Effect of Nitric Oxide Synthase Inhibition on Cerebral Blood Flow and Injury Volume During Focal Ischemia in Cats

Toshiaki Nishikawa, MD; Jeffrey R. Kirsch, MD; Raymond C. Koehler, PhD; David S. Bredt, PhD; Solomon H. Snyder, MD; Richard J. Traylor, PhD

Background and Purpose: We tested the hypothesis that inhibition of nitric oxide synthase activity in brain before ischemia alters cerebral blood flow and decreases brain injury after 4 hours of middle cerebral artery occlusion in cats.

Methods: Halothane-anesthetized cats underwent 4 hours of left middle cerebral artery occlusion after they were randomly assigned to receive either intravenous Nω-nitro-L-arginine methyl ester, at a dose that completely inhibited cortical nitric oxide synthase activity (10 mg/kg, n = 10), or an equal volume of diluent (10 mL saline, n = 10). Serial blood flow measurements were made with radiolabeled microspheres, and injury volume was measured by triphenyltetrazolium staining.

Results: Blood flow to caudate nucleus and inferior temporal cortex decreased to the same extent in both groups during middle cerebral artery occlusion. Somatosensory evoked potential amplitude was reduced to less than 10% of baseline values in both groups. Injury volume of ipsilateral caudate nucleus in cats pretreated with nitroarginine (52 ± 5%, mean ± SE) was less (P < .05) compared with the saline group (80 ± 4%), whereas ipsilateral cerebral hemispheric injury volume was similar between the two groups (30 ± 6% and 32 ± 4% of hemisphere in saline and nitroarginine groups, respectively).

Conclusions: These results suggest that inhibition of nitric oxide synthase decreases caudate injury volume at 4 hours of middle cerebral artery occlusion without an alteration in distribution of cerebral blood flow. (Stroke. 1993;24:1717-1724.)

Key Words • cerebral arteries • cerebral blood flow • evoked potentials, somatosensory • nitric oxide • cats

Experimental evidence has demonstrated a role for nitric oxide (NO) or NO-containing compounds in relaxing vascular smooth muscle,1 inhibiting platelet aggregation and adhesion,2 and mediating the cytoprotective effects of macrophages and neutrophils.3 Although in normal brain NO seems to be a nontoxic mediator of cerebral vasodilation,4 recent data suggest that NO may have neurotoxic effects if present in abnormally high concentrations.5,6 In endothelium and brain, ionized calcium is the intracellular messenger initiating the reduced nicotinamide-adenine dinucleotide-dependent oxidation of arginine to produce NO.8,9 It is well known that cerebral ischemia causes an increase in extracellular concentration of glutamate and aspartate, and these excitatory amino acids bind to and stimulate N-methyl-D-aspartate (NMDA) receptors in brain.10 Excessive activation of the NMDA receptors allows influx of ionized calcium into neurons, which may stimulate production of superoxide anion via a prostaglandin pathway and NO via stimulation of NO synthase.11,12 Stimulation of non-NMDA glutamate receptors is also thought to generate NO.13,14 Moreover, inhibition of NO synthase in neuronal culture ameliorates glutamate toxicity.15 One postulated mechanism of NO toxicity is that at high concentrations, superoxide anion and NO may react to form the peroxynitrite anion, which decomposes at acidic pH into strong oxidants.5,15 Thus, excessive NO generation might act as an agent of cell death in stroke by reacting with superoxide.

We tested the hypothesis that inhibiting NO synthase with Nω-nitro-L-arginine methyl ester (L-NAME) could ameliorate ischemic brain injury after 4 hours of focal cerebral ischemia in halothane-anesthetized cats. L-NAME was used in this study rather than Nω-monomethyl-L-arginine because L-NAME is more potent and not as rapidly metabolized.16 In addition, regional cerebral blood flow (CBF) and somatosensory evoked potentials (SEPs) were measured during cerebral ischemia to determine if these changes could account for any beneficial effect detected with drug treatment.

