The Effect of Nitric Oxide Synthase Inhibition on Infarct Volume After Reversible Focal Cerebral Ischemia in Conscious Rats

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Background and Purpose: Previous in vitro and in vivo studies of the effects of nitric oxide synthase inhibition in the central nervous system have yielded conflicting results concerning the role of nitric oxide in the events that lead to ischemic injury. In this study, we tested the hypothesis that preischemic inhibition of nitric oxide synthase increases infarct volume after reversible focal cerebral ischemia in rats.

Methods: N\textsuperscript{o}-nitro-l-arginine methyl ester hydrochloride 15 mg/kg IV or an equivalent volume of saline was administered to adult Wistar rats 15 minutes before middle cerebral artery occlusion by the intraluminal suture method. After 2 hours of ischemia, the suture was withdrawn, and rats were allowed to survive for 3 days. Areas of infarction in 10 hematoxylin-eosin-stained sections were measured and used to determine infarct volume.

Results: Administration of N\textsuperscript{o}-nitro-l-arginine methyl ester hydrochloride increased hemispheric infarct volume by 137% over control (60.9±30.5 to 144.3±19.6 mm\textsuperscript{3}, P<.05; mean±SEM). Cortical and subcortical infarct volumes were increased by 176% (33.8±21.9 to 93.3±15.2 mm\textsuperscript{3}, P<.05) and 103% (25.1±9.4 to 51.0±5.5 mm\textsuperscript{3}, P<.03), respectively.

Conclusions: Nitric oxide synthase inhibition increases infarct volume and decreases the variability of the response to middle cerebral artery occlusion in Wistar rats, a strain that is normally resistant to focal cerebral ischemic injury owing to extensive collateralization. The mechanism of the deleterious effect of nitric oxide synthase inhibition likely involves a more severe degree of blood flow reduction during and after middle cerebral artery occlusion, primarily by preventing the vasodilatory response of collateral vessels to proximal middle cerebral artery occlusion. Maintenance of nitric oxide synthase activity during and after focal cerebral ischemia appears to minimize ischemic injury. (Stroke. 1993;24:2023-2029.)

Key Words • cerebral ischemia • reperfusion • cerebral infarction • nitric oxide • rats

The exact role of nitric oxide (NO) in the pathogenesis of cerebral ischemic injury is poorly understood. In both cerebellar cell suspensions\textsuperscript{1} and cerebellar brain slices\textsuperscript{2} the increase in cyclic guanosine monophosphate (cGMP) after activation of N-methyl-D-aspartate receptors has been shown to be mediated by NO. In more recent studies, NO has been implicated as the neuronal second messenger that causes glutamate neurotoxicity both in vivo\textsuperscript{3} and in vitro.\textsuperscript{4} In contrast, NO production by cerebral vascular endothelial cells may be neuroprotective in the setting of focal cerebral ischemia by increasing blood flow to marginally ischemic tissues.\textsuperscript{5,6} We have recently shown\textsuperscript{7} that NO synthase inhibition decreases resting cerebral blood flow by approximately 30% within the autoregulatory range of mean arterial blood pressure (MAP) and causes ultrastructural changes in cerebral endothelial cells associated with increased vascular permeability. In addition, NO synthase inhibition not only attenuates early hyperemia but also severely depresses cortical cerebral blood flow (CBF) and prevents cortical CBF from returning to baseline after normotensive bilateral carotid artery occlusion.\textsuperscript{8} The purpose of this study was to examine the effect of NO synthase inhibition on infarct development after reversible focal cerebral ischemia in spontaneously breathing conscious rats.

