Nitric Oxide Synthase Inhibition Alters Cerebral Blood Flow and Oxygen Balance in Focal Cerebral Ischemia in Rats

Hwu Meei Wei, PhD; Oak Za Chi, MD; Xia Liu, MD; Arabinda K. Sinha, PhD; Harvey R. Weiss, PhD

Background and Purpose This study investigated whether the nitric oxide synthase inhibitor N\textsuperscript{\textdegree}O-nitro-L-arginine-methyl ester (L-NAME) would alter blood flow and oxygen balance in the ischemic cerebrocortex of isoflurane-anehydrated Long-Evans rats.

Methods Fifteen minutes after middle cerebral artery occlusion, L-NAME (1.5 mg/min per kilogram) was infused intravenously to the L-NAME group (n=14), and normal saline was given to the control group (n=14) for 45 minutes. In each group, regional cerebral blood flow was determined with \(^{14}\text{C}\)iodoantipyrine, and arterial and venous oxygen saturations were determined by microspectrophotometry.

Results In both groups regional cerebral blood flow of the ischemic cortex was significantly lower than that of the contralateral cortex (mean±SD) 55±13 versus 110±29 mL/min per 100 g in the control group and 35±13 versus 90±24 mL/min per 100 g in the L-NAME group). Compared with the blood flow in the ischemic cortex of the control group, L-NAME significantly reduced ischemic blood flow by 36%. Venous oxygen saturation was significantly increased in the ischemic cortex (41±1% versus 44±3%) but decreased in the contra-lateral cortex (65±3% versus 61±4%) by L-NAME. Calculated ischemic cortical oxygen consumption in the L-NAME group was 39% lower than that in the corresponding control group, whereas the difference was only 11% in the contralateral sides between groups. In both groups, the ratio of oxygen supply to consumption was lower in the ischemic than in the nonischemic regions. In the ischemic cortex, this ratio was significantly lower in the control group than in the L-NAME group (1.7±0.1 versus 1.9±0.1). In contrast, the ratio tended to be decreased by L-NAME in nonischemic regions.

Conclusions These observations suggest that despite a decrease in cerebral blood flow, inhibition of nitric oxide synthase mildly improves the oxygen supply and consumption balance in the ischemic cortex. (Stroke. 1994;25:445-450.)

Key Words • cerebral arteries • cerebral blood flow • nitric oxide • oxygen • rats

Nitric oxide (NO) is an important regulator of blood flow\textsuperscript{1,2} and also works as a neuronal messenger via cyclic GMP.\textsuperscript{3-5} Recent studies regarding the therapeutic utility of nitric oxide synthase (NOS) inhibitors in reducing ischemia-induced neuronal damage are very controversial. There is evidence that inhibition of NO synthesis increases\textsuperscript{6} and L-arginine, the precursor of NO, decreases\textsuperscript{7} focal ischemic infarction in rats. However, an in vivo study has demonstrated that inhibition of NOS reduces the volume of the lesions produced by occlusion of middle cerebral artery (MCA) in mice.\textsuperscript{8}

The possible neuroprotective effect of NO or NOS inhibitors in ischemia-induced neuronal damage could occur at the vascular and/or neuronal level. In either case, NO may affect the oxygen supply and consumption balance in the ischemic brain. During a stroke cerebral oxygen supply is decreased in relation to metabolic needs, i.e., an imbalance exists between oxygen supply and consumption. One of the neurotoxic effects of ischemic stroke may be related to the “hypermetabolism” of released excitatory amino acids, which could worsen the balance of oxygen supply and consumption in the ischemic area.\textsuperscript{9,10} It has recently been proposed that NO is associated with the neurotoxicity produced by the excitatory amino acid neurotransmitters in response to cerebral ischemia.\textsuperscript{11,12} Therefore, the level of NO in the ischemic brain may also influence the effect of excitatory amino acids on oxygen balance.

We hypothesized that an NOS inhibitor would worsen the balance of oxygen supply to consumption by reducing blood flow and/or increasing metabolic needs in the ischemic area of the brain. The effect of NO on cerebral oxygen balance in the in vivo ischemia brain has not been studied. The purpose of the present study was to test, using a microspectrophotometric technique, whether N\textsuperscript{\textdegree}O nitro-L-arginine-methyl ester (L-NAME), an NOS inhibitor, would alter the balance of oxygen supply and consumption in the focal ischemic area of the brain induced by occlusion of the MCA in the rat. We report that inhibition of NO synthesis decreases both blood flow and oxygen consumption but mildly increases venous oxygen saturation and improves the ratio of oxygen supply to consumption in ischemic brain.

