Effect of Systemic Nitric Oxide Synthase Inhibition on Optic Disc Oxygen Partial Pressure in Normoxia and in Hypercapnia

Ioannis K. Petropoulos,[1] Jean-Antoine C. Pournaras,[2] Alexandros N. Stangos,[1] and Constantin J. Pournaras[1]

Purpose. To investigate the effect of systemic nitric oxide synthase (NOS) inhibition on optic disc oxygen partial pressure (Po_2) in normoxia and hypercapnia.

Methods. Intervascular optic disc Po_2 was measured in 12 anesthetized minipigs by using oxygen-sensitive microelectrodes placed <50 μm from the optic disc. Po_2 was measured continuously during 10 minutes under normoxia, hyperoxia (100% O_2), carbogen breathing (95% O_2, 5% CO_2), and hypercapnia (increased inhaled CO_2). Measurements were repeated after intravenous injection of N^ω-nitro-L-arginine methyl ester (L-NAME) 100 mg/kg. Intravenous L-arginine 100 mg/kg was subsequently given to three animals.

Results. Before L-NAME injection, an increase was observed in optic disc Po_2 during hypercapnia ($\Delta Po_2 = 3.2 \pm 1.7$ mm Hg; 18%; $P = 0.001$) and carbogen breathing ($\Delta Po_2 = 12.8 \pm 5.1$ mm Hg; 69%; $P < 0.001$). Optic disc Po_2 in normoxia remained stable for 30 minutes after L-NAME injection (4% decrease from baseline; $P > 0.1$), despite a 21% increase of mean arterial pressure. Optic disc Po_2 increase under hypercapnia was blunted after L-NAME injection ($\Delta Po_2 = 0.6 \pm 1.1$ mm Hg; 3%; $P > 0.1$), and this effect was reversible by L-arginine. Moreover, L-NAME reduced the response to carbogen by 29% ($\Delta Po_2 = 9.1 \pm 4.4$ mm Hg; 49%; $P = 0.01$ versus before L-NAME). The response to hyperoxia was not affected.

Conclusions. Whereas systemic NOS inhibition did not affect optic disc Po_2 in normoxia, a blunting effect was noted on the CO_2-induced optic disc Po_2 increase. Nitric oxide appears to mediate the hypercapnic optic disc Po_2 increase. (*Invest Ophthalmol Vis Sci.* 2009;50:378–384) DOI:10.1167/iovs.08-2413

Similar to the cerebral arteries and retinal arterioles, the arterioles of the optic nerve head (ONH) are very sensitive to variations of oxygen (O_2) and carbon dioxide (CO_2) in the blood. Hyperoxia, or high oxygen partial pressure in the arterial blood (Pao_2), constricts cerebral arteries[1] and retinal arterioles,[2-5] leading to a decrease of cerebral,[1] retinal,[3-5] and ONH[6,7] blood flow. However, hyperoxia does not alter the tissue oxygen availability, as tissue oxygen partial pressure (Po_2) remains relatively stable in the inner retina[8,9] and at intervascular areas of the ONH.[10,11]

On the other hand, hypercapnia, or high carbon dioxide partial pressure in the arterial blood ($Paco_2$), dilates cerebral arteries[1,12] and retinal arterioles,[13-15] leading to an increase in cerebral,[1,12] retinal,[13-15] and ONH[6,7] blood flow. As a result, inhalation of CO_2 increases preretinal[16] and optic disc Po_2.[17] In addition, inhalation of carbogen (95% O_2, 5% CO_2), which increases both Pao_2 and $Paco_2$, also increases preretinal,[18,19] inner retinal,[20] and optic disc Po_2,[21] since the vasodilatory effect of elevated $Paco_2$ partially counterbalances the vasoconstriction induced by elevated Pao_2. Elevated $Paco_2$ increases tissue Po_2 through a dual mechanism: a rightward shift of the oxyhemoglobin dissociation curve,[22] which enhances oxygen release from hemoglobin (the Bohr effect), as well as a CO_2-induced arteriolar vasodilation.[13] Thus, a CO_2-induced Po_2 increase has been demonstrated at the level of the ONH.[17,21]

Nitric oxide (NO) has been reported to control CO_2-induced vasodilation in the cerebral[23] and the retinal circulation.[24] NO is constitutively synthesized from L-arginine by two isoforms of NO synthase (NOS): endothelial NOS, expressed in vascular endothelial cells[25] and in some neurons,[26] and neuronal NOS, expressed only in neurons.[26] In the ONH, NOS activity has been found in vascular endothelial cells[27-29] and sparsely in astrocytes[27] and the lamina cribrosa.[27] NO diffuses rapidly through membranes allowing the signal to spread from cell to cell, with concomitant relaxation of vascular smooth muscle cells.[26] Sodium nitroprusside, an NO donor, dilates retinal vessels.[30,31] Evidence suggests a role for NO in the control of basal arteriolar tone in the ONH in normoxia.[32] Furthermore, NOS inhibition by L-arginine analogues suppresses CO_2-induced vasodilation in the cerebral[23] and retinal circulation.[24] It is not known, however, whether this is also the case at the level of the ONH.

