J Anesth (2000) 14:24–29

Journal of
Anesthesia

Effects of nitric oxide synthase inhibition on extracellular glutamate and cerebral blood flow during forebrain ischemia-reperfusion in rat in vivo

TOSHIKO YUSA

Department of Anesthesiology, University of the Ryukyus, Faculty of Medicine, 207 Uehara, Nishihara-cho, Nakagami-gun, Okinawa 903-0215, Japan

Abstract

Purpose. To evaluate factors involved in global forebrain ischemia-reperfusion, the effects of the systemically administered NOS inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME), on changes in extracellular glutamate and cerebral blood flow (CBF) were studied during the early period of global forebrain ischemia-reperfusion, simultaneously measuring the glutamate released in the rat forebrain cortex and cortical CBF.

Methods. After injection of saline or L-NAME, forebrain ischemia-reperfusion was performed by bilateral carotid artery occlusion with controlled hemorrhagic hypotension (30 mmHg) for 10 min and reperfusion for 60 min. The microdialysis electrode and laser Doppler flowmetry were used for real-time monitoring of glutamate and CBF, respectively.

Results. During ischemia, glutamate increased linearly to over 100 μM and remained elevated 30 min after reperfusion in L-NAME-treated rats. In L-NAME-treated rats, CBF also remained significantly lower than baseline for 30–60 min after reperfusion, and glutamate was higher than in saline-treated rats throughout the experiment.

Conclusion. A remarkable linear increase in glutamate release was observed during ischemia. L-NAME did not prevent this dramatic glutamate accumulation, and moreover, its level increased during reperfusion. The decrease in CBF response after reperfusion might be a factor of the elevated glutamate after reperfusion due to a decrease in reuptake of glutamate.

Key words: Cerebral ischemia-reperfusion, Cerebral blood flow, Glutamate, NOS inhibitor, Rat

Introduction

Cerebral ischemic damage is associated with excessive release of excitatory amino acids (EAAs), such as glutamate and free radicals [1,2]. The neurotoxic effects of endogenous EAAs such as glutamate and aspartate have been elucidated. As one of the free radicals, nitric oxide (NO) has been shown to play an important role in the modulation of vascular tone and to be a physiological neurotransmitter or neuromodulator. Experimental evidence has demonstrated that NO is involved in *N*-methyl-D-aspartate (NMDA) receptor-mediated neurotoxicity in primary cortical culture [3] and in the neuronal death that occurs after focal cerebral ischemia [4,5]. Mechanisms by which NO could mediate cell damage include inhibition of iron-containing enzymes, production of toxic peroxynitrite or hydroxyl radical, mediation of thiol inactivation and protein ribosylation, and alteration of DNA synthesis leading to cell death [6]. Therefore, the effects of nitric oxide synthase (NOS) inhibitors on regulation of cerebral circulation and on cerebral ischemic damage have been investigated. There is, however, still substantial controversy regarding the role of NO in cerebral ischemia-reperfusion. Especially, in global forebrain ischemia, NOS inhibition did not attenuate the cerebral lesions [7,8], as was reported for a focal ischemic model [9]. A systemically administered NOS inhibitor suppressed the extracellular glutamate concentration in a focal ischemia model [9], but increased it in the reperfusion period after global forebrain ischemia [10].

In this study, to evaluate factors involved in global forebrain ischemia-reperfusion, the effects of the systemically administered NOS inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME), on changes in extracellular glutamate and cerebral blood flow (CBF) were studied during the early period of global forebrain ischemia-reperfusion, simultaneously measuring the glutamate released in the rat forebrain cortex and cortical CBF.

Received for publication on May 10, 1999; accepted on September 9, 1999

Materials and methods

All aspects of this study were approved by the Animal Care and Use Committee of University of the Ryukyus.

Preparation of the dialysis electrode for in vivo, real-time measurement of glutamate

The real-time measurement of glutamate level was done with a microdialysis biosensor system (Model EES-800, Eicom, Kyoto, Japan). The microdialysis biosensor was purchased from Syncopel International (London, UK). The structure and principles of the dialysis electrode for measuring glutamate were described by Walker et al. [11]. Briefly, the microdialysis biosensor consists of an electrochemical cell inside a microdialysis probe, and dialyzed L-glutamate is oxidized by L-glutamate oxidase, producing H_2O_2, which is detected on the platinum electrode amperometrically at +600 mV *vs* Ag/AgCl.

