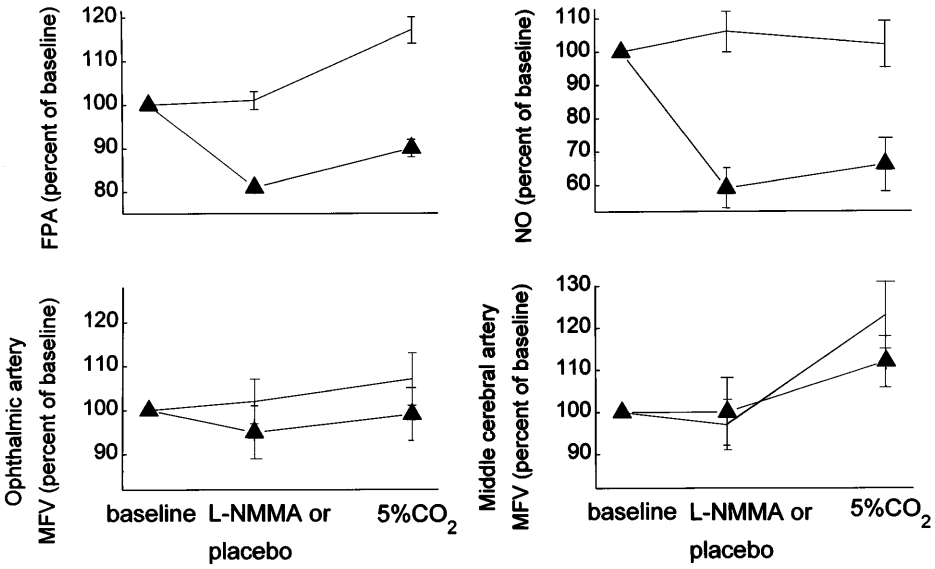

Fig. 3. Effect of L-NMMA (3 mg/kg for 5 min followed by 30 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 55 min, ▲) or placebo (no symbol) and a subsequent inhalation period of 5% CO_2 with 95% air on fundus pulsation amplitude (FPA), exhaled NO, and mean flow velocity (MFV) in OA and MCA (*protocol 1*, $n = 10$).

increased MAP 5 min after the start of drug infusion (Table 3). This effect was abolished by L-arginine administration ($P < 0.05$; Fig. 4). In contrast, neither L-NMMA nor L-arginine affected pulse rate.

Effects of Hypercapnia

CO_2 breathing increased FPA during all inhalation periods (16%, Fig. 3 and Tables 4 and 5, $P < 0.005$). A high reactivity to hypercapnia was also observed in the MCA (26%, $P < 0.01$) but not in the OA. Pretreatment with L-NMMA significantly reduced the change in P_{CO_2} in the MCA and in the choroid (Tables 4 and 5). However, this L-NMMA-induced effect was almost completely reversed by coadministration of L-arginine (Table 5). As expected, breathing 5% CO_2 with 95% air produced a significant increase in P_{CO_2} and a significant decrease in pH independent of the pretreatment (Tables 3 and 6). Hypercapnia did not affect systemic hemodynamics.

Effects of Hyperoxia

O_2 inhalation only slightly decreased FPA (−5%, $P < 0.05$, Fig. 5, Table 4). The effect of hyperoxia was more pronounced on MFV in the OA (−9%, $P < 0.05$) and on MFV in the MCA (−12%, $P < 0.05$). None of these

