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

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PURPOSE. To investigate the effect of systemic nitric oxide synthase (NOS) inhibition on optic disc oxygen partial pressure (PO2) in normoxia and hypercapnia.

METHODS. Intervascular optic disc PO2 was measured in 12 anesthetized minipigs by using oxygen-sensitive microelectrodes placed <50 μm from the optic disc. PO2 was measured continuously during 10 minutes under normoxia, hypercapnia (100% O2), carbogen breathing (95% O2, 5% CO2), and hypercapnia (increased inhaled CO2). Measurements were repeated after intravenous injection of Nω-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 PO2 during hypercapnia (ΔPO2 = 5.2 ± 1.7 mm Hg; 18%; P = 0.001) and carbogen breathing (ΔPO2 = 12.8 ± 5.1 mm Hg; 69%; P < 0.001). Optic disc PO2 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 PO2 increase under hypercapnia was blunted after L-NAME injection (ΔPO2 = 0.6 ± 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% (ΔPO2 = 9.1 ± 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 PO2 in normoxia, a blunting effect was noted on the CO2-induced optic disc PO2 increase. Nitric oxide appears to mediate the hypercapnic optic disc PO2 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 (O2) and carbon dioxide (CO2) in the blood. Hyperoxia, or high oxygen partial pressure in the arterial blood (PaO2), constricts cerebral arteries1 and retinal arterioles2–5 leading to a decrease of cerebral,1 retinal,6–7 and ONH8,9 blood flow. However, hyperoxia does not alter the tissue oxygen availability, as tissue oxygen partial pressure (PO2) remains relatively stable in the inner retina8,9 and at intervascular areas of the ONH.10,11

On the other hand, hypercapnia, or high carbon dioxide partial pressure in the arterial blood (PaCO2), dilates cerebral arteries1,12 and retinal arterioles,13–15 leading to an increase in cerebral,1,12 retinal,13–15 and ONH blood flow. As a result, inhalation of CO2 increases preretinal16 and optic disc PO2. In addition, inhalation of carbogen (95% O2, 5% CO2), which increases both PaO2 and PaCO2, also increases preretinal,18,19 inner retinal,20 and optic disc PO2,21 since the vasodilatory effect of elevated PaCO2 partially counterbalances the vasoconstriction induced by elevated PaO2. Elevated PaCO2 increases tissue PO2 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 CO2-induced arteriolar vasodilation.13 Thus, a CO2-induced PO2 increase has been demonstrated at the level of the ONH.17,18

Nitric oxide (NO) has been reported to control CO2-induced vasodilation in the cerebral19 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 cells25 and in some neurons,26 and neuronal NOS, expressed only in neurons.26 In the ONH, NOS activity has been found in vascular endothelial cells27–29 and sparsely in astrocytes27 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.30 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 CO2-induced vasodilation in the cerebral23 and the retinal circulation.4,4 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. PO2 measurements, which reflect tissue oxygen availability,16 were performed in this study. PO2 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 PO2 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.
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 atapropine (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% O₂ and 80% nitrous oxide (N₂O) 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.

In each experiment, with the purpose of obtaining a stable optic disc PO₂ recording over 10 minutes. Normoxia was again induced, and systemic hypercapnia was induced by adding 6% CO₂ to the gas mixture and adjusting N₂O downward accordingly (i.e., gas mixture: 6% CO₂ + 20% O₂ + 74% N₂O). Optic disc PO₂ recording was performed for 10 minutes, and thereafter the animal was allowed to return to normoxia. Each condition was confirmed by corresponding PaO₂, Paco₂, and pH measurements: PaO₂ had to be high in hyperoxia, Paco₂ had to be high in hypercapnia, and both PaO₂ and Paco₂ had to be high during carbogen breathing.

After several cycles of the above-described measurements, we proceeded to intravenous injection of Nω-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 PO₂ measurements were taken under systemic hyperoxia, carbogen breathing, and systemic hypercapnia, as described earlier.

In three animals, after optic disc PO₂ 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 PO₂ measurements were repeated under systemic hypercapnia.

N₂O was present in the breathing gas during normoxia, whereas it was adjusted downward whenever CO₂ 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 N₂O in the breathing gas during normoxia and had not found any differences in pH, Paco₂, PaO₂, or optic disc PO₂ (data not shown).