The microdialysis biosensor was filled with a perfusate of phosphate buffer solution (PBS) containing (mM) NaCl 146, KCl 2.7, $Na_2HPO_4{\cdot}7H_2O$ 4.3, KH_2PO_4 1.4, $CaCl_2$ 2.4, and was perfused from the inlet tube at a rate of 0.2 μl·min^{-1} via an EP 50 perfusion pump (Eicom). The microdialysis biosensor was prepared by immersing the membrane in a beaker of 5 mM *O*-phenylenediamine in PBS bubbled with 100% N_2 while constantly stirring the PBS. A voltage clamp was then switched on from −400 mV to +600 mV to induce electropolymerization onto the working electrode. The ascorbate calibration was carried out by ascorbate solution up to 250 μM to test *O*-phenylenediamine coverage in order to reduce the interference from compounds oxidized electrochemically by the working electrode, such as ascorbate.

The microdialysis biosensor set at +600 mV was perfused with PBS containing glutamate oxidase (5×10^{-2} M in PBS; Yamasa, Chiba, Japan), and then calibration in vitro was performed based on nanoamperes *vs* concentration of L-glutamate.

Animal preparation

Male Wistar rats weighing 250–300 g ($n = 10$), anesthetized with sodium pentobarbital (50 mg·kg^{-1}, i.p.), were mechanically ventilated with a small-animal respirator (Model SN-480-7, Shinano, Tokyo) after tracheostomy. Anesthesia was maintained with 0.5 % halothane in a mixture of air and oxygen and pancuronium bromide during experiments. A tail vein and both femoral arteries were cannulated for infusion of lactated Ringer's solution (3 ml·kg^{-1}), and for arterial blood pressure measurement and blood withdrawal or reperfusion, respectively. The carotid arteries were isolated bilaterally, separating them from the vagus nerve. Arterial blood samples of 400 μl were drawn to help adjust the respirator to maintain blood gas at $Pao_2 > 100$ mmHg and $Paco_2$ of 40 ± 3 mmHg. A thermocouple probe was placed in the rectum to record body temperature continuously, and the rectal temperature was maintained at 38 ± 0.3°C by a thermostatically controlled warming plate and an overhead incandescent bulb.

The head was fixed in a stereotaxic frame. The microdialysis biosensor, prepared as described above, was stereotactically inserted into the forebrain cortex (3.3 mm anterior, 2.8 mm lateral, to the bregma; 1.0 mm ventral from the dura). The laser Doppler flowmeter needle probe of 3 mm diameter (Advance, ALF 21, Tokyo, Japan) was placed at the contra-lateral forebrain cortex over the dura for continuous measurement of regional CBF. All animals were allowed to recover for at least 90 min from any damage caused by probe implantation.

Experimental protocol

The rats were divided into the L-NAME group ($n = 5$) and the saline group ($n = 5$). The systemic mean arterial pressure (MAP), CBF, and rectal temperature were continuously recorded. The microdialysis biosensor, prepared as described above, was perfused with PBS containing glutamate oxidase (5×10^{-2} M in PBS) at a constant flow rate of 0.2 μl·min^{-1} for real-time glutamate measurement.

After stabilization of the biosensor and measurements of control values, the rats received intravenously either L-NAME (30 mg·kg^{-1} of 50 mg·ml^{-1} solution) or saline (equivolume of L-NAME), and forebrain ischemia-reperfusion was performed 10 min later. Global forebrain ischemia was produced by occlusion of both carotid arteries with systemic hypotension. To induce hypotension, the heparinized rats were bled for 2–3 min from the femoral artery to an MAP of 30 mmHg. Immediately after the MAP reached 30 mmHg, both carotid arteries were occluded by tapes. MAP was maintained at 30 mmHg by withdrawing additional blood or reperfusion blood from a syringe. Ischemia was maintained for 10 min, and then the cerebral circulation was restored for 60 min by reperfusing blood and unclamping the carotid arteries.