Materials and Methods

Twenty female cats weighing 2.7 to 4.2 kg were used for this experiment. After anesthesia was induced with halothane in oxygen, cats were orally intubated and
mechanically ventilated (model 665, Harvard Apparatus, South Natick, Mass) to maintain Pao₂ at approximately 35 to 40 mm Hg. Anesthesia was maintained with halothane (0.5 to 1.5%) in oxygen-enriched air (FiO₂ 0.35 to 0.40). Anesthetic concentration was not altered during the experimental protocol. Pancuronium bromide (0.2 mg/kg IV) was administered, as a single dose, for muscle relaxation to prevent movement during electrocautery and muscle artifact during evoked potential monitoring.

Both femoral veins were catheterized for infusion of lactated Ringer’s solution and drugs. Catheters were placed in the descending aorta via a femoral artery for blood pressure measurement, arterial blood gas sampling, and for withdrawal of reference blood samples during injection of radiolabeled microspheres. After left thoracotomy, a catheter was inserted into the left atrium for injection of radiolabeled microspheres. The cat was turned prone and its head positioned in a stereotaxic frame approximately 4 cm higher than its heart. A thermistor (Mon-A-Therm, LaBarge, St Louis, Mo) placed in the right temporal epidural space was used to measure brain temperature. Epidural temperature was maintained at 38.0±0.5°C using a warmed water blanket and a heating lamp. The left middle cerebral artery (MCA) was exposed by a transorbital approach using microsurgical techniques.17 To produce focal ischemia, the MCA was permanently occluded near its origin from the intracranial carotid artery using a microvascular clip for 4 hours.

Arterial blood pressure was measured continuously with a Statham pressure transducer. Arterial pH, Paco₂, and Pao₂ were measured with a self-calibrating Radiometer electrode system (ABL 3; Copenhagen, Denmark). Hemoglobin and arterial oxygen content were measured with a hemoximeter (model OSM3, Radiometer). Blood glucose was measured with a glucose analyzer (model 2300A, Yellow Springs Instruments, Yellow Springs, Ohio). A multichannel signal averager (model CA-1000, Nicolet Biomedical Instruments, Madison, Wis) was used to measure SEPs with foreleg stimulation as previously described.17,18 The amplitude to the peak of the first major negative wave was measured from the peak of the preceding positive wave.

Regional CBF was measured with radiolabeled microspheres (16±0.5 μm diameter; Du Pont-NEN Products, Boston, Mass) using the reference withdrawal method.19 Six radioactive isotopes (153Gd, 114In, 113Sn, 103Ru, 99Nb, 46Sc) were injected in random sequence in each animal. Approximately 1.5×10⁴ microspheres were injected into the left atrium over a 20-second period, followed by a 5-mL saline flush. The reference blood sample was withdrawn from the aorta at 1.94 mL/min beginning 30 seconds before the injection and continuing for 90 seconds after the saline flush.

Four additional cats were used to determine the optimal dose of L-NAME to produce 100% inhibition of NO synthase activity within 60 minutes of administration and lasting for the 4-hour experimental protocol. Cats were prepared in a fashion identical to that of the injury volume protocol except that blood flow and injury volume were not measured. Cats received either saline, 1, 5, or 10 mg/kg L-NAME intravenously, and brain samples were taken through a craniotomy before drug injection at 1 hour and at 4 hours after drug adminis-

Brain homogenates were analyzed for maximal NO synthase activity by measuring conversion of ³H-arginine to ³H-citrulline under optimal conditions,20 and values were expressed as percentage of baseline activity (before drug injection). With 1 mg/kg of L-NAME, NO synthase activity was inhibited 67% at 1 hour and 31% at 4 hours (n=1). With 5 mg/kg, activity was inhibited 100% at 1 hour and 85% at 4 hours (n=1). No further animals were administered 1 or 5 mg/kg because we wanted to find a dose that would consistently cause 100% inhibition of NO synthase activity. Using 10 mg/kg, activity was inhibited by 100% at both 1 and 4 hours (n=2). With these preliminary data we chose to use the 10-mg/kg dose in the remainder of the study.

For the injury protocol each cat randomly received either 10 mL of 0.9% saline (n=10) or 10 mg/kg of L-NAME in 0.9% saline 10 mL (n=10) over 30 minutes by continuous intravenous infusion after baseline measurement. The microvascular clip was placed on the left MCA 30 minutes after saline or L-NAME administration. SEPs were measured before and at 30 minutes after saline or L-NAME and every 30 minutes during the 4-hour ischemia. Arterial blood gas and CBF measurements were made before and at 30 minutes after saline or L-NAME and at 30 minutes, 2, 3, and 4 hours after left MCA occlusion.