Statistics

Optic disc PO₂, PaO₂, Paco₂, and pH, as well as their differences from baseline (optic disc ΔPO₂, ΔPaO₂, ΔPaco₂, and ΔpH), are expressed as the mean ± 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 PaO₂, PaCO₂, 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 (PaO₂ = 103.6 ± 17.9 mm Hg; PaCO₂ = 35.4 ± 2.4 mm Hg; pH = 7.47 ± 0.04; n = 12), mean optic disc PO₂ recorded in intervascular areas of 12 eyes was 18.3 ± 4.0 mm Hg, a level similar to those described previously in minipigs.10,11,17,21

Measurements under Systemic Hyperoxia (100% O₂).

The inhalation of 100% O₂ induced a mean increase in optic disc PO₂ of ΔPO₂ = 3.9 ± 1.6 mm Hg, or 22% (n = 11; Fig. 1) after 7 minutes. Under systemic hyperoxia, mean optic disc PO₂ increased from 18.6 ± 4.2 to 22.5 ± 4.9 mm Hg and that difference, although moderate, was statistically significant (Table 1; P < 0.001) but disproportional to a substantial increase.

In the presence or absence of N₂O in the breathing gas during normoxia and hypercapnia, the mean PaO₂ and PaCO₂ differed by a factor of 2 or more. These differences were also statistically significant (Table 1; P < 0.001) but not proportional to a substantial increase.

In the case of systemic hyperoxia, the mean optic disc PO₂ increased by 6.4 ± 1.6 mm Hg during systemic hypercapnia. This increase was statistically significant (Table 1; P < 0.001) but disproportional to a substantial increase.
in PaO2 (ΔPaO2 = 353.6 ± 83.2 mm Hg, or 365%; ΔPaCO2 = −2.9 ± 3.6 mm Hg; ΔpH = 0.03 ± 0.03; n = 11), as previously described.21

Measurements during Carbogen Inhalation (95% O2, 5% CO2). The inhalation of carbogen induced a mean increase in optic disc PO2 of ΔPO2 = 12.8 ± 5.1 mm Hg, or 69% (n = 12; Fig. 1) after 7 minutes, similar to that described previously.21 Mean optic disc PO2 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 PaO2 and PaCO2 increased significantly (ΔPaO2 = 364.4 ± 59.2 mm Hg, or 355%; ΔPaCO2 = 11.5 ± 2.9 mm Hg, or 33%; n = 12). The CO2 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 CO2 in the inhaled gas induced a mean increase in optic disc PO2 of ΔPO2 = 3.2 ± 1.7 mm Hg, or 18% (n = 9; Fig. 2) after 7 minutes. Under systemic hypercapnia, mean optic disc PO2 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 PaCO2 (ΔPaCO2 = −6.6 ± 5.3 mm Hg, or −6%; ΔPaCO2 = 15.0 ± 5.6 mm Hg, or 42%; n = 9). The CO2 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 PO2 under normoxia was performed for 30 minutes after the onset of injection.

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

![Graph showing optic disc PO2 measurements](image)

**FIGURE 1.** Continuous typical recordings of optic disc PO2 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 PO2 variation (ΔPO2) 7 minutes after the onset of each condition. P, post hoc comparisons by Bonferroni t-test between baseline means and means at 7 minutes. Car- bogen breathing induced a greater increase in optic disc PO2 than did hyperoxia, because PaCO2 reached higher levels during carbogen breathing.

<table>
<thead>
<tr>
<th>Inhaled gas</th>
<th>n</th>
<th>PaO2 (mm Hg)</th>
<th>PaCO2 (mm Hg)</th>
<th>pH</th>
<th>ΔPO2 0–7 min</th>
<th>t-test 0–7 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperoxia</td>
<td>11</td>
<td>450.7 ± 72.4</td>
<td>32.5 ± 3.2</td>
<td>7.49 ± 0.07</td>
<td>3.9 ± 1.6 (22%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Carbogen</td>
<td>12</td>
<td>467.0 ± 61.5</td>
<td>46.5 ± 2.8</td>
<td>7.39 ± 0.03</td>
<td>12.8 ± 6.1 (69%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**TABLE 1.** Summary of Optic Disc PO2 Measurements before and after l-NAME Intravenous Injection in Different Conditions of Gas Inhalation