Statistical analysis

Statistical analysis was performed with StatView computer software. The data during the course of ischemia-reperfusion were analyzed by two-way repeated-measures analysis of variance. The Wilcoxon signed rank test was used for statistical analyses *vs* baseline

values. The unpaired *t*-test was used to compare the two groups.

Results

The results are shown in Figs. 1–3. The baseline values of MAP were 118.9 ± 21.5 mmHg in the saline group ($n = 5$) and 122.8 ± 10.5 mmHg in the L-NAME group ($n = 5$), and did not differ significantly between the groups. In the L-NAME group, MAP was significantly higher than in the saline group after L-NAME injection. Within 10 min after reperfusion, MAP returned to the baseline level in both groups (Fig. 1).

The baseline values of CBF were 31.8 ± 10.3 ml·min^{-1}·100 g^{-1} in the saline group ($n = 5$) and 32.6 ± 12.9 ml·min^{-1}·100 g^{-1} in the L-NAME group ($n = 5$), and did not differ significantly between the groups. The data are presented as percentages of baseline, because the laser Doppler flowmeter does not provide accurate measurements of absolute regional CBF values. In the L-NAME group, CBF was significantly lower than in the saline group after L-NAME injection and remained lower than in the saline group throughout the experiment. Within 20 min after reperfusion, CBF returned to the baseline level in the saline group but remained significantly lower for 30–60 min after reperfusion in the L-NAME group (Fig. 2).

The baseline values of extracellular glutamate were 21.6 ± 8.5 μM in the saline group ($n = 5$) and 21.5 ± 5.4 μM in the L-NAME group ($n = 5$), and did not differ significantly between the groups. The glutamate levels increased linearly markedly from the start of ischemia to immediately after reperfusion in both groups. The

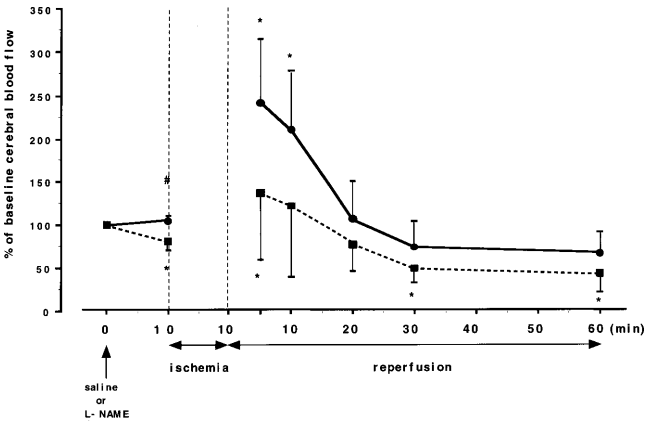

Fig. 2. Time-course changes in CBF during forebrain ischemia and reperfusion with and without intravenous N^G-nitro-L-arginine methyl ester (*L-NAME*) administration. The baseline values are 31.8 ± 10.3 ml·min^{-1}·100 g^{-1} in the saline group ($n = 5$) and 32.6 ± 12.9 ml·min^{-1}·100 g^{-1} in the L-NAME group ($n = 5$). They did not differ significantly between the groups. Data are presented as percentages of the baseline and means ± SD. *$P < 0.05$ *vs* baseline; #$P < 0.05$ between the two groups. During ischemia, CBF measured by laser Doppler flowmeter is near zero. *Circles*, saline group; *squares*, L-NAME group