Changes in vessel diameter are technically difficult to demonstrate in the ONH. Po_2 measurements, which reflect tissue oxygen availability,[16] were performed in this study. Po_2 measurements have the advantage of providing information regarding the metabolic status and the needs of the ONH in the normal state and in disease. We conducted this study to investigate the effect of systemic NOS inhibition on optic disc Po_2 in normoxia and in hypercapnia.

The data have been published in part in abstract form (Petropoulos IK, et al. *IOVS* 2005;46:ARVO E-Abstract 3908).

MATERIALS AND METHODS

Experiments were conducted in one eye of 12 minipigs (Arare Animal Facility, Geneva, Switzerland) weighing 10 to 12 kg. The advantage of experimenting on the minipig is the anatomic similarity of its optic nerve to the optic nerve of primates,[33] excepting that minipig retinal arteries arise from the ciliary circulation.[33] All the experiments were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Corresponding author: Constantin J. Pournaras, Department of Ophthalmology, University Hospitals of Geneva, 22 Alcide-Jentzer Street, CH-1211 Geneva 14, Switzerland; constantin.pournaras@hcuge.ch.

Animal Preparation

Minipigs were prepared for the experiments according to the following protocol: After premedication with intramuscular injection of 2 mL (10 mg) of the tranquilizer midazolam maleate (Dormicum; Roche Pharma, Reinach, Switzerland), 3 mL (120 mg) of the tranquilizer azaperone (Stresnil; Janssen Pharmaceutica, Beerse, Belgium), and 1 mL (0.5 mg) of atropine, anesthesia was induced with 2 to 3 mg of thiopental sodium (Pentothal; Abbott AG, Baar, Switzerland) injected into an ear vein. Analgesia was induced with 2 mL (100 μg) of fentanyl (Sintenyl; Sintetica SA, Mendrisio, Switzerland), and curarization was performed with 2 mL (4 mg) of pancuronium bromide (Pavulon; Organon SA, Pfäffikon, Switzerland). The animals were intubated and artificially ventilated. After arterial, venous, and bladder catheterization, the anesthesia, analgesia, and myorelaxation were maintained throughout the experiment by continuous perfusion of thiopental, fentanyl, and pancuronium, respectively.

Each animal was ventilated at approximately 18 strokes/min, with a continuous flow of 20% O_2 and 80% nitrous oxide (N_2O) through a variable-volume respirator. Systolic and diastolic arterial blood pressure was monitored through the femoral artery with a transducer. Temperature was maintained between 36°C and 37°C with a thermal blanket. Pao_2, $Paco_2$, and pH were measured intermittently from the same artery with a blood gas analyzer (Labor-system; Flukiger AG, Menziken, Switzerland) and kept under control by adjusting ventilatory rate, stroke volume, and composition of the inhaled gas.

A head-holder was used to avoid movements from respiration. Upper and lower eyelids were removed as well as a rectangular area of skin surrounding the eye; the bulbar conjunctiva was detached; the sclera was carefully cleaned to 5 mm from the limbus; the superficial scleral vessels were thermocauterized; the globe was fixed with a metal ring sutured around the limbus; and a sclerotomy 2 to 3 mm posterior to the limbus was performed. A small contact lens with a flat exterior surface was placed on the cornea. The pupil was dilated with 1% atropine eye drops, and the fundus was observed with an operating microscope (Carl Zeiss Surgical GmbH, Oberkochen, Germany).

Instruments for Po_2 Measurement

Optic disc Po_2 measurements were obtained using double-barreled, recess-type, oxygen-sensitive microelectrodes with a 10-μm tip diameter, as previously described.[9] The microelectrodes were prepared in our laboratory according to a technique described in detail in previous papers.[34,35] They were calibrated before insertion into the eye and again after withdrawal in a buffered saline solution at 35°C, which was equilibrated with air. The microelectrodes were mounted on an electronic micromanipulator[36] and they were inserted into the vitreous cavity through the sclerotomy. On visualization of the fundus using the operating microscope, the microelectrode tip was positioned at a distance of less than 50 μm from the optic disc, over intervascular areas, as follows: When the microelectrode tip touched the optic disc surface, the reference barrel recorded a sudden negative DC potential shift of several millivolts. At the moment of contact, the position display was zeroed and the microelectrode was then withdrawn less than 50 μm from the optic disc surface. The reference electrode was placed under the skin of the neck.