At 4 hours of ischemia, the cats were killed with intravenous potassium chloride. The brain was removed and cut immediately into 12 uniform coronal sections 3 mm thick. The freshly cut sections were immersed in a 1% solution of 2,3,5-triphenyltetrazolium chloride (TTC; Sigma Chemical Co, St Louis, Mo) in 0.9% saline at 37°C for 30 minutes and turned every 10 minutes. Viable brain areas appear dark red as a result of reduction of TTC by mitochondrial enzymes, whereas injured areas appear white.21,22 Both sides of each section were photographed in color, and the areas of ischemic injury of the cerebral hemisphere and caudate nucleus were measured separately by digital planimetry.17,18,23 The areas of the anterior and posterior surfaces of each section were averaged. The product of this averaged area and the section thickness was calculated for each section, and the volume of injury was obtained by summing these products from all 12 sections. The slices of brain were placed in 10% buffered formalin for 1 to 2 days. Ipsilateral and contralateral temporal and parietal lobe of the middle four slices were subsequently sectioned into three cortical gray matter regions (inferior temporal, lateral temporal parietal, and superior parietal) and one white matter region. Injury volume within each of these areas was also recorded. Brain was also sectioned to determine regional CBF to the right and left caudate nucleus, right and left hippocampus, and posterior fossa (brain stem and cerebellum). The arterial reference samples and weighed tissue specimens were counted in a Packard multichannel autogamma scintillation spectrometer (model 5530, Minaxi, Downers Grove, Ill). The overlap of activity among isotopes was corrected by differential spectroscopy, and blood flow was calculated by the reference sample technique.17,18

Values are expressed as mean±SE. Statistical comparison to assess changes in measured physiological variables within groups was performed by analysis of variance. The effect of experimental manipulation on
TABLE 1. Mean Arterial Blood Pressure, pHa, PaCO₂, PaO₂, Arterial Hemoglobin, and Glucose Concentrations

<table>
<thead>
<tr>
<th>Table 1.</th>
<th>Mean Arterial Blood Pressure, pHa, PaCO₂, PaO₂, Arterial Hemoglobin, and Glucose Concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Saline or L-NAME</strong></td>
<td><strong>Administration</strong></td>
</tr>
<tr>
<td><strong>Before</strong></td>
<td><strong>After</strong></td>
</tr>
<tr>
<td><strong>MABP, mm Hg</strong></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>98±6</td>
</tr>
<tr>
<td>L-NAME</td>
<td>102±2</td>
</tr>
<tr>
<td><strong>pHa</strong></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>7.41±0.01</td>
</tr>
<tr>
<td>L-NAME</td>
<td>7.40±0.01</td>
</tr>
<tr>
<td><strong>PaCO₂, mm Hg</strong></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>37±0.7</td>
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<tr>
<td>L-NAME</td>
<td>38±0.7</td>
</tr>
<tr>
<td><strong>PaO₂, mm Hg</strong></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>161±7</td>
</tr>
<tr>
<td>L-NAME</td>
<td>169±7</td>
</tr>
<tr>
<td><strong>Hemoglobin, g/dL</strong></td>
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</tr>
<tr>
<td>Saline</td>
<td>9.7±0.4</td>
</tr>
<tr>
<td>L-NAME</td>
<td>9.7±0.5</td>
</tr>
<tr>
<td><strong>Glucose, mg/dL</strong></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>122±14</td>
</tr>
<tr>
<td>L-NAME</td>
<td>127±11</td>
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</tbody>
</table>

Values are mean±SE. L-NAME indicates N⁴-nitro-L-arginine methyl ester, 10 mg/kg IV; L-MCAO, left middle cerebral artery occlusion; and MABP, mean arterial blood pressure.

*P<.05 compared with presaline or pre-L-NAME value; †P<.05 compared with saline group.

blood flow within each group was determined with paired t tests with Bonferroni correction. Differences between groups were determined with Student’s t test. Pearson correlations were used to determine the relation between injury volume and blood flow to each region. Statistical differences were considered significant at P<.05.