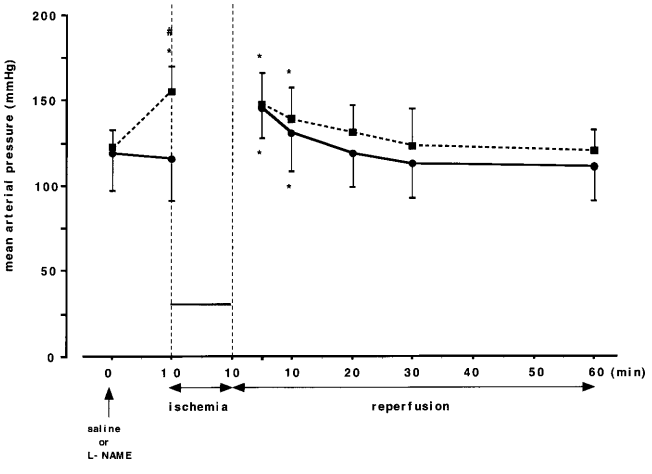

Fig. 1. Time-course changes in systemic MAP during forebrain ischemia and reperfusion with and without intravenous N^G-nitro-L-arginine methyl ester (*L-NAME*) administration. The baseline values are 118.9 ± 21.5 mmHg in the saline group ($n = 5$) and 122.8 ± 10.5 mmHg in the L-NAME group ($n = 5$). They did not differ significantly between the groups. Data are means ± SD. *$P < 0.05$ *vs* baseline; #$P < 0.05$ between the two groups. *Circles*, saline group; *squares*, L-NAME group

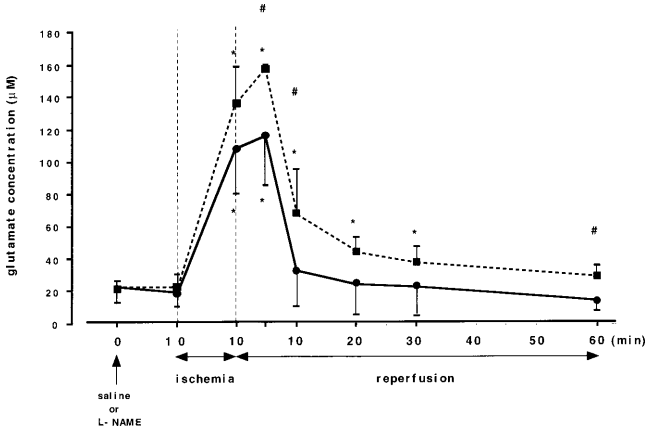

Fig. 3. Time-course changes in extracellular glutamate release in forebrain cortex during forebrain ischemia-reperfusion with and without intravenous N^G-nitro-L-arginine methyl ester (*L-NAME*) administration. The baseline values of glutamate were 21.6 ± 8.5 μM in the saline group ($n = 5$) and 21.5 ± 5.4 μM in the L-NAME group ($n = 5$). They did not differ significantly between the groups. Data are means ± SD. The maximum glutamate levels immediately after reperfusion were 116.3 ± 30.8 μM in the saline group and 157.6 ± 2.9 μM in the L-NAME group. *$P < 0.05$ *vs* baseline; #$P < 0.05$ between the two groups. *Circles*, saline group; *squares*, L-NAME group

maximum glutamate levels immediately after reperfusion were 116.3 ± 30.8 μM in the saline group and 157.6 ± 2.9 μM in the L-NAME group, and differed significantly between the groups. The glutamate levels were lower in the saline group than in the L-NAME group throughout the experiment, and differed significantly during the first 10 and 60 min after reperfusion. In the saline group the glutamate level decreased nearly to the baseline level within 10 min after reperfusion, but in the L-NAME group it remained significantly higher than baseline until 30 min after reperfusion (Fig. 3).

Discussion

Extracellular accumulation of EAAs causes damage to neuronal cells [6,12]. The activation of the glutamate receptor substantially increases calcium conductance, thereby contributing to neuronal injury. Cerebral ischemia results in energy failure (ATP depletion) within several minutes and is associated with a sudden, rapid increase in extracellular glutamate. As the pathway by which glutamate is released during ischemia, two different pools of glutamate exist within the synaptosomes. The extracellular glutamate accumulated during transient ischemia is constituted mainly of that released from the vesicular neurotransmitter pools in presynaptic terminals, and is a Ca^{2+}- and ATP-dependent process. After complete energy failure, glutamate release is mediated by the second cytoplasmic metabolic pool. Glutamate efflux from the metabolic pool is induced by reversal of the Na^+ cotransport system of glutamate in the plasma membrane provoked by depolarization caused by the limitation in ATP for Na^+K^+-ATPase during ischemia, whereas the vesicular release of transmitter is inhibited by the lack of ATP. In synaptosomes in vitro, Ca^{2+}-dependent glutamate release occurs in the first 2 min after the start of anoxia, and then is inhibited because of ATP depletion. However, Ca^{2+}-independent release of glutamate is activated during anoxia as a consequence of the decrease in ATP level and the change in ion gradient across the plasma membrane [13].