Experimental Procedure

Each experiment began with baseline continuous optic disc Po_2 recordings under systemic normoxia (i.e., breathing of 20% O_2 + 80% N_2O). When a stable, continuous recording was achieved, systemic hyperoxia was induced by 100% O_2 breathing and optic disc Po_2 was measured for 10 minutes. At the next step, normoxia was again induced, with the purpose of obtaining a stable optic disc Po_2 recording for at least 10 minutes. This recording was considered the baseline before subsequent inhalation of carbogen (95% O_2 + 5% CO_2) and optic disc Po_2 recording over 10 minutes. Normoxia was again induced, a stable optic disc Po_2 recording for at least 10 minutes was achieved, and systemic hypercapnia was induced by adding 6% CO_2 to

the gas mixture and adjusting N_2O downward accordingly (i.e., gas mixture: 6% CO_2 + 20% O_2 + 74% N_2O). Optic disc Po_2 recording was performed for 10 minutes, and thereafter the animal was allowed to return to normoxia. Each condition was confirmed by corresponding Pao_2, $Paco_2$, and pH measurements: Pao_2 had to be high in hyperoxia, $Paco_2$ had to be high in hypercapnia, and both Pao_2 and $Paco_2$ had to be high during carbogen breathing.

After several cycles of the above-described measurements, we proceeded to intravenous injection of N^{ω}-nitro-L-arginine methyl ester (L-NAME; Fluka Chemie GmbH, Buchs, Switzerland) 100 mg/kg over 10 minutes. The same experimental procedure was then repeated, and optic disc Po_2 measurements were taken under systemic hyperoxia, carbogen breathing, and systemic hypercapnia, as described earlier.

In three animals, after optic disc Po_2 measurements under different gas conditions before and after L-NAME injection, we proceeded to intravenous injection of L-arginine (Fluka Chemie GmbH) 100 mg/kg over 10 minutes to reverse the action of L-NAME. After this injection, optic disc Po_2 measurements were repeated under systemic hypercapnia.

N_2O was present in the breathing gas during normoxia, whereas it was adjusted downward whenever CO_2 was added to the gas mixture to achieve hypercapnia. It was absent during hyperoxia or carbogen breathing. However, these adjustments are not considered to have affected the results, since we had previously tested the effect of the presence or absence of N_2O in the breathing gas during normoxia and had not found any differences in pH, $Paco_2$, Pao_2, or optic disc Po_2 (data not shown).

Statistics

Optic disc Po_2, Pao_2, $Paco_2$, and pH, as well as their differences from baseline (optic disc ΔPo_2, ΔPao_2, $\Delta Paco_2$, and ΔpH), are expressed as the mean $\pm$ SD. For each result presented, n represents the number of minipigs studied. In many cases, measurements from different locations on the optic disc of the same eye were obtained. In these cases, the responses from different locations in an individual eye were averaged to a value representing that eye in statistical comparisons.

Values were excluded when Pao_2, $Paco_2$, and/or pH were out of the expected range for the specific gas condition studied in each case. This explains an $n < 12$ appearing in some results.

Repeated-measures analysis of variance (ANOVA) was used to test the effect of hyperoxia, carbogen breathing, and hypercapnia at four predetermined time points (2, 5, 7, and 10 minutes). Post hoc comparisons at the same time points were performed using paired Student's t-test with Bonferroni correction for multiple comparisons. Unpaired Student's t-test with Bonferroni correction was used to compare the response to each gas condition at 7 minutes before and after L-NAME injection. $P < 0.05$ defined statistically significant differences. Data were analyzed using commercial software (SPSS, ver. 15.0 for Windows; SPSS, Chicago, IL).

RESULTS

Measurements before Intravenous Injection of L-NAME

Measurements under Systemic Normoxia. Under systemic normoxia (Pao_2 = 103.6 $\pm$ 17.9 mm Hg; $Paco_2$ = 35.4 $\pm$ 2.4 mm Hg; pH = 7.47 $\pm$ 0.04; n = 12), mean optic disc Po_2 recorded in intervascular areas of 12 eyes was 18.3 $\pm$ 4.0 mm Hg, a level similar to those described previously in minipigs.[10,11,17,21]