Methods

Twenty-four fasted adult male Wistar rats (Charles River Laboratories, Inc, Wilmington, Mass) weighing 261 to 304 g were used in this study. Anesthesia was induced with isoflurane 3.5% in a mixture of 70% nitrous oxide and 30% oxygen and was maintained with isoflurane 1.0% to 1.5% in a mixture of 70% nitrous oxide and 30% oxygen via a face mask throughout the surgical preparation and initial induction of middle cerebral artery (MCA) occlusion. Temperature probes were inserted in the rectum and the left temporalis muscle, and heating lamps were used to maintain rectal and temporalis temperatures at 37° to 38°C (Mon-a-therm 7000; Mallinckrodt Inc, St Louis, Mo). The femoral vein was cannulated for drug and fluid admin-
istration. Through a midline neck incision the left common carotid artery was carefully dissected free from surrounding nerves and fascia from its bifurcation to the base of the skull. After cauterizing the occipital artery and ligating the distal external carotid artery, a 4-0 monofilament nylon suture (Harvard Apparatus; South Natick, Mass) was inserted under direct vision using an operating microscope into the proximal external carotid artery, through the internal carotid artery, and into the circle of Willis, effectively occluding the MCA.9,10 The suture, the tip of which had been rounded by heating, was inserted 18 mm from the bifurcation of the common carotid artery and was doubly ligated to the remaining stump of the external carotid artery. Beginning 15 minutes before MCA occlusion, NG-nitro-L-arginine methyl ester hydrochloride (L-NAME) 15 mg/kg (n=8) or an equivalent volume of 0.9% saline on the day of the experiment, and the pH of the solution was adjusted to 7.0 using 4N NaOH. After the intraluminal suture was placed, the neck incision was closed with staples, and the venous catheter and the temperature probes were removed. The duration of this phase of the experiment was less than 30 minutes. The animals were returned to their cages and allowed to recover with free access to food and water for the next 115 minutes. Rats that did not demonstrate a right upper extremity paresis during this recovery period were excluded from the study (n=0).

After 115 minutes of MCA occlusion, rats were reanesthetized with the same anesthetic combination, temperature probes were reinserted, and the intraluminal suture was carefully removed. The temperature probes were then removed, the neck incision was closed with silk suture, and the animals were allowed to recover and survive for 3 days.

On each postoperative day, the rats were weighed and assessed for right upper extremity motor deficit using the following scale: 0, normal; 1, asymmetry of extension or abduction of the right upper extremity when lifted by the tail; and 2, circling to the right during locomotion. After a 3-day survival, rats were reanesthetized and transcardially perfused with 0.9% saline at 140 cm water pressure for 2 minutes, followed by fixation with formaldehyde 37.5%:acetic acid/methanol (1:1:8) for 5 minutes. After the head had been in fixative for 24 hours, the brain was removed and processed for paraffin embedding. Ten-micron-thick sections were cut in the coronal plane and stained with hematoxylin and eosin. The areas of infarction at 10 levels throughout the brain were traced and measured, and the volume of infarction was calculated by numerical integration using a personal computer software program by an investigator who was blinded to the treatment group (J.W.K.). The areas of infarction included pancellular necrosis as well as dense areas of eosinophilic, shrunken neurons along the edges of the infarct.

Physiological measurements were made in a parallel group of seven rats receiving L-NAME 15 mg/kg (n=3) or an equivalent volume of 0.9% saline (n=4) 15 minutes before a 2-hour period of MCA occlusion. The anesthetic management and the method of MCA occlusion were exactly as described above. MAP and heart rate were measured via an indwelling femoral arterial catheter connected to a precalibrated Statham pressure transducer (model P23XL; Viggo-Spectramed, Inc, Oxnard, Calif) and recorded continuously (model RS3400; Gould, Inc, Valley View, Ohio). Serial measurements were made of arterial blood gases and pH (model ABL 330; Radiometer America, Inc, Westlake, Ohio) and plasma glucose and lactate (model 2300 Stat; Yellow Springs Instrument Co, Inc, Yellow Springs, Ohio). Rectal and temporalis muscle temperatures were measured as described above and maintained at 37° to 38°C using heating lamps. After 15 minutes of MCA occlusion, the femoral catheters were shortened, tied off, and tucked under the skin. The animals were allowed to recover for the next 90 minutes, at which time they were reanesthetized for further physiological monitoring and removal of the intraluminal suture. Fifteen minutes after removal of the suture, the animals were killed with an overdose of isoflurane.

Temperatures, MAP, blood gases, glucose, lactate, daily weight changes, and infarct areas and volumes were analyzed by repeated-measures analysis of variance (ANOVA) and by Student's t test using the statistical software STATVIEW II (Abacus Concepts, Inc, Berkeley, Calif). Motor scores were analyzed using Kruskal-Wallis. Differences were considered significant at P<.05.