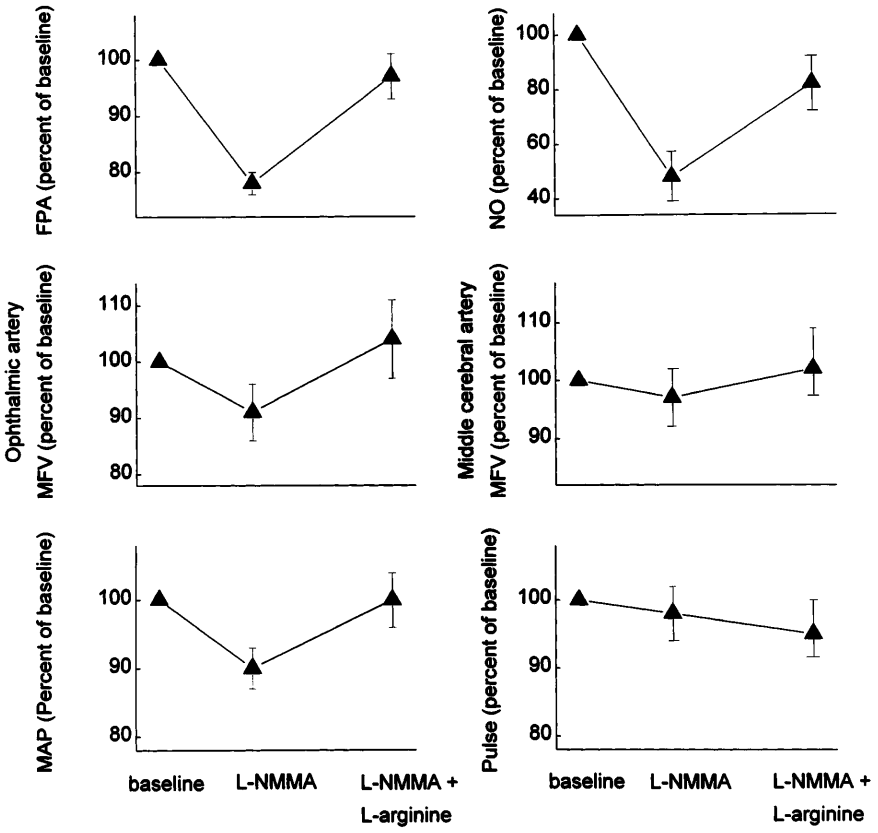

Fig. 4. Effect of L-NMMA (3 mg/kg for 5 min followed by 30 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 55 min, ▲) and coadministration of L-NMMA with L-arginine on FPA, exhaled NO, MFV in OA and MCA, mean arterial pressure (MAP), and pulse rate (*protocol 2*, $n = 8$).

Table 3. *Effect of L-NMMA or placebo and subsequent 15-min breathing periods of 5% CO_2 + 95% air and 100% O_2 on blood gas parameters and systemic hemodynamics (protocol 1)*

	Baseline	L-NMMA or Placebo	5% CO_2 + 95% Air	Resting Period	100% O_2
Time, min	−10	5	20	35	50
L-NMMA treatment					
P_{O_2}, mmHg	not done	94.0 ± 3.5	102.9 ± 12.0	93.7 ± 3.3	537.8 ± 17.7*
P_{CO_2}, mmHg	not done	37.8 ± 1.2	48.2 ± 0.9*	38.8 ± 1.2	35.2 ± 1.2
pH	not done	7.40 ± 0.01	7.33 ± 0.01*	7.40 ± 0.01	7.42 ± 0.01*
MAP, mmHg	75.7 ± 2.5	84.6 ± 2.6	88.1 ± 2.8	79.1 ± 2.6	78.5 ± 2.3
Pulse rate, min^{-1}	57.9 ± 4.1	52.1 ± 3.4	59.7 ± 3.0	56.2 ± 3.3	53.8 ± 3.7
Placebo treatment					
P_{O_2}, mmHg	not done	91.0 ± 2.3	101.8 ± 2.1	88.6 ± 2.2	530.4 ± 8.8*
P_{CO_2}, mmHg	not done	38.0 ± 0.9	48.4 ± 0.8*	37.5 ± 0.9	36.1 ± 1.4
pH	not done	7.39 ± 0.01	7.34 ± 0.02*	7.39 ± 0.01	7.41 ± 0.01*
MAP, mmHg	79.0 ± 2.8	79.7 ± 2.3	82.8 ± 2.8	77.2 ± 2.2	77.2 ± 2.6
Pulse rate, min^{-1}	61.6 ± 4.4	56.4 ± 13.0	60.0 ± 3.2	58.1 ± 3.8	54.0 ± 8.8

Values are means ± SE; n = 10. L-NMMA, N^G-monomethyl-L-arginine; MAP, mean arterial pressure. *Significant difference vs. preinhalation measurements ($P < 0.05$).

effects was significantly influenced by L-NMMA administration. Breathing of 100% O_2 significantly increased exhaled NO during L-NMMA by 32% ($P < 0.05$) and during placebo by 38% ($P < 0.005$). Breathing 100% O_2 caused an increase in P_{O_2} and a decrease in pH (Table 3). Systemic hemodynamic parameters were not altered by hyperoxia.