Materials and Methods Twenty-eight adult male Long-Evans rats weighing 300 to 400 g were used in this study. They were divided into two
groups of 14 each: the control group and the L-NAME group. Half the animals in each group were used to determine cerebral blood flow, and the other half were used to determine the arterial and venous oxygen saturations.

Animals were anesthetized with 1.4% isoﬂurane and mechanically ventilated. Two femoral veins and one femoral artery were cannulated. The femoral artery catheter was connected to a Statham P23Db pressure transducer and a Beckman R-411 recorder to obtain heart rate and blood pressure. This catheter was also used to obtain arterial blood samples for analysis of P02 and PC02 on a blood gas analyzer. Surgical procedures for MCA exposure and ligation were modiﬁed from those of Tamura et al. Briefly, an incision was made near the superior and posterior margins of the temporals muscle. The infratemporal fossa was exposed. A hole was drilled at the junction between the medial wall and the roof of the infratemporal fossa. The artery was occluded as close to the base of the skull as possible.

Fifteen minutes after MCA occlusion, L-NAME was infused into one of the venous canulas at a rate of 1.7 mL/h (1.5 mg/min per kilogram) for 45 minutes. The same amount of normal saline solution without L-NAME was infused in the control group. Body temperature was monitored and maintained at 37°C with a servocontrolled rectal thermistor probe and a heating lamp.

One hour after MCA occlusion, half the rats in each group were infused with 15 μCi of [14C]iodoantipyrine into the other venous cannula for the determination of regional cerebral blood flow (rCBF). When the isotope entered the venous circulation the arterial catheter was cut to a length of 20 mm to minimize smearing in the sampling catheter. Blood samples (20 μL) were obtained from the arterial catheter approximately every 3 seconds during the next minute. At the moment the last sample was obtained the decapitated head was frozen in liquid nitrogen. While frozen, the brain was sampled from four brain regions: ischemic cortex, contralateral cortex, basal ganglia, and pons. Blood and tissue samples were then placed in a tissue solubilizer, and 24 hours later they were put in a counting fluid. These samples were counted on a liquid scintillation counter. The isotope counts were quench corrected.

Regional cerebral blood flow determinations were made using the equation

\[ \text{Ci(T)} = \lambda K \int_0^T C_i(t) e^{-Kt} \, dt \]

where \( \text{Ci(T)} \) equals the tissue concentration of the [14C]iodoantipyrine at the time of decapitation; \( \lambda \) equals the tissue to blood partition coefficient; \( C_i \) is the arterial concentration of the tracer; and \( t \) equals time. \( K \) is deﬁned as follows:

\[ K = mF/W, \]

where \( m \) is the constant related to diffusion and \( F/W \) equals the blood flow per unit mass of tissue. The \( \lambda \) value of 0.8 calculated by Sakurada et al. was used. rCBF was expressed in milliliters per minute per 100 g. Regional cerebral vascular resistance (rCVR) was calculated as the ratio of mean arterial blood pressure to rCBF and expressed in mm Hg · mL⁻¹ · min · 100 g⁻¹.