All methods were approved by the Animal Research Committee of the University of Miami School of Medicine.

Results

Temporalsis temperatures were not different between groups at the time of placement (L-NAME: 37.8±0.2°C versus saline: 37.8±0.4°C, mean±SEM; not significant [NS]) or removal (L-NAME: 37.9±0.2°C versus saline: 37.9±1.0°C; NS) of the intraluminal suture. In the parallel group of rats, L-NAME increased baseline MAP from 117±8 mm Hg to 137±12 mm Hg before MCA occlusion (P<.05), whereas saline did not change MAP significantly (113±1 mm Hg to 109±4 mm Hg; NS). MAP remained higher in L-NAME–treated rats throughout the monitoring period during ischemia and early reperfusion (Table 1).

Arterial PCO2 (Paco2) was not significantly different between groups except at 5 minutes after MCA occlusion, when L-NAME–treated rats had a lower Paco2 than did controls (32.6±0.3 mm Hg versus 42.8±2.3 mm Hg; P<.05). By the end of the ischemic period Paco2 was not different between the two groups.

Mean right upper extremity motor deficit scores were significantly higher in L-NAME–treated rats compared with controls on each day after ischemia (Table 2). In addition, body weight decreased significantly in L-NAME–treated rats, whereas it did not change in controls during the survival period (Table 2).

Compared with controls, L-NAME–treated rats developed larger infarct volumes that were more consistent in size in the total cerebral hemisphere (144.3±19.6 versus 60.9±30.5 mm3; P<.05), cortex (93.3±15.2 versus 33.8±21.9 mm3; P<.05), and subcortex (51.0±5.5 versus 25.1±9.4 mm3; P<.03; Fig 1). These represent increases over saline-treated rats of 137%, 176%, and 103%, respectively. L-NAME increased infarct volume to the greatest extent in the cortex. In controls, infarct volume was more variable in the cortex than in the subcortex. Figs 2 through 4 illustrate the rostrocaudal distribution...
of infarct areas in the two groups. Four of 8 L-NAME–treated rats had small, sometimes multiple intraparenchymal hemorrhages (3-in striatum, 1-in cortex), whereas only 1 of 7 saline-treated rats had a striatal hemorrhage. In addition, unilateral CA1 hippocampal necrosis was seen in 2 L-NAME–treated and 0 saline–treated rats.

**Discussion**

This study showed that L-NAME 15 mg/kg, when given before reversible MCA occlusion, increases infarct volume in spontaneously breathing, conscious Wistar rats. These findings are in agreement with those of Yamamoto et al., who administered N-omega-nitro-L-arginine (NNA) 2.4 mg/kg over 1 hour to spontaneously hypertensive rats immediately after permanent occlusion of the MCA. In their study, NO synthase inhibition increased infarct volume by 32%, an effect that was completely prevented by the coadministration of the nitric oxide precursor L-arginine.11,12 Morikawa et al also found that L-arginine decreased infarct volume after permanent MCA occlusion in spontaneously hypertensive rats. In contrast, Dawson et al13 administered L-NAME 30 mg/kg 30 minutes before and 30 minutes after permanent MCA occlusion in Sprague-Dawley rats and found no difference in the volume of ischemic damage; however, these investigators measured injury after only 4 hours of ischemia, which may not have been long enough to allow for the full development of infarction. In another study in which systemic variables such