DISCUSSION

Effects of L-NMMA and L-Arginine

The selected dose of L-NMMA significantly reduced FPA. This might be caused by a particularly high reactivity of the choroidal vascular bed to changes in NO production, which has already been observed in animals (4) and humans (23, 24). Hence it can be speculated that a high local NO generation may be necessary to maintain the high perfusion level in this vascular bed. The effect of L-NMMA on MFV in the OA was smaller and only reached the level of significance in the second study. In contrast, NO synthase inhibition did not affect MFV in the MCA. This is somewhat unexpected, because animal experiments have shown that administration of NO synthase inhibitors reduces CBF (5). However, it must be kept in mind that Doppler sonography accurately determines BFV in the MCA but not vessel diameter. Therefore, an estimation of total flow is not necessarily possible (16), and we cannot exclude that L-NMMA reduced MCA diameter and total blood flow in this artery. The effect of L-NMMA on MAP was small but significant. An increase in BP following comparable doses has already been observed previously (13, 23, 24, 32). L-NMMA also exerted a significant effect on exhaled NO, which argues that the chosen dose was appropriate to partially inhibit NO synthase.

Table 4. *Reactivity to hypercapnia and hyperoxia during administration of L-NMMA or placebo (protocol 1)*

	Placebo	L-NMMA	P Value
r_{CO_2}			
Fundus pulsation amplitude	1.75 ± 0.37	1.21 ± 0.33	0.031
Mean flow velocity in OA	0.47 ± 0.36	0.36 ± 0.42	0.538
Mean flow velocity in MCA	2.05 ± 0.26	1.15 ± 0.39	0.014
r_{O_2}			
Fundus pulsation amplitude	−0.019 ± 0.009	−0.014 ± 0.004	0.342
Mean flow velocity in OA	−0.027 ± 0.012	−0.019 ± 0.012	0.482
Mean flow velocity in MCA	−0.052 ± 0.017	−0.043 ± 0.012	0.644

Values are means ± SE; n = 10. r_{CO_2}, r_{O_2}, reactivity to hypercapnia and hyperoxia, respectively.

All hemodynamic effects of L-NMMA were reversed by coadministration of L-arginine. This is particularly important, because concerns regarding the specificity of L-NMMA as an inhibitor of NO synthase have been raised (11). Hence the results of *protocol 2* provide evidence that the hemodynamic effects induced by L-NMMA were due to the NO-blocking properties of the drug and not to other metabolic mechanisms.

Effects of Hypercapnia

Several animal studies suggest an attenuation of hypercapnia-induced vasodilation by NO synthase inhibition. Results supporting this theory have been re-

Table 5. *Reactivity to hypercapnia at baseline, during L-NMMA administration, and during coadministration of L-NMMA and L-arginine (protocol 2)*

	Baseline	L-NMMA	P Value*	L-NMMA + L-Arginine	P Value†
Fundus pulsation amplitude	1.82 ± 0.42	1.12 ± 0.39	0.009	1.68 ± 0.26	0.064
Mean flow velocity in OA	0.24 ± 0.51	0.29 ± 0.43	0.698	0.47 ± 0.33	0.357
Mean flow velocity in MCA	1.98 ± 0.33	1.21 ± 0.50	0.041	2.06 ± 0.47	0.034

Values are means ± SE; n = 8. *L-NMMA vs. placebo. †L-NMMA vs. L-NMMA + L-arginine.

Table 6. *Effect of breathing periods of 5% CO_2 + 95% air on blood gas parameters and systemic hemodynamics at baseline, during administration of L-NMMA, and during coadministration of L-NMMA and L-arginine (protocol 2)*

	Baseline	5% CO_2 + 95% Air	5% CO_2 + 95% Air + L-NMMA	5% CO_2 + 95% Air + L-NMMA + L-arginine
Time, min	−10	5	50	95
P_{O_2}, mmHg	92.8±3.8	99.9±5.6	101.2±9.3	94.9±7.7
P_{CO_2}, mmHg	39.2±1.3	50.3±2.0*	49.6±1.5*	47.5±0.9*
pH	7.41±0.01	7.33±0.01*	7.33±0.01*	7.34±0.01*
MAP, mmHg	81.3±2.2	85.1±3.6	89.9±2.6*	78.9±3.3
Pulse rate, min^{-1}	58.2±5.3	60.7±2.4	57.3±5.8	54.9±2.8

Values are means ± SE; $n = 8$. *Significant difference vs. baseline.

ported in rats (13, 30), in cats, in dogs, and in rabbits (5, 11). In primates, a role of NO in hypercapnia-induced vasodilation has been observed in the cortex (19, 29).