Results

There were no differences between the two groups in baseline values of any physiological variables before the intravenous administration of saline or L-NAME (Table 1). Mean arterial blood pressure increased by 33±3% after L-NAME 10 mg/kg (P<.05) and was higher in the L-NAME group than in the saline group during 240 minutes of left MCA occlusion. Although PaO₂ decreased slightly in both groups, values remained above 100 mm Hg. Other physiological variables were not affected by time and were comparable between groups during the experimental protocol.

Preischemia blood flow to caudate nucleus decreased after L-NAME but not after saline administration (Fig 1). During 4 hours of MCA occlusion, blood flow to ipsilateral caudate nucleus was reduced to similar levels in both groups. In the contralateral caudate nucleus, blood flow remained lower in the L-NAME group, but flows were unchanged from the immediate preischemic values in the respective groups.

During left MCA occlusion, both groups demonstrated sustained reduction in blood flow to all ipsilateral structures except the hippocampus in the saline group. The greatest reduction in cortical CBF was in the inferior portion of the temporal lobe. The reduction was less in superior parietal cortex and intermediate in lateral cortex. There was no difference between groups in CBF in ipsilateral region during ischemia except hippocampus, which demonstrated lower flow in L-NAME-treated cats (Table 2). Blood flow to contralateral regions and posterior fossa was not decreased by MCA occlusion. However, in each of these regions

![Fig 1. Line graph depicts blood flow to caudate nucleus before and 30 minutes after intravenous N⁴-nitro-L-arginine methyl ester (L-NAME) 10 mg/kg or 0.9% saline, and 30, 120, 180, and 240 minutes of left middle cerebral artery occlusion (L-MCAO). Values are mean±SE ipsilateral (IPSI) and contralateral (CONTRA) to L-MCAO. *P<.05 compared with presischemic baseline values. †P<.05 compared with saline-treated group. n=10, saline; n=10, L-NAME.](https://stroke.ahajournals.org/doi/10.1161/01.STR.0000162949.63473.40)
TABLE 2. Regional Blood Flow Before and After Intravenous Administration of 0.9% Saline (10 mL) or \textit{N}*-Nitro-L-Arginine Methyl Ester (10 mg/kg in Saline 10 mL) and During Left Middle Cerebral Artery Occlusion

<table>
<thead>
<tr>
<th>Regional Blood Flow, mL · min⁻¹ · 100 g⁻¹</th>
<th>Saline or L-NAME Administration</th>
<th>L-MCAO, h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Ipsilateral inferior temporal cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>140±22</td>
<td>142±28</td>
</tr>
<tr>
<td>L-NAME</td>
<td>146±12</td>
<td>96±7*</td>
</tr>
<tr>
<td>Contralateral inferior temporal cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>164±25</td>
<td>177±38</td>
</tr>
<tr>
<td>L-NAME</td>
<td>168±13</td>
<td>100±8*</td>
</tr>
<tr>
<td>Ipsilateral lateral temporoparietal cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>150±24</td>
<td>155±31</td>
</tr>
<tr>
<td>L-NAME</td>
<td>154±12</td>
<td>100±6*</td>
</tr>
<tr>
<td>Contralateral lateral temporoparietal cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>172±26</td>
<td>191±39</td>
</tr>
<tr>
<td>L-NAME</td>
<td>177±13</td>
<td>108±7*†</td>
</tr>
<tr>
<td>Ipsilateral superior parietal cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>193±32</td>
<td>222±47</td>
</tr>
<tr>
<td>L-NAME</td>
<td>210±22</td>
<td>123±10*</td>
</tr>
<tr>
<td>Contralateral superior parietal cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>199±29</td>
<td>233±43</td>
</tr>
<tr>
<td>L-NAME</td>
<td>213±21</td>
<td>127±6*†</td>
</tr>
<tr>
<td>Ipsilateral hippocampus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>83±9</td>
<td>85±15</td>
</tr>
<tr>
<td>L-NAME</td>
<td>95±10</td>
<td>60±5*</td>
</tr>
<tr>
<td>Contralateral hippocampus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>87±11</td>
<td>85±12</td>
</tr>
<tr>
<td>L-NAME</td>
<td>99±10</td>
<td>63±7*</td>
</tr>
<tr>
<td>Total cerebellum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>117±18</td>
<td>120±22</td>
</tr>
<tr>
<td>L-NAME</td>
<td>124±11</td>
<td>84±7*</td>
</tr>
<tr>
<td>Posterior fossa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>112±17</td>
<td>118±23</td>
</tr>
<tr>
<td>L-NAME</td>
<td>112±8</td>
<td>69±6*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. L-NAME indicates \textit{N}*-nitro-L-arginine methyl ester; L-MCAO, left middle cerebral artery occlusion. n = 10, saline; n = 10, L-NAME.