The other half of the animals in each group were used to determine arterial and venous oxygen saturations after 1 hour of MCA ligation. Details of this technique have been published previously. Briefly, the head was frozen in liquid nitrogen as soon as the rat was decapitated. The frozen brain, stored in liquid nitrogen until analyzed, was cut into wafers at -20°C. The following four regions were isolated and examined: ischemic cortex, contralateral cortex, basal ganglia, and pons. The regions were mounted with embedding medium in a microtome-cryostat. Twenty-micrometer sections were obtained on the microtome-cryostat at -35°C under a nitrogen atmosphere. The sections were transferred to precooled glass slides and covered with degassed silicone oil and a coverslip. These slides were placed on a microspectrophotometer fitted with a nitrogen-flushed cold stage to obtain readings of optical density at 568, 523, and 560 nm. This three-wavelength method corrects for the light scattering in the frozen blood. Only vessels in the transverse section (~40 μm) were studied so that the path of light only traversed the blood. Readings were obtained to determine oxygen saturation in five arteries and eight veins in each region. The oxygen content of blood was determined by multiplying the percent oxygen saturation by the hemoglobin concentration times 1.36, the maximal binding capacity of hemoglobin for oxygen per gram. The difference between the average arterial and venous oxygen contents (regional oxygen extraction) was then obtained. Using the Fick principle, we calculated the oxygen consumption for each region as the product of average flow and oxygen extraction. The ratio of oxygen supply to consumption was determined by dividing oxygen supply by consumption: CaO₂ × rCBF/rCBF × (CaO₂ - CVO₂), where CaO₂ and CVO₂ are arterial and venous O₂ content. This equation for the ratio of oxygen supply to consumption becomes SaO₂/(SaO₂ - SvO₂), where SaO₂ and SvO₂ are the percent oxyhemoglobin in the arterial and venous blood, respectively.

ANOVA was applied for the various measurements performed to determine the difference between brain regions and groups. Post hoc multiple comparisons were made using Duncan's post hoc procedure. All values are expressed as mean±SD. A value of \( P<0.05 \) was considered statistically significant.

Results

Hemodynamic and blood gas parameters for the two groups are presented in Table 1. The values for heart rate and blood gas data were not different between the groups, and these data were within the normal range for the anesthetized rat. L-NAME signiﬁcantly increased mean arterial blood pressure by 45%.

Values for rCBF are presented in Fig 1. The cerebral blood flow of the ischemic cortex was 50% lower than that of the contralateral cortex in the control group (55±13 versus 110±29 mL/min per 100 g), while cerebral vascular resistance was doubled by ischemia in this group (1.87±0.62 versus 0.94±0.33 mm Hg · mL⁻¹ · min · 100 g). In the L-NAME group, the blood flow of the ischemic cortex was decreased to 39% that of the contralateral cortex (35±13 versus 90±24 mL/min per 100 g), and resistance was increased to 2.70±0.80 (4.19±1.34 versus 1.55±0.43 mm Hg · mL⁻¹ · min · 100 g). Compared with the clotting time

**TABLE 1. Hemodynamic and Blood Gas Parameters for Rats 1 Hour After Unilateral Middle Cerebral Artery Occlusion**

<table>
<thead>
<tr>
<th></th>
<th>Control (n=14)</th>
<th>L-NAME (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure, mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>117±11</td>
<td>155±14*</td>
</tr>
<tr>
<td>Diastolic</td>
<td>83±11</td>
<td>127±11*</td>
</tr>
<tr>
<td>Mean</td>
<td>94±11</td>
<td>136±11*</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>322±35</td>
<td>347±35</td>
</tr>
<tr>
<td>PaO₂, mm Hg</td>
<td>95±11</td>
<td>95±10</td>
</tr>
<tr>
<td>Paco₂, mm Hg</td>
<td>39±3</td>
<td>39±2</td>
</tr>
<tr>
<td>pH, U</td>
<td>7.36±0.03</td>
<td>7.34±0.07</td>
</tr>
<tr>
<td>Hemoglobin, g/100 mL</td>
<td>14.3±0.4</td>
<td>14.7±0.9</td>
</tr>
</tbody>
</table>

L-NAME indicates N²-nitro-L-arginine-methyl ester; bpm, beats per minute.

*Significantly different from corresponding data in control group.
the control group, blood flow in the ischemic cortex of the L-NAME group was significantly reduced, a decrease of 36%. There were no statistical differences in the rCBF of the nonischemic regions between the groups (rCBF decreased 18% to 23% in the presence of L-NAME). However, L-NAME significantly increased rCVR in all measured areas. In both groups there were no regional differences in blood flow and vascular resistance of the nonischemic regions.

In both groups regional cerebral venous oxygen saturation was significantly decreased in the ischemic cortex compared with those of the nonischemic brain regions (Table 2). Venous oxygen saturation of the ischemic cortex of the L-NAME group was significantly higher than that of the same region of the control group. However, L-NAME significantly reduced the venous oxygen saturation in nonischemic regions. There were no significant differences in arterial oxygen saturation between any treatment or region (Table 2). In both groups, oxygen extraction in ischemic cortex was significantly higher than those in the nonischemic regions (Table 2). Except for the basal ganglia where L-NAME caused a significantly higher oxygen extraction, there were no differences in oxygen extraction between the groups.