Our results indicate that inhibition of NO synthase attenuates hypercapnia-induced cerebral and ocular hemodynamic effects in man. During placebo infusion, we observed a high reactivity in the MCA and the choroid to increased P_{CO_2}. In contrast, the response to hypercapnia was much lower in the OA. This is in keeping with previous studies (9) and argues that certain brain regions are more responsive to P_{CO_2} than others. A high reactivity in the MCA, however, has unequivocally been shown, and recent investigations argue that there is also a high reactivity in the choroid (22, 25, 27). In the MCA and the choroid, hypercapnia-induced vasodilation was significantly blunted during administration of L-NMMA (Fig. 3, Table 4). Because this L-NMMA-induced effect was reversed by L-arginine, our findings demonstrate that partial inhibition of NO synthase was responsible for the blunting of hypercapnia-induced hemodynamic effects (Table 4). However, as discussed in detail by Iadecola et al. (11), NO may not be the final mediator acting on vascular smooth muscles to produce vasodilation during hypercapnia but may have a permissive role.

Effects of Hyperoxia

Hyperoxia caused a significant reduction in blood flow velocities and fundus pulsation. The lower reactivity in the choroid compared with the OA or the MCA is in agreement with previous findings (22, 25, 27). Administration of L-NMMA did not affect hyperoxia-induced hemodynamic effects. In newborn pigs it has been shown that hyperoxia does not lead to a decrease in choroidal blood flow because of increased NO synthesis (8). Our experiments indicate that this phenomenon is not present in humans. However, during hyperoxia we observed a significant increase in exhaled NO after pretreatment with placebo or L-NMMA, respectively. It has already been observed in buffer-perfused rabbit lungs that hypoxia decreases exhaled NO (6). In the same study, NO originating from sites with ready access to the gaseous space decreased in response to hypoxia, whereas intravascular NO production was unchanged. In the isolated pig lung, hypoxia produced an increase in pulmonary vascular resistance and a drop in exhaled NO levels (3). Hence our results argue for a role of NO in the regulation of pulmonary vasoactivity in response to changes in P_{O_2}.

Study Limitations

Hypercapnia-induced hemodynamic effects were blunted but not abolished by administration of L-NMMA. This might have at least two reasons. On the one hand, there is experimental evidence that NO-independent components are involved in cerebrovasodilation elicited by hypercapnia, which are likely to be more important at very high P_{CO_2} (12). However, any study in human volunteers is, for ethical reasons, limited to moderate increases in P_{CO_2}, and a dose-response curve cannot be obtained. On the other hand, it is evident from our measurements of exhaled NO that

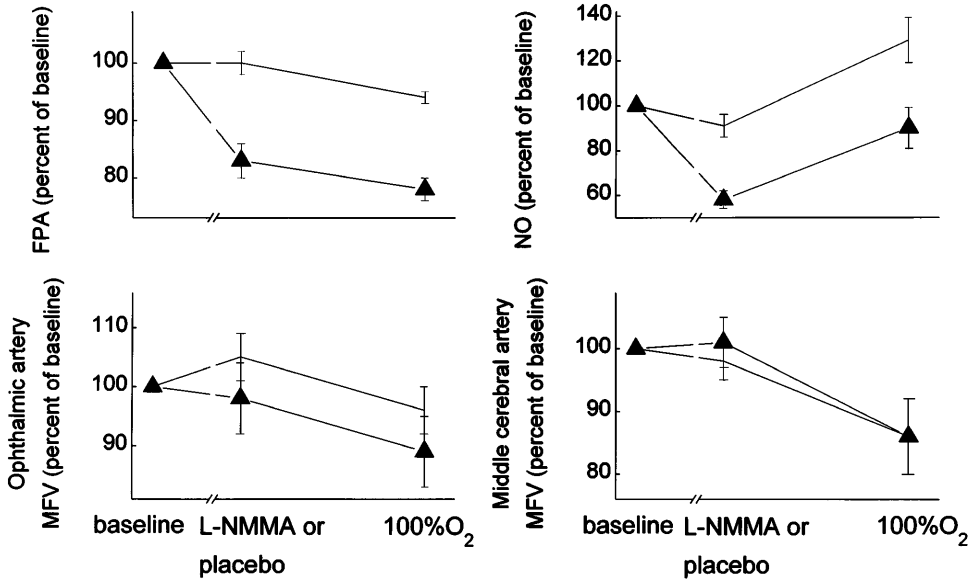

Fig. 5. Effect of L-NMMA (3 mg/kg for 5 min followed by 30 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 55 min, ▲) or placebo (no symbol) and a subsequent inhalation period of 100% O_2 on FPA, exhaled NO, and MFV in OA and MCA (*protocol 1*, $n = 8$).

the chosen dose of L-NMMA did not completely inhibit NO synthase in our study (Figs. 3 and 4).