Measurements under Systemic Hyperoxia (100% O_2). The inhalation of 100% O_2 induced a mean increase in optic disc Po_2 of ΔPo_2 = 3.9 $\pm$ 1.6 mm Hg, or 22% (n = 11; Fig. 1) after 7 minutes. Under systemic hyperoxia, mean optic disc Po_2 increased from 18.6 $\pm$ 4.2 to 22.5 $\pm$ 4.9 mm Hg and that difference, although moderate, was statistically significant (Table 1; $P < 0.001$) but disproportional to a substantial increase
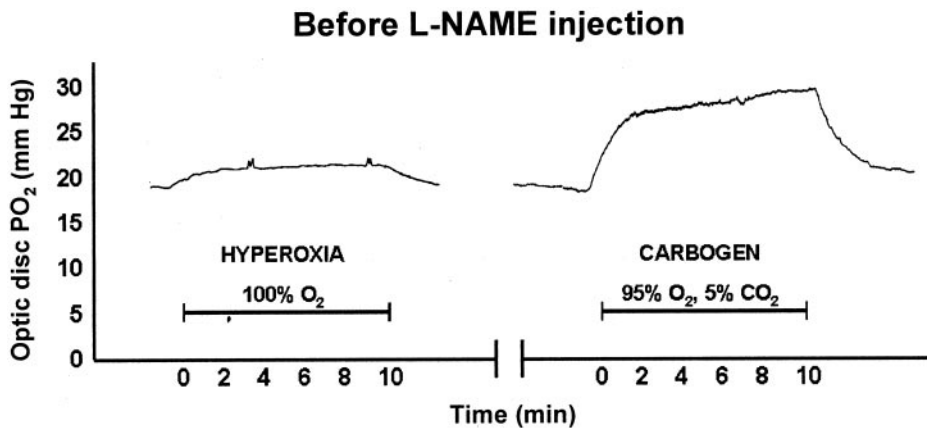
FIGURE 1. Continuous typical recordings of optic disc Po_2 during hyperoxia (*left*) or carbogen breathing (*right*) before injection of L-NAME. The corresponding table shows the mean ± SD of the blood gas levels and of optic disc Po_2 variation (ΔPo_2) 7 minutes after the onset of each condition. *P*, post hoc comparisons by Bonferroni *t*-test between baseline means and means at 7 minutes. Carbogen breathing induced a greater increase in optic disc Po_2 than did hyperoxia, because $Paco_2$ reached higher levels during carbogen breathing.

in Pao_2 (ΔPao_2 = 353.6 ± 83.2 mm Hg, or 365%; $\Delta Paco_2$ = −2.9 ± 3.6 mm Hg; ΔpH = 0.03 ± 0.03; n = 11), as previously described.[21]

Measurements during Carbogen Inhalation (95% O_2, 5% CO_2). The inhalation of carbogen induced a mean increase in optic disc Po_2 of ΔPo_2 = 12.8 ± 5.1 mm Hg, or 69% (n = 12; Fig. 1) after 7 minutes, similar to that described previously.[21] Mean optic disc Po_2 increased significantly from 18.4 ± 5.1 to 31.2 ± 9.9 mm Hg (Table 1; P < 0.001). Under the effect of carbogen, both Pao_2 and $Paco_2$ increased significantly (ΔPao_2 = 364.4 ± 59.2 mm Hg, or 355%; $\Delta Paco_2$ = 11.5 ± 2.9 mm Hg, or 33%; n = 12). The CO_2 increase in the blood reduced pH from 7.47 ± 0.03 to 7.39 ± 0.03 (ΔpH = −0.08 ± 0.02; n = 12).

Measurements under Systemic Hypercapnia. The increase of CO_2 in the inhaled gas induced a mean increase in optic disc Po_2 of ΔPo_2 = 3.2 ± 1.7 mm Hg, or 18% (n = 9; Fig. 2) after 7 minutes. Under systemic hypercapnia, mean optic disc Po_2 increased from 17.8 ± 1.9 to 20.9 ± 2.3 mm Hg and that difference, although moderate, was statistically significant (Table 1; P = 0.001) after a significant increase in $Paco_2$ (ΔPao_2 = −6.6 ± 5.3 mm Hg, or −6%; $\Delta Paco_2$ = 15.0 ± 5.6 mm Hg, or 42%; n = 9). The CO_2 increase in the blood reduced pH from 7.47 ± 0.03 to 7.38 ± 0.05 (ΔpH = −0.09 ± 0.03; n = 9).

Measurements after Intravenous Injection of L-NAME

Measurements under Systemic Normoxia. In every case, intravenous injection of L-NAME lasted 10 minutes, and continuous monitoring of arterial blood pressure and optic disc Po_2 under normoxia was performed for 30 minutes after the onset of injection.

In all cases (n = 12), L-NAME injection induced a long-lasting increase in arterial blood pressure. Mean arterial blood

TABLE 1. Summary of Optic Disc Po_2 Measurements before and after L-NAME Intravenous Injection in Different Conditions of Gas Inhalation

| | n | Mean (SD) Optic Disc PO_2 (mm Hg) | | | | | ANOVA | Post hoc 0–7 min (P) | Mean (SD) ΔPo_2 % 0–7 min |
		0 min	2 min	5 min	7 min	10 min			
Before L-NAME									
Hyperoxia	11	18.6 (4.2)	21.5 (4.6)	22.3 (4.6)	22.5 (4.9)	23.2 (5.0)	S	<0.001	22 (10)
Carbogen	12	18.4 (5.1)	25.7 (7.9)	29.9 (9.5)	31.2 (9.9)	33.1 (11.1)	S	<0.001	69 (16)
Hypercapnia	9	17.8 (1.9)	18.8 (1.7)	20.5 (2.4)	20.9 (2.3)	22.6 (1.1)	S	0.001	18 (10)
After L-NAME									
Hyperoxia	10	18.5 (2.7)	21.1 (3.0)	23.0 (3.6)	23.2 (4.0)	23.7 (4.0)	S	0.001	26 (12)*
Carbogen	12	18.8 (3.0)	23.6 (5.5)	27.0 (5.5)	27.9 (6.6)	29.2 (5.7)	S	<0.001	49 (22)†
Hypercapnia	9	19.3 (0.7)	19.5 (1.4)	19.8 (2.0)	19.9 (1.9)	20.0 (2.7)	NS	0.177 (NS)	3 (6)‡