*P<.05 compared with pre-L-NAME value; †P<.05 compared with saline group.

Blood flow was decreased by L-NAME throughout the protocol (Table 2). Baseline amplitude (before saline, 105±15 μV; before L-NAME, 105±13 μV) and latency (before saline, 12.6±0.3 milliseconds; before L-NAME, 12.8±0.3 milliseconds) of the primary cortical SEP were not different between groups. Amplitude of the SEP was also not altered by saline or L-NAME (Fig 2). During left MCA occlusion, ipsilateral SEP amplitudes were suppressed to the same extent (<10% of baseline values) in the saline and L-NAME groups. However, SEP amplitude over the contralateral somatosensory cortex showed no change during 240 minutes of left MCA occlusion in both groups. In addition, all cats had normal latency of
the wave measured over the second cervical vertebra throughout the protocol.

Injured volume of left caudate nucleus in cats pretreated with L-NAME (126±15 mm³) was less (P<.05) than compared with cats pretreated with saline (185±18 mm³), whereas left cerebral hemispheric injury volume was similar between the two groups (saline, 2467±585 mm³; L-NAME, 2792±356 mm³) (Fig 3). Within each coronal section, the volume of injury was measured in the inferior, intermediate, and superior portions of cortex where blood flow was measured individually. Most of the injury was in inferior cortex (Table 3), where blood flow was lowest. Some of the injury extended into lateral cortex, where blood flow was intermediate. When analyzed separately, there was no effect of L-NAME on injury volume in individual cortical regions (Table 3). In each region there was a significant negative correlation between blood flow during ischemia and injury volume, which was not different between groups. When injury volume was analyzed separately for each of 12 coronal sections, there was no difference between groups (Fig 4). No cat in either group demonstrated injury in the right caudate nucleus or right hemisphere.

**Table 3. Injury Volume to Cortical Regions Ipsilateral to Middle Cerebral Artery Occlusion**

<table>
<thead>
<tr>
<th>Injury Volume</th>
<th>mm³</th>
<th>% of Region</th>
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</thead>
<tbody>
<tr>
<td>Inferior temporal cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>988±172</td>
<td>61±9</td>
</tr>
<tr>
<td>L-NAME</td>
<td>1240±143</td>
<td>81±9</td>
</tr>
<tr>
<td>Lateral temporoparietal cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>25±18</td>
<td>14±10</td>
</tr>
<tr>
<td>L-NAME</td>
<td>40±14</td>
<td>25±8</td>
</tr>
<tr>
<td>Superior parietal cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>L-NAME</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are mean±SEM. L-NAME indicates N°-nitro-L-arginine methyl ester. There was no difference between groups for any region.

**Discussion**

This study demonstrates that intravenous administration of L-NAME before prolonged MCA occlusion in cats results in protection from injury in the caudate nucleus but not in cerebral hemispheres. Protection from injury does not appear to be due to a favorable redistribution of blood flow during MCA occlusion in spite of increased arterial pressure. Decreased injury volume in L-NAME-pretreated cats was not associated with electrophysiological evidence of protection.

In rats, others have demonstrated that L-NAME (3 mg/kg but not 1 mg/kg) reduced the amount of brain injury resulting from permanent MCA occlusion. Likewise, in mice, systemic administration of N°-nitro-L-arginine resulted in reduction in cerebral infarction from permanent MCA occlusion. However, in both studies efficacy of NO synthase inhibition was not determined, nor was there any attempt to control arterial blood gases that affect CBF. In the current study arterial blood gases and brain temperature were controlled and arterial pressure, SEPs, and regional CBF were monitored.