**Table 1. Physiological Data Obtained Before, During, and After 120 Minutes of MCA Occlusion**

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Treatment</th>
<th>MAP, mm Hg</th>
<th>pH</th>
<th>Paco₂, mm Hg</th>
<th>Glucose, mg/dL</th>
<th>Lactate, mmol/L</th>
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<tbody>
<tr>
<td>−15</td>
<td>Saline</td>
<td>113.0±1.2</td>
<td>7.44±.01</td>
<td>41.8±1.2</td>
<td>NM</td>
<td>NM</td>
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<tr>
<td></td>
<td>L-NAME</td>
<td>117.3±7.9</td>
<td>7.49±.03</td>
<td>36.0±2.0</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>108.8±3.8</td>
<td>7.44±.01</td>
<td>39.4±3.2</td>
<td>128.8±1.7</td>
<td>1.9±3.3</td>
</tr>
<tr>
<td>0</td>
<td>L-NAME</td>
<td>136.7±11.7*</td>
<td>7.48±.03</td>
<td>35.0±0.9</td>
<td>124.5±26.5</td>
<td>2.5±0.8</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>118.8±8.4</td>
<td>7.41±.01</td>
<td>42.8±2.3</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>5</td>
<td>L-NAME</td>
<td>146.0±8.3</td>
<td>7.51±.00*</td>
<td>32.6±0.3*</td>
<td>NM</td>
<td>NM</td>
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<tr>
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<td>7.45±.00</td>
<td>39.7±2.1</td>
<td>135.0±5.2</td>
<td>2.5±0.6</td>
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<tr>
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<td>L-NAME</td>
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<td>7.49±.02*</td>
<td>35.0±2.0</td>
<td>132.3±21.1</td>
<td>2.5±0.1</td>
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<tr>
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<td>112.8±7.5</td>
<td>7.41±.01</td>
<td>44.8±1.3</td>
<td>135.5±2.4</td>
<td>1.8±0.4</td>
</tr>
<tr>
<td>120</td>
<td>L-NAME</td>
<td>131.7±7.4</td>
<td>7.45±.02</td>
<td>41.0±1.3</td>
<td>130.7±13.5</td>
<td>2.6±0.5</td>
</tr>
</tbody>
</table>

MCA indicates middle cerebral artery; MAP, mean arterial pressure; L-NAME, N-omega-nitro-L-arginine methyl ester hydrochloride; NM, not measured. Values are mean ± SEM (n).

*P<.05 vs saline controls.

| Table 2. Body Weight and Right Upper Extremity Motor Deficit Score |
|-------------------|-------------------|-----------------|-----------------|-----------------|
|                   | n    | Baseline | Day 1      | Day 2      | Day 3      |
| **Body weight, g**|      |          |            |            |            |
| Saline            | 7    | 294±3    | 296±6      | 296±10     | 302±13     |
| L-NAME            | 8    | 281±5    | 259±4*     | 257±8*     | 260±12*    |
| **Motor deficit score** |
| Saline            | 7    | NM       | 0.71±.29   | 0.43±.30   | 0.29±.29   |
| L-NAME            | 8    | NM       | 2.00±.00†  | 1.50±.27†  | 1.25±.31†  |

L-NAME indicates N-omega-nitro-L-arginine methyl ester hydrochloride; NM, not measured. For definition of motor deficit score, see "Methods." Values are mean±SEM.

*P<.05 vs baseline.

†P<.03, †P<.005 vs saline controls.
as blood pressure and arterial blood gases were not measured, N\textsuperscript{0}-nitro-L-arginine 1 mg/kg decreased infarction measured 7 days after permanent MCA occlusion in mice.\textsuperscript{3}

Our study is unique in several ways. First, we studied Wistar rats, a strain that is known to be relatively resistant to focal cerebral ischemia, developing smaller infarct volumes and greater variance after MCA occlusion than do Sprague-Dawley, Fischer, and spontaneously hypertensive rat strains.\textsuperscript{14} If this resistance to focal ischemia is due to greater functional vasodilatory reserve, then one would expect that inhibition of NO synthase would increase ischemic injury by decreasing blood flow to the penumbral zone.\textsuperscript{14-16} The small dose of NNA used by Yamamoto et al\textsuperscript{8} increased infarction without decreasing CBF in nonischemic controls, although it did increase cerebral vascular resistance. We previously reported\textsuperscript{7} that L-NAME 15 mg/kg decreases CBF by 27\% in nonischemic Wistar rats and that this decrease is sustained for at least 1 hour after the dose. Based on these findings, we believe that NO synthase inhibition as achieved in the present study increases the severity and distribution of ischemia during MCA occlusion in Wistar rats. Recently, Buxton et al\textsuperscript{17} found that the alkyl esters of arginine, including L-NAME, are competitive antagonists of muscarinic membrane receptors located in several tissues in the body, including vascular endothelium. Blockade of acetylcholine-induced vascular relaxation would further reduce blood flow to the ischemic tissue. The effects of L-NAME in this study may have been due to a combination of NO synthase inhibition and muscarinic receptor blockade.