Data are expressed as the mean ± SD. The results were evaluated by repeated-measures ANOVA followed by post hoc analysis by Bonferroni *t*-test between baseline (0 min) and 7 min. A statistically nonsignificant Po_2 increase was observed after 7 min of hypercapnia after L-NAME injection. Moreover, comparing the response to CO_2 before and after L-NAME injection, a statistically significant reduction in the response to carbogen and to hypercapnia was noted after L-NAME injection. These results illustrate the blunting effect of NO synthase inhibition on CO_2-induced optic disc Po_2 increase. *n*, number of minipigs; S, statistically significant difference; NS, statistically non-significant difference.
  * P = 0.366 (NS) versus hyperoxia before L-NAME.
  † P = 0.01 (S) versus carbogen before L-NAME.
  ‡ P < 0.001 (S) versus hypercapnia before L-NAME.
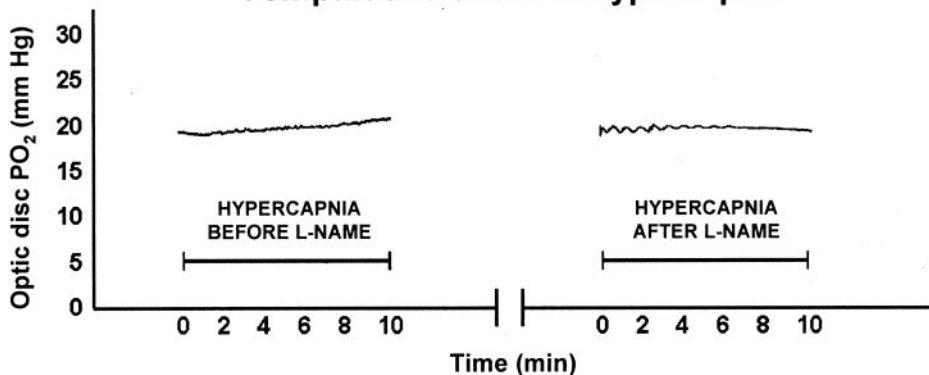
Comparative effect of hypercapnia

FIGURE 2. Continuous typical recordings of optic disc Po$_2$ during hypercapnia before (*left*) and after (*right*) L-NAME injection. The corresponding table shows the mean ± SD of the blood gas levels and of optic disc Po$_2$ variation (ΔPo$_2$) 7 minutes after the onset of each condition. *P*, post hoc comparisons by Bonferroni *t*-test between baseline means and means at 7 minutes. Hypercapnia before L-NAME injection induced a significant 18% increase of optic disc Po$_2$, whereas hypercapnia after L-NAME injection did not change optic disc Po$_2$ significantly, due to a blocking effect on CO$_2$-induced Po$_2$ increase induced by L-NAME.

Inhaled gas	n	PaO$_2$	PaCO$_2$	pH	ΔPO$_2$ 0-7 min	P 0-7 min
Hypercapnia before L-NAME	9	106.3 ± 9.3	51.0 ± 5.7	7.38 ± 0.05	3.2 ± 1.7 (18%)	0.001
Hypercapnia after L-NAME	9	101.6 ± 4.6	51.6 ± 6.4	7.35 ± 0.05	0.6 ± 1.1 (3%)	0.177

pressure was 70 ± 17 mm Hg at the onset of injection, increased moderately to 74 ± 21 mm Hg at 5 minutes of injection (5% increase from baseline; $P > 0.1$), became 83 ± 25 mm Hg at 10 minutes of injection (19% increase from baseline; $P < 0.05$), and peaked to 86 ± 26 mm Hg at 30 minutes after the onset of injection (21% increase from baseline; $P < 0.005$). Thereafter, mean arterial blood pressure decreased and returned to baseline at 60 to 65 minutes after the onset of injection, without significant variation thereafter.

Optic disc Po$_2$ under normoxia did not change significantly at any moment during or after L-NAME injection (Fig. 3). Optic disc Po$_2$ was 19.2 ± 3.7 mm Hg at the onset of injection, became 18.9 ± 2.8 mm Hg at 5 minutes of injection (2% decrease from baseline; $P > 0.5$), remained at 18.9 ± 3.1 mm

Optic disc PO$_2$ recording during 30 minutes of normoxia after L-NAME injection
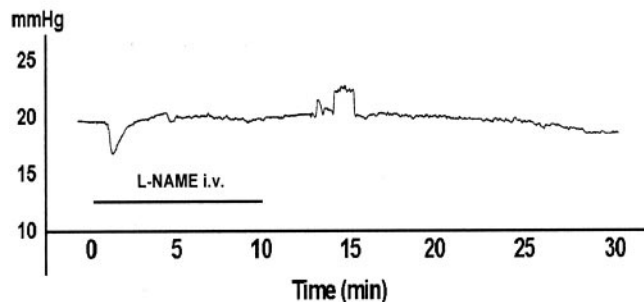