<table>
<thead>
<tr>
<th>Before l-NAME</th>
<th>Mean (SD) Optic Disc PO2 (mm Hg)</th>
<th>ANOVA Post hoc 0–7 min</th>
<th>Mean (SD) ΔPO2 % 0–7 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperoxia</td>
<td>11</td>
<td>18.6 (4.2)</td>
<td>21.5 (4.6)</td>
</tr>
<tr>
<td>Carbogen</td>
<td>12</td>
<td>18.4 (5.1)</td>
<td>25.7 (7.9)</td>
</tr>
<tr>
<td>Hypercapnia</td>
<td>9</td>
<td>17.8 (1.9)</td>
<td>18.8 (1.7)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>After l-NAME</th>
<th>Mean (SD) Optic Disc PO2 (mm Hg)</th>
<th>ANOVA Post hoc 0–7 min</th>
<th>Mean (SD) ΔPO2 % 0–7 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperoxia</td>
<td>10</td>
<td>18.5 (2.7)</td>
<td>21.1 (3.0)</td>
</tr>
<tr>
<td>Carbogen</td>
<td>12</td>
<td>18.8 (3.0)</td>
<td>23.6 (5.5)</td>
</tr>
<tr>
<td>Hypercapnia</td>
<td>9</td>
<td>19.3 (0.7)</td>
<td>19.5 (1.4)</td>
</tr>
</tbody>
</table>

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 PO2 increase was observed after 7 min of hypercapnia after l-NAME injection. Moreover, comparing the response to CO2 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 CO2-induced optic disc PO2 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.
Measurements during Systemic Hyperoxia (100% O₂).
The inhalation of 100% O₂ after i-NNAME injection induced a mean increase in optic disc PO₂ of ΔPO₂ = 4.8 ± 2.3 mm Hg, or 26% (n = 10; Fig. 4), after 7 minutes. Under systemic hyperoxia, mean optic disc PO₂ 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 PaO₂ (ΔPaO₂ = 376.2 ± 64.7 mm Hg, or 361%; ΔPaCO₂ = −4.0 ± 4.0 mm Hg; ΔpH = 0.03 ± 0.03; *n* = 10). The response to hyperoxia after i-NNAME injection was not significantly different from the response to hyperoxia before i-NNAME injection (Table 1; *P* = 0.366).

Measurements during Carbogen Inhalation (95% O₂, 5% CO₂). The inhalation of carbogen after i-NNAME injection induced a mean increase in optic disc PO₂ of ΔPO₂ = 9.1 ± 4.4 mm Hg, or 49% (n = 12; Fig. 4), after 7 minutes. Mean optic disc PO₂ 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 PaO₂ and PaCO₂ increased significantly (ΔPaO₂ = 392.3 ± 71.8 mm Hg, or 178%; ΔPaCO₂ = 11.8 ± 3.2 mm Hg, or 35%; *n* = 12). The CO₂ 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 i-NNAME injection was significantly lower than the response to carbogen before i-NNAME injection (Table 1; *P* = 0.01), as i-NNAME reduced the response to carbogen by 29% on average.

Measurements under Systemic Hypercapnia. The increase in CO₂ in the inhaled gas after i-NNAME injection induced a mean increase in optic disc PO₂ of ΔPO₂ = 0.6 ± 1.1 mm Hg, or only 3% (n = 9; Fig. 2), after 7 minutes. Under systemic hypercapnia, mean optic disc PO₂ 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 PaCO₂ (ΔPaCO₂ = −6.4 ± 3.7 mm Hg, or −6%; ΔPaCO₂ = 15.8 ± 5.4 mm Hg, or 44%; *n* = 9). The CO₂ 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

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**FIGURE 3.** Continuous typical recordings of optic disc PO₂ during 30 minutes of normoxia after intravenous injection of i-NNAME. Optic disc PO₂ did not change significantly at any moment during or after i-NNAME 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 i-NNAME injection. A brief decrease in optic disc PO₂ lasting less than 1 minute was recorded at the very beginning of each i-NNAME injection, apparently caused by a transient decrease in MAP due to the shock of the bolus injection.
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 $P_{O_2}$ 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 $P_{O_2}$ under hypercapnia, since the observed pattern of optic disc $P_{O_2}$ 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 $P_{O_2}$ 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 $P_{O_2}$ increase, demonstrating NO as a mediator of CO$_2$-induced $P_{O_2}$ increase at the level of the ONH.