Second, rats in this study were allowed to awaken immediately after MCA occlusion, thereby minimizing the cumulative dose of anesthetic. The volatile anesthetics have been shown to inhibit NO synthase,\textsuperscript{18-20} and perhaps this accounts for the smaller difference in infarct volumes between nitro-arginine–treated and placebo-treated rats in previous studies. Dawson et al\textsuperscript{13} maintained anesthesia with halothane 0.75\% to 1.0\% in a mixture of 70\% nitrous oxide and 30\% oxygen. Before MCA occlusion, MAP was approximately 82 mm Hg and increased to only 95 mm Hg after L-NAME 30 mg/kg. These blood pressures are clearly in the hypotensive range and are consistent with a cumulative effect of halothane on systemic vascular resistance.

Third, we studied the effect of NO synthase inhibition in a model of reversible focal ischemia. In this setting, one would expect NO synthase inhibition to delay or prevent the reestablishment of blood flow to ischemic areas. This has been demonstrated by Prado et al,\textsuperscript{5} who found that L-NAME 30 mg/kg given before 20 minutes of bilateral carotid artery occlusion attenuated early hyperemia and severely depressed cortical CBF during recirculation. However, the presence of intraparenchymal hemorrhages in half of the L-NAME–treated rats
in the present study suggested that some reperfusion of the ischemic core did in fact occur. Based on this finding, CBF in the L-NAME–treated rats may not have returned to a sufficient level to prevent secondary ischemic injury after removal of the intraluminal suture. It is possible that the higher MAP in L-NAME–treated rats contributed to the development of the intraparenchymal hemorrhages. However, since the MAP did not exceed the upper limit of autoregulation in the parallel group of rats studied, we do not believe that the small increase in MAP by L-NAME can account for the increase in infarct volume measured in this study. In our previous study, L-NAME 15 mg/kg increased MAP by only 15% in nitrous oxide–anesthetized Wistar rats (from 125±3 mm Hg to 144±4 mm Hg). Moreover, Yamamoto et al. found that phenylephrine-induced hypertension did not increase infarct volume over normotensive controls, nor did they report the presence of hemorrhages within infarcts. A more likely explanation for the worse outcome of L-NAME–treated rats is the effect of NO synthase inhibition on the structure and function of cerebral endothelial cells, eg, formation of endothelial microvilli, pinocytotic uptake of horseradish peroxidase, enlargement of perivascular spaces, stasis of red blood cells, and constriction of blood vessels characterized by irregular contours. It is interesting that L-NAME was associated with a lower Paco₂ early after MCA occlusion (Table 1). While the likelihood of a type I error is high because of the small number of animals studied, the lower Paco₂ in the L-NAME–treated rats could reflect a more severe or more widespread reduction of CBF, since the acidosis that accompanies brain ischemia is known to increase minute ventilation. We did not intubate and mechanically ventilate the rats in this study because to do so would likely have required muscle relaxation and a greater cumulative dose of anesthetic. In addition, spontaneously breathing rats maintained Paco₂ between 35 and 45 mm Hg in our pilot studies using this model.

Another possible explanation for the deleterious effect of NO synthase inhibition on infarct development involves the function of circulating platelets. Radomski et al. report that NO inhibits platelet adhesion to collagen fibrils and endothelial cell matrix, probably mediated via activation of guanylate cyclase and elevation of platelet cGMP concentration. The method of producing reversible MCA occlusion in the present study likely involves significant injury to the vessel lining by the intraluminal suture, which, particularly in the presence of L-NAME, could serve as a nidus for platelet adhesion and subsequent release of thromboemboli. This may explain the hippocampal injury in two L-NAME–treated rats, since the

![Fig 3. Bar graph showing the rostrocaudal distribution of the area of cerebral cortical infarction (mean±SEM) at 10 levels in saline-treated rats (hatched bars; n=7) and N⁶-nitro-L-arginine methyl ester hydrochloride (L-NAME)–treated rats (solid bars; n=8). *P<.05, **P<.03.](http://stroke.ahajournals.org/)

![Fig 4. Bar graph showing the rostrocaudal distribution of the area of subcortical infarction (mean±SEM) at 10 levels in saline-treated rats (hatched bars; n=7) and N⁶-nitro-L-arginine methyl ester hydrochloride (L-NAME)–treated rats (solid bars; n=8). *P<.03.](http://stroke.ahajournals.org/)
anterior choroidal artery, which supplies the anterior hippocampus, usually originates from the internal carotid artery posterior to the origin of the MCA. Injury to the endothelial lining of the internal carotid artery at or before the anterior choroidal artery could be a source of thromboemboli.