FIGURE 3. Continuous typical recordings of optic disc Po$_2$ during 30 minutes of normoxia after intravenous injection of L-NAME. Optic disc Po$_2$ did not change significantly at any moment during or after L-NAME injection, despite a progressive increase of mean arterial blood pressure (MAP), which showed a peak 21% increase on average 30 minutes after the onset of L-NAME injection. A brief decrease in optic disc Po$_2$ lasting less than 1 minute was recorded at the very beginning of each L-NAME injection, apparently caused by a transient decrease in MAP due to the shock of the bolus injection.

Hg at 10 minutes of injection (2% decrease from baseline; $P > 0.1$), and was recorded at 18.5 ± 2.8 mm Hg 30 minutes after the onset of injection (4% decrease from baseline; $P > 0.1$). Levels of Pao$_2$, Paco$_2$, and pH were within normal ranges during this period.

Measurements under Systemic Hyperoxia (100% O$_2$). The inhalation of 100% O$_2$ after L-NAME injection induced a mean increase in optic disc Po$_2$ of ΔPo$_2$ = 4.8 ± 2.3 mm Hg, or 26% ($n = 10$; Fig. 4), after 7 minutes. Under systemic hyperoxia, mean optic disc Po$_2$ increased from 18.5 ± 2.7 to 23.2 ± 4.0 mm Hg and that difference was statistically significant (Table 1; $P = 0.001$) but was again disproportional to a substantial increase in Pao$_2$ (ΔPao$_2$ = 376.2 ± 64.7 mm Hg, or 361%; ΔPaco$_2$ = −4.0 ± 4.0 mm Hg; ΔpH = 0.03 ± 0.03; $n = 10$). The response to hyperoxia after L-NAME injection was not significantly different from the response to hyperoxia before L-NAME injection (Table 1; $P = 0.366$).

Measurements during Carbogen Inhalation (95% O$_2$, 5% CO$_2$). The inhalation of carbogen after L-NAME injection induced a mean increase in optic disc Po$_2$ of ΔPo$_2$ = 9.1 ± 4.4 mm Hg, or 49% ($n = 12$; Fig. 4), after 7 minutes. Mean optic disc Po$_2$ increased significantly from 18.8 ± 3.0 to 27.9 ± 6.6 mm Hg (Table 1; $P < 0.001$). Under the effect of carbogen, both Pao$_2$ and Paco$_2$ increased significantly (ΔPao$_2$ = 392.3 ± 71.8 mm Hg, or 378%; ΔPaco$_2$ = 11.8 ± 3.2 mm Hg, or 33%; $n = 12$). The CO$_2$ increase in the blood reduced pH from 7.46 ± 0.05 to 7.37 ± 0.05 (ΔpH = −0.08 ± 0.02; $n = 12$). However, the response to carbogen after L-NAME injection was significantly lower than the response to carbogen before L-NAME injection (Table 1; $P = 0.01$), as L-NAME reduced the response to carbogen by 29% on average.

Measurements under Systemic Hypercapnia. The increase in CO$_2$ in the inhaled gas after L-NAME injection induced a mean increase in optic disc Po$_2$ of ΔPo$_2$ = 0.6 ± 1.1 mm Hg, or only 3% ($n = 9$; Fig. 2), after 7 minutes. Under systemic hypercapnia, mean optic disc Po$_2$ increased from 19.3 ± 0.7 to 19.9 ± 1.9 mm Hg, but that difference was not statistically significant (Table 1; $P = 0.177$) despite a significant increase in Paco$_2$ (ΔPao$_2$ = −6.4 ± 3.7 mm Hg, or −6%; ΔPaco$_2$ = 15.8 ± 5.4 mm Hg, or 44%; $n = 9$). The CO$_2$ increase in the blood reduced pH from 7.44 ± 0.06 to 7.35 ± 0.05 (ΔpH = −0.09 ± 0.04; $n = 9$). In addition, the response to hypercapnia after