Using brain microdialysis in vivo, extracellular glutamate increases dramatically in the hippocampus and striatum during several minutes of transient cerebral ischemia [14,15]. Also, the time-course relationship between the release of glutamate from neurotransmitter pools in the striatum and from metabolic pools in the four-vessel occlusion-reperfusion model in the rat was reported with the dialysis electrode technique, the same method employed in this study [16]. A sharp and rapid elevation of glutamate release took place approximately 1 min later (first phase). Glutamate release continued to rise throughout the ischemic period, shifting to a continuous and gradual increase during the second phase. Ninety percent of the increase in extracellular glutamate produced by ischemic insult was cleared within approximately 10 min of reperfusion. These time-courses are consistent with glutamate release in this study. Excitatory neurotransmitters at high extraneuronal concentrations are important causes of neuronal injury after cerebral ischemia, and glutamate becomes cytotoxic at extracellular concentrations of ≈100 μM [17]. The maximal glutamate concentration immediately after reperfusion in this study was above this cytotoxic level (Fig. 3).

The physiological roles of NO in the central nervous system are also complex. NO has been implicated in cerebral vasodilation, long-term potentiation, and excitotoxicity. Ionotropic glutamate receptor agonists, such as NMDA, (+)-α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA), and kinate, have been shown to activate NOS by calcium influx from the extracellular space triggered by NMDA or non-NMDA receptors. Activation of metabotropic glutamate receptor by trans-(±)-l-amino-(1S,3R)-cyclopentanedicarboxylic acid (ACPD) also results in NOS activation [18]. The direct infusion of NMDA, AMPA, and ACPD through a microdialysis probe into the cerebellum of conscious rat results in a dose-dependent increase in the total levels of NO end products in the dialysate, suggesting NO production in vivo [19]. Moreover, inducible NOS has been identified in neuronal astrocytes and microglia in the brain [20] and is responsible for the synthesis of large amounts of NO in response to stimuli such as lipopolysaccarides and cytokines.

The experimental evidence has demonstrated that NO released by NOS-containing neurons mediates excitatory neurotoxicity [3] and is involved in NMDA receptor-mediated neurotoxicity [21] and in the neuronal death that occurs after focal cerebral ischemia [4,5]. In the rat, neurologic lesions in the cerebral cortex produced by cerebral ischemia-reperfusion were accompanied by an increase in tissue levels of NO [22,23] and NOS activity in the brain [22,24].

Thus, many previous studies have investigated the effects of NOS inhibitors on the regulation of cerebral circulation and on cerebral ischemic damage. However, the role of NO as a neuromodulator remains controversial. NOS inhibition can decrease infarct size in focal brain ischemia [5], but in global forebrain ischemia, NOS inhibition aggravated the hippocampal lesions [7,8]. A systemically administered NOS inhibitor suppressed extracellular glutamate concentration in a focal ischemia model [9], but increased it in the perfusion period after global forebrain ischemia [10]. In this study, extracellular glutamate was also significantly higher during the first 10 and 60 min after reperfusion in L-

NAME-treated rats compared with saline-treated control rats (Fig. 3). In an experiment similar to this study, in which the duration of forebrain ischemia produced by systemic hypotension (MAP, 35 mmHg) and occlusion of both carotid arteries was 15 min, the glutamate concentration remained elevated during reperfusion [10]. Thus, the degree of elevated glutamate concentration on reperfusion might be related to the extent of the ischemic insult.

The mechanisms of this postischemic increase in glutamate concentration after NOS inhibition are unclear. Regarding the effect of NO on glutamate level, the extracellular glutamate and aspartate originating from a Ca^{2+}-independent pool during forebrain ischemia is not modulated by NO, whereas the accumulated glutamate from enhanced vesicular release in high-K^+ depolarization is modulated by NO [25]. Thus, in this study, glutamate levels after 10 min of ischemia, when glutamate release originated from an Ca^{2+}-independent pool, did not differ significantly between the groups. Other possible effects of NOS inhibition on postischemic increase in glutamate concentration are the following. First, NO may act on presynaptic terminals to inhibit the release of glutamate. Because the NMDA receptor is inactivated by reaction of NO with a thiol group in its redox site [26], NOS inhibition may antagonize NO-mediated NMDA receptor inactivation. Second, NO may also play a role in scavenging reactive oxygen species such as superoxide and hydrogen peroxide [27]. Because free radicals promote release of glutamate [28], decrease in the scavenging of reactive oxygen species by NOS inhibition would antagonize NO-mediated inhibition of glutamate release in the presynaptic site. The failure of glutamate reuptake after reperfusion must also be considered.