On the other hand, NO synthase inhibition in the setting of reversible MCA occlusion might be expected to decrease reperfusion injury mediated by free radical species. Hydroxyl radical production is believed to increase in the presence of NO during reoxygenation of ischemic tissue. In support of this theory, myocardial reperfusion injury was markedly reduced in piglets receiving L-NAME 4 mg/kg, protection that was lost by the addition of L-arginine.

According to the literature on the pathophysiology of NO in the central nervous system, NO has paradoxical effects in the setting of brain ischemia. In vitro studies of “brain cell” cultures, “pure” neuronal cultures, and brain slices clearly show that NO, produced either directly from sodium nitroprusside or indirectly via activation of N-methyl-D-aspartate receptors, injures brain cells. On the other hand, none of the in vivo studies in which systemic variables were measured and controlled support the hypothesis that NO synthase inhibition protects the brain from ischemic injury. Indeed, we demonstrated a marked increase in ischemic damage in the presence of NO synthase inhibition. Based on the present study and other in vivo studies, we conclude that the effects of NO in the artificial microenvironment of the tissue-culture setting do not correlate with its effects in the intact living brain, where the spatial arrangement of individual types of cells, the supply of substrate, and the removal of toxins are vastly different. When taken from their natural microenvironment in the brain and grown in tissue culture, neurons may synthesize NO that cannot be buffered or absorbed by neighboring nonneuronal cells such as smooth muscle cells, astrocytes, and endothelial cells, in which NO production and subsequent activation of guanylate cyclase may have a beneficial effect during ischemia.

In summary, this study clearly demonstrated that L-NAME 15 mg/kg increased functional and histopathologic damage after 2 hours of reversible MCA occlusion in rats. Maintenance of NO synthase activity appears to improve outcome after focal cerebral ischemia/reperfusion injury, particularly in animal strains that have greater cerebral vasodilatory reserve.

Acknowledgments

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26. Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci U S A. 1990;87:1620-1624.
The purpose of the experiments described in this interesting article was to examine the effect of nitric oxide synthase inhibition on infarct development after reversible focal cerebral ischemia (middle cerebral artery occlusion) in spontaneously breathing conscious rats. The authors found that nitric oxide synthase inhibition with $N^\omega$-nitro-L-arginine methyl ester hydrochloride (L-NAME) inhibition worsens focal ischemia/reperfusion injury, increases infarct volume, and decreases the variability of the response to middle cerebral artery occlusion in rats. They conclude that the maintenance of nitric oxide synthase activity during and after focal cerebral ischemia appears to minimize ischemic-reperfusion injury. The authors' results support the increasing role for nitric oxide in the regulation of the cerebral circulation, and more importantly support the role of nitric oxide as a potential agent in minimizing ischemic injury. However, it would have been appropriate under these conditions for the authors to use L-arginine to demonstrate that the change in infarct volume was prevented by coadministration of this substance. While the present article does not precisely address the mechanism by which nitric oxide minimizes the ischemia/reperfusion injury, it certainly implicates this agent as a participant. Over the last years, tens, perhaps hundreds, of agents have been demonstrated as neuroprotective in one animal model or another or in cell culture. Most of these agents, however, do not afford complete protection, and it seems clear that a single neuroprotective agent is not going to be found that will cure all evils of ischemia/reperfusion injury. More likely, there are multiple mechanisms for injury, ie, calcium channels, excitatory amino acids, free radicals, lipid peroxidation, and so forth. Whatever agent or agents we eventually find to ameliorate the ill effects of neurological injury resulting from ischemia/reperfusion, it is likely that it will be a “cocktail” agent that has all or some of the above qualities included, or perhaps effective treatment may be a matter of simultaneous or sequential treatment with several different pharmacological agents. Nevertheless, nitric oxide, as demonstrated by the authors in the present article, clearly seems to be involved and no doubt will be part of the cocktail, or treatment regimen, that is formulated.

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