After L-NAME injection

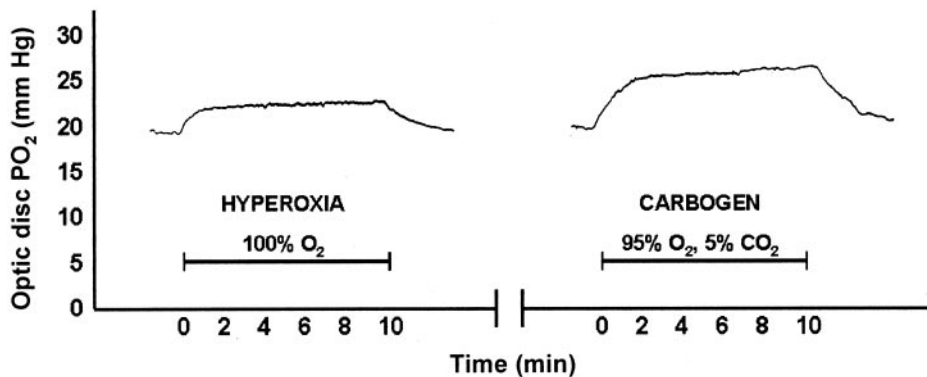| Inhaled gas	n	PaO₂	PaCO₂	pH	ΔPO₂ 0-7 min	P 0-7 min
Hyperoxia	10	480.6 ± 63.8	32.5 ± 3.3	7.49 ± 0.06	4.8 ± 2.3 (26%)	0.001
Carbogen	12	496.0 ± 71.5	47.3 ± 2.5	7.37 ± 0.05	9.1 ± 4.4 (49%)	<0.001

FIGURE 4. Continuous typical recordings of optic disc Po_2 during hyperoxia (*left*) or carbogen breathing (*right*) after L-NAME injection. The corresponding table shows the mean ± SD of the blood gas levels and of optic disc Po_2 variation (ΔPo_2) 7 minutes after the onset of each condition. *P*, post hoc comparisons by Bonferroni *t*-test between baseline means and means at 7 minutes. Carbogen breathing induced a greater increase in optic disc Po_2 than did hyperoxia, as $Paco_2$ reached higher levels during carbogen breathing. However, the difference in the two responses was lower than in Figure 1 (i.e., before L-NAME injection), as L-NAME reduced the response to carbogen by 29%, on average.

L-NAME injection was significantly lower than the response to hypercapnia before L-NAME injection (Table 1; $P < 0.001$).

In three minipigs that received L-NAME, after Po_2 measurements performed according to the protocol, intravenous injection of L-arginine was administered. In all three minipigs, L-arginine reversed the effect of L-NAME on the increase in optic disc Po_2 under hypercapnia, since the observed pattern of optic disc Po_2 variation under hypercapnia after L-arginine injection was identical with that observed under hypercapnia before L-NAME injection (data not shown).

DISCUSSION

In the present study, systemic NOS inhibition with intravenous injection of L-NAME was shown not to affect baseline optic disc Po_2 in normoxia, but to be capable of attenuating the response to hypercapnia and to carbogen breathing. These results indicate a blunting effect of systemic NOS inhibition on CO_2-induced optic disc Po_2 increase, demonstrating NO as a mediator of CO_2-induced Po_2 increase at the level of the ONH.

Baseline measurements before L-NAME injection confirmed the existence of a CO_2-induced Po_2 increase at the level of the ONH, as previously shown.[21] Systemic hyperoxia induced a marked increase in Pao_2 but only a moderate, yet statistically significant, increase in optic disc Po_2, which was then regulated to stable levels. This was apparently the result of arteriolar vasoconstriction at the level of the ONH, a mechanism that prevents excessive ONH Po_2 increase. In contrast, carbogen breathing induced a marked increase of both Pao_2 and $Paco_2$, as well as a marked increase in optic disc Po_2 due to the Bohr effect and because, most probably, the vasodilatory effect of elevated $Paco_2$ partially counterbalanced the vasoconstriction due to elevated Pao_2. Furthermore, systemic hypercapnia induced a marked increase in $Paco_2$ and a linear, moderate but statistically significant increase in optic disc Po_2 due to the Bohr effect and because of the vasodilatory effect of elevated $Paco_2$.

Injection of L-NAME blunted the CO_2-induced increase in optic disc Po_2. A nonsignificant 3% increase in optic disc Po_2 in response to hypercapnia after L-NAME injection versus a significant 18% increase in response to hypercapnia before L-NAME injection was noted. This effect was reversible when L-arginine,

an NO donor, was injected, confirming that the lack of NO was responsible for the blunting effect of L-NAME on the CO_2-induced increase in optic disc Po_2. Moreover, a 49% increase in optic disc Po_2 in response to carbogen after L-NAME injection versus a 69% increase in response to carbogen before L-NAME injection was observed, despite comparable $Paco_2$ levels between these two conditions. L-NAME reduced the response to carbogen by 29% on average. These observations showed that the presence of NO is necessary to enable the maximum CO_2-induced increase in optic disc Po_2, indicating NO as a mediator of that increase.

The blunting effect of L-NAME on the response to CO_2 noted in the present study is in accordance with the results of previous animal studies in neural tissue showing a hypercapnia-induced increase in NO concentration in the brain[37] as well as a reduction through NOS inhibition of CO_2-induced retinal vasodilation[24] and of CO_2-induced retinal[24] and cerebral[23,38,39] blood flow increase. In addition, Schmetterer et al.[40] showed a blunting effect of NOS inhibition on CO_2-induced increase in blood velocities in the middle cerebral artery and the ophthalmic artery in humans, an effect reversed by L-arginine. Thus, regarding neural tissue oxygen availability, there is growing evidence of an important role of NO in the response to hypercapnia. According to Iadecola and Zhang,[41] NO may act as a permissive factor in hypercapnia by facilitating the action of other vasodilators. Further studies are needed to confirm this assumption at the level of the ONH.