NO has a neuroprotective effect in ischemia, increasing CBF by vasodilation, inhibiting platelet aggregation. In this study, the hyperemia that occurred immediately after ischemia was attenuated, and thereafter CBF was depressed by L-NAME (Fig. 2), suggesting that NO may be a mediator of these postischemic changes in CBF. Previous findings suggested that endothelial NOS was protective during ischemia, but neuronal and inducible NOS might be cytotoxic [4,29]. The decrease in CBF response after reperfusion by inhibition of endothelial NOS might contribute to a decrease in the reuptake of glutamate after reperfusion.

Systemic administration of a nonspecific NOS inhibitor such as L-NAME results in a decrease in global CBF (Fig. 2) and worsens the global ischemic insults and outcome [7,8]. In focal ischemia, however, the decrease in global CBF may not be critical, and L-NAME attenuates the cerebral lesions [9]. The use of a systemic low-dose or locally administered NOS inhibitor may decrease its systemic effects. Low-dose L-NAME ($0.1\,mg \cdot kg^{-1}$ bolus followed by $0.01\,mg \cdot kg^{-1} \cdot min^{-1}$) reduced the infarct volume in a focal ischemic rat model [30]. The local administration of the NOS inhibitor L-NAME via a striatal microdialysis probe in a global ischemic rat model resulted in a significant attenuation of peak glutamate release [31]. However, the continuously administered local L-NAME dose of $0.2\,mg \cdot ml^{-1}$ at the rate of $1\,\mu l \cdot min^{-1}$ used in this report is approximately the extracellular L-NAME concentration when an L-NAME dose of $30\,mg \cdot kg^{-1}$ is administered systemically. Thus, the systemic L-NAME dose of $30\,mg \cdot kg^{-1}$ used in this study may be necessary to decrease glutamate release through its direct modulatory effect.

In conclusion, by real-time monitoring of extracellular glutamate release using the microdialysis electrode technique, a remarkable linear increase in glutamate release was demonstrated in the forebrain cortex during global forebrain ischemia. NOS inhibition by L-NAME did not prevent this dramatic ischemia-induced accumulation of glutamate, and moreover, increased its level during reperfusion. In L-NAME-treated rats, the hyperemia that occurred immediately after reperfusion was attenuated, and the decrease in CBF response after reperfusion might be a factor in the elevated glutamate concentration after reperfusion due to a decrease in reuptake of glutamate.

Acknowledgment. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan (No. 08671757).

References

1. Choi DW (1993) Nitric oxide: foe or friend to the injured brain? Proc Natl Acad Sci USA 90:9741–9743
2. Lipton SA, Rosenberg PA (1994) Excitatory amino acids as a final common pathway for neurologic disorders. N Engl J Med 330:613–622
3. Dawson VL, Dawson TM, London ED, Bredt DS, Snyder SH (1991) Nitric oxide mediates glutamate neurotoxicity in primary cortical cultures. Proc Natl Acad Sci USA 88:6368–6371
4. Huang Z, Huang PL, Panahian N, Dalkara T, Fishman MC, Moskowitz MA (1994) Effects of cerebral ischemia in mice deficient in neuronal nitric oxide synthase. Science 265:1883–1885
5. Nowicki JP, Duval D, Poignet H, Scatton B (1991) Nitric oxide mediates neuronal death after focal cerebral ischemia in the mouse. Eur J Pharmacol 204:339–340
6. Dawson VL, Dawson TM (1996) Nitric oxide in neuronal degeneration. Proc Soc Exp Biol Med 211:33–40
7. Moncada C, Lekieffre D, Arvin B, Meldrum B (1992) Effect of NO synthase inhibition on NMDA- and ischaemia-induced hippocampal lesions. Neuroreport 3:530–532
8. Sancesario G, Iannone M, Morello M, Nisticò G, Bernardi G (1994) Nitric oxide inhibition aggravates ischemic damage of hippocampal but not of NADPH neurons in gerbils. Stroke 25:436–444
9. Buisson A, Margaill I, Callebert J, Plotkine M, Boulu RG (1993) Mechanisms involved in the neuroprotective activity of a nitric