Moreover, it must be kept in mind that L-NMMA is a nonspecific NO synthase inhibitor acting on endothelial and neuronal NO synthase. Hence the present study does not provide evidence of the source of NO to permit vasodilation. Recent investigations in the rat, however, argue that most of NO released during hypercapnia is produced by neuronal NO (31). Additionally, L-NMMA blocks ATP-sensitive potassium channels, and this blocking effect is reversed by L-arginine, which may contribute to the blunting of hypercapnia-induced changes in CBF (17). However, other investigators did not observe any effect of ATP-sensitive potassium channel blockers on increased CBF during high P_{CO_2} (21). Nevertheless, it is well established that L-arginine has many biological actions that are independent of the NO pathway. Hence a vasodilator response in the cerebral and ocular circulation is not necessarily caused by an increase in local NO synthase (23). In the present study, L-arginine almost completely reversed the L-NMMA-induced reduction in exhaled NO, which argues that L-arginine partially counteracted NO synthase inhibition.

Several methodological limitations have to be considered for interpreting the results obtained in the cerebral and ocular circulation. As mentioned above, the Doppler sonographic method measures BFV rather than blood flow. This limitation has been discussed in detail by Kontos (16) for the MCA and in principle also applies for the OA. Regarding the FPA measurements, it has to be pointed out that only pulsatile blood flow is assessed. Hence, the validity of the technique for estimations of total blood flow depends on changes in flow pulsatility. However, in the OA, supplying the choroid, we did not find a significant change in the ratio of pulsatile to steady blood flow (data not shown). Moreover, an increase in vascular resistance, which likely occurs during administration of L-NMMA, should lead to an increased flow pulsatility. Hence, under such circumstances FPA measurements rather underestimate effects on total blood flow.

In consideration of these limitations, our results cannot generally be extrapolated to cerebral and ocular blood flow. However, transcranial Doppler flow velocities correlate well with total blood flow when flow is altered by CO_2 variations (2). During administration of L-NMMA, we cannot exclude that vessel diameter changes occurred. Because NO synthase inhibitors are vasoconstrictors in cerebral arteries, it may well be that CBF was decreased during L-NMMA, although we did not observe changes in BFV. Hence, Doppler sonographic measurements rather underestimate the effect of L-NMMA on CBF as well as the blunting of hypercapnia-induced CBF changes.

L-NMMA blunted the response to hypercapnia as evidenced from MFV measurements in the MCA and fundus pulsation measurements. Because these methods are based on different principles in assessing hemodynamic parameters, it is most likely that NO synthase inhibitors also attenuate hypercapnia-induced increases in MCA and choroidal blood flow.

Because NO is short lived, diffusible, and highly reactive, it is difficult to directly measure local NO generation in vivo. Hence, we measured NO in exhaled air as an indicator of NO production, and the observed 40–50% decrease does not necessarily reflect a 40–50% inhibition in NO synthase. It is unlikely that exhaled NO is an appropriate index of NO synthase inhibition at the level of cerebral and ocular circulation. However, together with the finding that L-arginine infusions in healthy volunteers increase exhaled NO (15, 23), our results indicate that this method is at least a semiquantitative tool for the characterization of NO production in vivo.

Conclusions

We have shown that partial inhibition of NO synthase by L-NMMA blunts hemodynamic effects in the MCA and the choroid induced by moderate hypercapnia. This effect of L-NMMA is reversed by L-arginine. In contrast, hyperoxia-induced hemodynamic effects are not influenced by NO synthase inhibition. These results support the concept that NO has a role in hypercapnia-induced vasodilation in man.

Perspectives

The present study is one of the first attempts to demonstrate a role of NO in the human cerebral and ocular circulation. However, a multitude of questions regarding the importance of NO in these vascular beds remains. It is not clear from the present study to what extent NO is involved in the maintenance of basal CBF in humans. Moreover, on the basis of animal studies, a potential role of the inducible isoform of NO synthase in the pathophysiology of cerebral and ocular vascular disease can be assumed, but no data concerning these phenomena in humans are available. Clearly, the role of NO in the regulation of blood flow and metabolism has to be further elucidated before therapeutic regimen in cerebral and ocular vascular disease can be directed to the L-arginine-NO pathway.

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