Optic disc Po_2 under normoxia did not change significantly during or after L-NAME injection, even though MAP increased by 21% from baseline. This is in accordance with the study of Bouzas et al.[11] in minipigs: intervascular ONH Po_2 200 μm deep in the tissue remained stable during and after intravenous injection of nitro-L-arginine, even though arterial blood pressure increased by 28%. Moreover, in the inner retina of minipigs, Donati et al.[42] found no effect of intravenous injection of nitro-L-arginine on arteriolar diameter, whereas local juxta-arteriolar application of the same NOS inhibitor induced transient vasoconstriction, suggesting a role of NO of neuronal origin from the glial cells surrounding the retinal arterioles in maintaining the basal retinal arteriolar tone. Studies exploring the effect of systemic NOS inhibition on ONH blood flow are somewhat conflicting: A decrease in ONH blood flow assessed

by different methods was reported in rabbits[43,44] and in humans,[32] whereas Buerk et al.[45] report variable changes of baseline ONH blood flow after NOS inhibition in cats, suggesting that other autoregulatory mechanisms compensated for the effects of NOS inhibition in the cat ONH. Thus, NO may not control basal arteriolar tone in the ONH, or other autoregulatory mechanisms may intervene to keep ONH Po$_2$ stable during NOS inhibition. These potential mechanisms may be insufficient in the presence of hypercapnia.

The molecular relation between CO$_2$ and the L-arginine–NO pathway in the retinal and ONH circulation is quite challenging but is still open to research. It is generally believed that after an increase in Pa$_{CO_2}$, CO$_2$ diffuses readily into the interstitial space and the cytoplasm of periarteriolar glial cells and/or arteriolar endothelial cells where, at the catalyzing effect of carbonic anhydrase, the production of H$^+$ lowers the interstitial and intracellular pH.[46] This probably acts as a stimulus to increase the cytosolic free Ca^{2+}, part of which is bound to calmodulin, which in turn activates NOS to produce NO from L-arginine.[26] Donati et al.[42] have presented evidence that Müller cells may be a source of NO, since these cells have a significant rate of arginine biosynthesis. NO activates guanylyl cyclase to increase the concentration of cGMP in the vascular smooth muscle cells[26,47] and in capillary pericytes.[30] This lowers the intracellular Ca^{2+} concentration by activating Ca^{2+}-sensitive K$^+$ channels and inhibiting the release of Ca^{2+} from the sarcoplasmic reticulum, thus inducing relaxation of the vascular smooth muscle cells of the arteriolar wall.[47]

In a previous study, prostaglandins have also been shown to mediate a CO$_2$-induced Po$_2$ increase at the level of the ONH.[17] The L-arginine–NO pathway and the prostaglandin pathway may act in parallel, synergistically, or they may interact. Evidence of this interaction exists in the literature. NO can activate cyclooxygenase (COX),[48] and it can also react with superoxide to form peroxynitrite, with subsequent lipid peroxidation and liberation of arachidonic acid.[49] Moreover, NOS/COX cross talk has been described wherein NO activates COX-1 but inhibits COX-2-derived prostaglandin production.[50] Conversely, prolonged hypercapnia can increase retinal blood flow in piglets by PGE$_2$-mediated increased expression of eNOS mRNA.[51] Further research is needed to demonstrate similar mechanisms at the level of the ONH.

The role of the L-arginine-NO pathway in the CO$_2$-induced Po$_2$ increase at the level of the ONH raises clinical interest in the presence of glaucomatous and/or ischemic ONH disease. A relative vasoconstriction has been shown at the level of the ONH in patients with glaucoma that can be partially reversed by hypercapnia.[52] However, a possible increase in NOS activity due to hypercapnia at the level of the ONH may expose the ONH to excessive levels of NO, which in turn could be destructive to the retinal ganglion cells through the formation of peroxynitrite, which may trigger apoptosis.[53] This adverse event should be taken into account if CO$_2$ is to be used for the treatment of glaucoma. In addition, polymorphism of the endothelial NOS gene has been proposed as an important risk factor in the development of nonarteritic anterior ischemic neuropathy.[54] Potential use of CO$_2$ to increase oxygen availability may not be effective in these patients.

In conclusion, the results of the present study showed that systemic NOS inhibition with L-NAME in minipigs does not reduce optic disc Po$_2$ in normoxia but exerts a blunting effect on CO$_2$-induced optic disc Po$_2$ increase. Evidence is thus given that the L-arginine-NO pathway mediates the increase in Po$_2$ due to hypercapnia at the level of the ONH, as this reaction is believed to happen in the inner retina and in the brain. Since prostaglandins are also mediators of the increase, studies are needed to elucidate the NO-prostaglandin interaction in neural tissue.

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