Although it is possible that L-NAME protected brain from injury by redistributing flow without an increase in...
whole caudate flow, this is unlikely because inhibition of NO synthase is associated with a decreased CBF in this and other studies. In addition, if the mechanism of protection for L-NAME was related to elevation of arterial pressure and blood flow promotion, we would have anticipated a greater amelioration of injury in cortical gray matter, where collateral blood supply is greater than in caudate nucleus, which is an end-artery region. L-NAME has also recently been found to be a muscarinic receptor antagonist. However, this effect would more likely be detrimental to neurological recovery since others have found that parasympathetic denervation increases infarction volume after MCA occlusion and electrical stimulation of the cerebellar fastigial nucleus, which increases CBF by a muscarinic mechanism, reduces ischemic infarction elicited by MCA occlusion in the rat. We speculate that the mechanism for protection results from inhibition of NO synthesis but without a beneficial effect on blood flow. Possible sites of NO production include vascular endothelium, perivascular neurons, or astrocytes. An important role for NO production by astrocytes and/or neurons is suggested by the finding of improved injury volume in caudate but not cortex since Breidt et al have demonstrated higher NO synthase activity in striatum compared with cortex. Confirmation of the NO hypothesis would rest on being able to demonstrate that l-arginine treatment could prevent any protective role of L-NAME.

Another possible explanation for the difference in therapeutic efficacy for L-NAME in caudate compared with cortex may be that blood flow to ipsilateral caudate nucleus during MCA occlusion was approximately 25 mL/min per 100 g in both L-NAME and saline groups, whereas in cortex blood flow was approximately 20 mL/min per 100 g in L-NAME-treated animals and 30 mL/min per 100 g in saline-treated animals. Since volume of injury in both cortex and caudate is inversely related to blood flow to that region, it is possible that cortical blood flow effects of L-NAME may have counteracted a potential beneficial effect on cortical parenchyma.

Focal ischemia is associated with excitatory amino acid–mediated brain injury. The increase in excitatory amino acids stimulates both NMDA and non-NMDA glutamate receptors and is thought to stimulate NO synthase activity. Stimulation of NO synthase presumably results in increased NO production and neurotoxicity via a mechanism that has not been clearly elucidated but may involve an interaction of NO with superoxide anion. In a previous study we demonstrated an increase in flow during MCA occlusion in cats treated with conjugated superoxide dismutase, which was associated with decreased injury volume in caudate nucleus. Although it is possible that NO may be directly toxic to cells by reacting with nonheme iron-sulfur complex, this seems unlikely because NO is also a natural mediator of vasodilation for agents that act via an endothelial-dependent process. Nonetheless, both NO and excitatory amino acids appear to be linked to the mechanism of injury after focal cerebral ischemia. An alternative explanation for protection by L-NAME may be via an effect on cerebral metabolism. For example, other drugs (e.g., barbiturates) that decrease cerebral metabolism have been demonstrated to protect brain during focal ischemia. We believe this is an unlikely mechanism for the beneficial effects of L-NAME because we have previously demonstrated reduced CBF during inhibition of NO synthase activity without a decrease in O2 consumption.

Inhibition of NO synthase activity may not be protective in all types of ischemia. Indeed, in gerbil systemic administration of L-NAME resulted in delayed electrocortical recovery after transient forebrain ischemia. However, efficacy of NO synthase inhibition was not determined, and physiological variables were not recorded. These authors also did not determine the effect of L-NAME on electrocortical activity in control animals to determine baseline effects. In our study, the lack of effect of L-NAME on SEPs during 4 hours of ischemia may be related to the lack of effect on hemispheric injury volume. This is consistent with the correlation that has been demonstrated between cortical injury and SEPs.

More recently, others have demonstrated that administration of Nω-nitro-L-arginine to spontaneously hypertensive rats was associated with a worsening of cortical injury volume after MCA occlusion. Furthermore, in this study the detrimental effect of NO synthase inhibition could be prevented with L-arginine but not D-arginine. Unlike cats in our study and normotensive rats in other studies, inhibition of NO synthase in hypertensive rats did not appear to reduce CBF. This observation suggests a different effect of NO synthase inhibition on brain in spontaneously hypertensive rats.