Regional cerebral oxygen consumption was calculated as a product of the mean of the regional blood flow and the mean of the oxygen extraction. In the control group oxygen consumption was 5.7 mL of oxygen per minute per 100 g in ischemic cortex, 6.5 in contralateral cortex, 7.0 in basal ganglia, and 8.4 in pons. The difference in oxygen consumption between ischemic and contralateral cortex was 11% in the control group. In the L-NAME group the oxygen consumption of the ischemic cortex (3.5 mL of oxygen per minute per 100 g) was 61% of that in the corresponding control group and was more than 40% lower than the values for contralateral cortex (5.8), basal ganglia (6.4), and pons (8.0) of the same group.

In both groups the ratio of oxygen supply to consumption in the ischemic cortex was significantly lower than those in the nonischemic regions (Fig 2). L-NAME significantly increased this ratio in the ischemic cortex (1.7±0.1 in the control group and 1.9±0.1 in the L-NAME group) and lowered it in the basal ganglia (3.2±0.4 in the control group and 2.7±0.2 in the L-NAME group).

**Discussion**

Our data demonstrated that an NOS inhibitor, L-NAME, decreased blood flow and oxygen consumption but increased venous oxygen saturation in an ischemic brain region. We found that MCA occlusion induced a significant increase in regional oxygen extraction that helped maintain oxygen consumption in the ischemic area of the control group. L-NAME mildly but significantly increased the ratio of oxygen supply to consumption in the ischemic region. Calculated oxygen extraction...
consumption was lowered 39% by L-NAME in the ischemic region, and this effect may not be due to flow limitation because of the increased venous oxygen saturation in the presence of L-NAME.

L-NAME is known to inhibit NOS in the endothelium and certain central neuronal and glial cells. The dosage used in this study was based on our unpublished data and the experience of other investigators. L-NAME increased systemic arterial blood pressure by 45% in this study, which was comparable to recently reported data using N^G-monomethyl-L-arginine as an NOS inhibitor. This increase in blood pressure indicated vasoconstriction of the systemic resistance vessels and was considered to be due to inhibition of NO synthesis in the systemic vascular endothelium. Increasing evidence demonstrates that NO also plays a role in maintaining basal cerebral blood flow and in decreasing cerebral vascular resistance during hypoxia, hypercapnia, and neurally induced relaxation. However, baseline blood flow was not reduced in control brain regions, which was in contrast to data presented by other investigators.

We have reported that phenylephrine, which raises blood pressure to an extent similar to that of L-NAME, also increases cerebral blood flow in isoflurane-anesthetized rats. It is likely that cerebral autoregulation is impaired by isoflurane and that this limits the flow decrement caused by increased resistance in the ischemic region. The same but opposite effect of L-NAME may limit blood supply to the ischemic region by a direct vascular effect. NO is also known to inhibit platelet aggregation, which helps to prevent the blockage of microvessels during ischemia. An in vivo study demonstrates that inhibition of NO synthesis increases focal ischemic infarct size in the rat, suggesting that dilatation of vessels adjacent to NOS-containing neurons may preserve blood supply in the presence of ischemia. Other in vivo studies also show that NO may play a beneficial role during ischemia.

Recent studies further support a role of NO as a neuronal messenger. NO and NOS inhibitors have no effects on cerebral metabolism under basal conditions. However, NOS inhibitors have been reported to increase cerebral vascular resistance and to lower cerebral metabolic rate in hyperglycemic conditions. The advantage of this study is that our microspectrophotometric technique provides one of the few methods of quantitatively determining oxygen consumption in ischemia. Measurements with deoxyglucose uptake are limited by potential alterations in the relationship of cerebral metabolism under hypoglycemic and hypoxic conditions. The advantage of this study is that we have measured approximately 2 mmol/L NO being produced in ischemic cortex during the ischemic period. The decrease in oxygen consumption in ischemic brain treated with L-NAME also may be due in part to the decreased demand for oxygen to produce NO. Alternatively, the differential effects of L-NAME on cerebral blood flow and oxygen consumption in the ischemic versus nonischemic areas may be due to reduced perfusion pressure or other ischemia-induced factors that increase the ability of L-NAME or any other vasoconstrictors to decrease flow in the ischemic area.