Baseline measurements before l-NAME injection confirmed the existence of a CO$_2$-induced $P_{O_2}$ increase at the level of the ONH, as previously shown. Systemic hypercapnia induced a marked increase in $P_{O_2}$ but only a moderate, yet statistically significant, increase in optic disc $P_{O_2}$, 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 $P_{O_2}$ increase. In contrast, carbogen breathing induced a marked increase of both $P_{O_2}$ and $P_{CO_2}$, as well as a marked increase in optic disc $P_{O_2}$ due to the Bohr effect and because, most probably, the vasodilatory effect of elevated $P_{CO_2}$ partially counterbalanced the vasoconstriction due to elevated $P_{O_2}$. Furthermore, systemic hypercapnia induced a marked increase in $P_{CO_2}$ and a linear, moderate but statistically significant increase in optic disc $P_{O_2}$ due to the Bohr effect and because of the vasodilatory effect of elevated $P_{CO_2}$.

Injection of l-NAME blunted the CO$_2$-induced increase in optic disc $P_{O_2}$. A nonsignificant 3% increase in optic disc $P_{O_2}$ 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 $P_{O_2}$. Moreover, a 49% increase in optic disc $P_{O_2}$ in response to carbogen after l-NAME injection versus a 69% increase in response to carbogen before l-NAME injection was observed, despite comparable $P_{CO_2}$ 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 $P_{O_2}$, 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 as well as a reduction through NOS inhibition of CO$_2$-induced retinal vasodilation and of CO$_2$-induced retinal and cerebral blood flow increase. In addition, Schmetterer et al. 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, 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 $P_{O_2}$ 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. in minipigs: intravascular ONH $P_{O_2}$ 200 µm deep in the tissue remained stable during and after intravenous injection of nitro-arginine, even though arterial blood pressure increased by 28%. Moreover, in the inner retina of minipigs, Donati et al. found no effect of intravenous injection of nitro-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 with the laser Doppler flowmeter was noted during systemic NOS inhibition, whereas a pressor effect of NOS inhibition was observed with the end-tidal P$_{CO_2}$ method.
by different methods was reported in rabbits\textsuperscript{33,44} and in humans,\textsuperscript{52} whereas Buerk et al.\textsuperscript{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\textsubscript{2} stable during NOS inhibition. These potential mechanisms may be insufficient in the presence of hypercapnia.

The molecular relation between CO\textsubscript{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 Paco\textsubscript{2}, CO\textsubscript{2} diffuses readily into the interstitial space and the cytoplasm of periarriolar glial cells and/or arteriolar endothelial cells where, at the catalyzing effect of carbonic anhydrase, the production of H\textsuperscript{+} lowers the interstitial and intracellular pH.\textsuperscript{46} This probably acts as a stimulus to increase the cytosolic free Ca\textsuperscript{2+}, part of which is bound to calmodulin, which in turn activates NOS to produce NO from l-arginine.\textsuperscript{26} Donati et al.\textsuperscript{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\textsuperscript{36,47} and in capillary pericytes.\textsuperscript{38} This lowers the intracellular Ca\textsuperscript{2+} concentration by activating Ca\textsuperscript{2+}-sensitive K\textsuperscript{+} channels and inhibiting the release of Ca\textsuperscript{2+} from the sarcoplasmic reticulum, thus inducing relaxation of the vascular smooth muscle cells of the arteriolar wall.\textsuperscript{47}

In a previous study, prostaglandins have also been shown to mediate a CO\textsubscript{2}-induced PO\textsubscript{2} increase at the level of the ONH.\textsuperscript{15} 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),\textsuperscript{48} and it can also react with superoxide to form peroxynitrite, with subsequent lipid peroxidation and liberation of arachidonic acid.\textsuperscript{49} Moreover, NOS/COX cross talk has been described wherein NO activates COX-1 but inhibits COX-2-derived prostaglandin production. Conversely, prolonged hypercapnia can increase retinal blood flow in piglets by PGE\textsubscript{2}-mediated increased expression of eNOS mRNA.\textsuperscript{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\textsubscript{2}-induced PO\textsubscript{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.\textsuperscript{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.\textsuperscript{53} This adverse event should be taken into account if CO\textsubscript{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.\textsuperscript{54} Potential use of CO\textsubscript{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\textsubscript{2} in normoxia but exerts a blunting effect on CO\textsubscript{2}-induced optic disc PO\textsubscript{2} increase. Evidence is thus given that the l-arginine–NO pathway mediates the increase in PO\textsubscript{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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