Injury volume was estimated with TTC staining. TTC acts as a proton acceptor for mitochondrial oxidative cellular metabolism. When compared with light microscopy TTC tended to overestimate ischemic volumes of hemispheres and cortex and underestimate ischemic volume in caudate for rat exposed to 4 hours of MCA occlusion. The rationale for using a 4-hour period of ischemia was to obtain an early indicator of injury and to avoid potential detrimental effects of prolonged halothane anesthesia during MCA occlusion. In another study TTC staining was found to correlate with histopathology best in cats when the time of ischemia was greater than 2 hours and there was at least 2 hours of reperfusion. However, in this study, cats exposed to 4 hours of MCA occlusion did not demonstrate any evidence of brain injury by TTC staining, which makes the results not directly comparable to our results. More recently, Bederson et al demonstrated that there was good correlation between TTC staining and histopathological evaluation in rats exposed to 1 to 2 hours or 5 to 6 hours of MCA occlusion but not to 3 hours of MCA occlusion (sample size for 3 hours of only 3). Nonetheless, since Cole et al have demonstrated that the histochemical abnormality revealed by TTC staining may not necessarily represent irreversible infarction when it is used for paradigms of short ischemic periods (3 hours in their study), we cannot be certain that the eventual injury, measured at a later time, would not be similar between groups. Therefore, our results may only indicate that rate of caudate injury development is altered by treatment with L-NAME. However, an alteration in rate of injury may also have important clinical implications because it may increase the window of opportunity for use of other therapeutic agents.

The mechanism for reduction of CBF with L-NAME appears to be related to decreased tonic release of NO.
Inhibition of NO synthase results in decreased CBF in rats and piglets. Possible sites of action of L-NNAME-mediated inhibition of NO synthase include vascular endothelial, perivascular neurons, or astrocytes. The effect of L-NNAME may be accentuated in animals in this study because of baseline halothane anesthesia. Halothane is a potent cerebral vasodilator. Like NO, halothane appears to cause cerebral vasodilation via an increase in cyclic guanosine 3',5'-monophosphate. Likewise, preliminary work from our laboratory suggests that halothane-induced vasodilation can be blocked by L-NNAME.

Cerebral injury detected by TTC staining has been used in rats and cats to determine extent of brain injury after focal ischemia. A deficiency in TTC staining should not be assumed to be an absolute indicator of cerebral injury. Therefore, an absolute infarct size may be overestimated if some neurons that are incapable of being stained but are functionally recoverable exist. However, the significant difference in caudate nucleus volume of approximately 30% between the two groups in the current experiment is unlikely to be explained by higher sensitivity of TTC staining compared with other techniques.

In conclusion, these data indicate that L-NNAME treatment before 4 hours of focal ischemia can alleviate acute neuronal injury of caudate nucleus in cats anesthetized with halothane. Our data support the hypothesis that NO may be important in the mechanism of brain injury from focal ischemia. However, because we did not also find protection in cerebral cortex, we cannot exclude other mechanisms that are specific for the caudate nucleus.

Acknowledgments

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**Editorial Comment**

The accompanying article by Nishikawa and colleagues is commendable for several of the following reasons. First, the work is well executed and in its approach emphasizes the complex actions of nitric oxide (NO) inhibition on the pathophysiology of ischemia. Rather than relying on published reports, the authors wisely chose the dosage of Nω-nitro-l-arginine methyl ester (L-NAME) based on their own assessment of brain NO synthase activity after the administration of L-NAME at three different dosages. They then selected a dosage that blocked activity for the duration of study by 100%. This is a key step, which deserves emphasis and emulation by others working in the field. Its omission may well cause avoidable discrepancies and inaccurate conclusions. In my own laboratory, Irikura recently observed that little or no inhibition of the regional cerebral blood flow response was recorded to whisker stimulation if enzyme inhibition was not at least 50% to 60% that of control (achieved by topical application of l-nitroarginine). Other physiological or pathophysiological responses may require a greater or lesser degree of enzyme inhibition, and this information would be important to know.

The report of Nishikawa et al is also important because it raises the interesting possibility that protection by NO synthase inhibitors may be demonstrated best when blood flow values are equivalent in control and treated groups. Tissue outcome in some regions of cortex supports this possibility. Obviously more work will be needed to clarify this issue.

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T Nishikawa, J R Kirsch, R C Koehler, D S Bredt, S H Snyder and R J Traystman

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