Nitric oxide has been recently implicated as a key mediator of N-methyl-D-aspartate (NMDA) receptor-associated glutamate neurotoxicity in primary neuronal culture. NMDA receptor-induced elevation of intracellular Ca^2+ can rapidly activate NOS. Therefore, both NO and excitatory amino acids levels may be significantly elevated under ischemic conditions. Previous studies suggest that the disturbance in cerebral energy metabolism between supply and demand may be one of the factors responsible for the neuronal damage in cerebral ischemia. Because both NO and excitatory amino acids levels may be significantly elevated under ischemic conditions, the relation between ischemia-induced neurotoxicity and NO-dependent alteration in oxygen balance should be considered. Our data demonstrated that L-NAME reduced both cerebral blood flow (36%) and oxygen consumption (39%) and mildly improved the balance of oxygen supply to consumption in the ischemic area. Flow limitation may not be the major cause for the L-NAME-induced decrease in oxygen consumption because venous oxygen saturation is increased in the presence of L-NAME. Our data suggest that L-NAME may exert a direct metabolism-attenuating effect at the neuronal and glial levels in the ischemic areas. Alternatively, the decrease in oxygen consumption by L-NAME could at least in part be due to the limitation of blood flow. The small increase in venous oxygen saturation may be due to the decrease in perfused surface area and/or the transit time of blood in the capillary level caused by a severe precapillary vascular constriction in the presence of L-NAME.

Studies performed by other investigators to evaluate the effects of NOS inhibitors on ischemia-induced infarction are very controversial. Although our study demonstrated that the ratio of oxygen supply to consumption in the ischemic cortex slightly increased from 1.7±0.1 to 1.9±0.1 with L-NAME, the physiological contribution of this small increase is questionable. The improved oxygen balance with L-NAME in the ischemic area may be simply due to cell death. It could also be related to the attenuated metabolic needs. The differential effects of L-NAME in ischemic versus nonischemic areas on blood flow, oxygen consumption, venous oxygen saturation, and the ratio of oxygen supply to consumption may result from both the vascular and nonvascular actions of NO. At the vascular level NO production may be increased in response to ischemic challenge and may thus play a role in maintaining the blood supply of the ischemic area. At the neuronal and glial levels NO may enhance metabolic needs, and this
action may be related to the excessively released excitatory amino acids during ischemia or other NO-mediated neuronal effects. Since venous oxygen saturation increased in the ischemic area, the reduction in oxygen consumption may not be due to flow limitation.

References


Editorial Comment

Nitric oxide (NO) (or endothelial-derived relaxing factor) plays an important role in regulation of the cerebral circulation under normal conditions. During ischemia, increased production of nitric oxide may have both protective and cytotoxic effects. Beneficial effects of NO include maintenance of cerebral blood flow, inhibition of aggregation and adherence of platelets, and inhibition of N-methyl-D-aspartate receptors that may mediate cellular damage. Detrimental effects of excessive production of NO are possible because high concentrations of NO are cytotoxic. Studies using cultured neurons suggest that glutamate-induced neurotoxicity and neuronal damage due to hypoxia may be mediated by NO. In the present study, Wei et al examined the hypothesis that inhibition of NO synthase using Nomega-nitro-L-arginine-methyl ester (L-NAME) would worsen the balance between oxygen delivery and oxygen consumption in ischemic regions of the brain. Although administration of L-NAME produced an additional decrease in cerebral blood flow in the ischemic cortex, L-NAME produced modest improvement in the balance between oxygen supply and oxygen consumption. The influence of NO during cerebral ischemia is potentially complex and its underlying mechanisms are poorly understood at present. The present study provides additional insight into the influence of NO and
acute administration of an inhibitor of NO synthase during cerebral ischemia.

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Nitric oxide synthase inhibition alters cerebral blood flow and oxygen balance in focal cerebral ischemia in rats.

H M Wei, O Z Chi, X Liu, A K Sinha and H R Weiss

Stroke. 1994;25:445-449
doi: 10.1161/01.STR.25.2.445

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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