oxide synthase inhibitor during focal cerebral ischemia. J Neurochem 61:690–696
10. Zhang J, Benveniste H, Klitzman B, Piantadosi CA (1995) Nitric oxide synthase inhibition and extracellular glutamate concentration after cerebral ischemia/reperfusion. Stroke 26:298–304
11. Walker MC, Galley PT, Errington ML, Shorvon SD, Jefferys JGR (1995) Ascorbate and glutamate release in the rat hippocampus after perforant path stimulation: a "dialysis electrode" study. J Neurochem 65:725–731
12. Rothman SM, Olney JW (1986) Glutamate and the pathophysiology of hypoxic-ischaemic brain damage. Ann Neurol 19:105–111
13. Sánchez-Prieto J, González P (1988) Occurrence of a large Ca^{2+}-independent release of glutamate during anoxia in isolated nerve terminals (synaptosomes). J Neurochem 50:1322–1324
14. Benveniste H, Drejer J, Schousboe A, Diemer NH (1984) Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. J Neurochem 43:1369–1374
15. Globus MY-T, Busto R, Dietrich WD, Martinez E, Valdes I, Ginsberg MD (1988) Effect of ischemia on the in vivo release of striatal dopamine, glutamate, and γ-aminobutyric acid studied by intracerebral microdialysis. J Neurochem 51:1455–1464
16. Asai S, Iribe Y, Kohno T, Ishikawa K (1996) Real time monitoring of biphasic glutamate release using dialysis electrode in rat acute brain ischemia. Neuroreport 7:1092–1096
17. Choi DW, Koh J-Y, Peters S (1988) Pharmacology of glutamate neurotoxicity in cortical cell culture: attenuation by NMDA antagonists. J Neurosci 8:185–196
18. Yamada K, Nabeshima T (1997) Two pathways of nitric oxide production through glutamate receptors in the rat cerebellum in vivo. Neurosci Res 28:93–102
19. Garthwaite J (1991) Glutamate, nitric oxide and cell-cell signalling in the nervous system. Trends Neurosci 14:60–67
20. Bredt DS, Hwang PM, Snyder SH (1990) Localization of nitric oxide synthase indicating a neural role for nitric oxide. Nature 347:768–770
21. Dawson VL, Dawson TM, Bartley DA, Uhl GR, Snyder SH (1993) Mechanisms of nitric oxide-mediated neurotoxicity in primary brain cultures. J Neurosci 13:2651–2661
22. Kader A, Frazzini VI, Solomon RA, Trifiletti RR (1993) Nitric oxide production during focal cerebral ischemia in rats. Stroke 24:1709–1716
23. Kumura E, Yoshimine T, Iwatsuka K, Yamanaka K, Tanaka S, Hayakawa T, Shiga T, Kosaka H (1996) Generation of nitric oxide and superoxide during reperfusion after focal cerebral ischemia in rats. Am J Physiol 270: C748–C752
24. Zhang ZG, Chopp M, Zaloga C, Pollock JS, Förstermann U (1993) Cerebral endothelial nitric oxide synthase expression after focal cerebral ischemia in rats. Stroke 24:2016–2022
25. Ghribi O, Callebert J, Plotkine M, Boulu RG (1994) L-NAME modulates glutamate accumulation induced by K^+-depolarization but not by forebrain ischaemia in the rat striatum. Neurosci Lett 174:34–38
26. Lipton SA, Choi Y-B, Pan Z-H, Lei SZ, Chen H-SV, Sucker NJ, Loscalzo J, Singel DJ, Stamler JS (1993) A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. Nature 364:626–632
27. Wink DA, Hanbauer I, Krishna MC, DeGraff W, Gamson J, Mitchell JB (1993) Nitric oxide protects against cellular damage and cytotoxicity from reactive oxygen species. Proc Natl Acad Sci USA 90:9813–9817
28. Pellegrini-Giampietro DE, Cherici G, Alesiani M, Carlà V, Moroni F (1988) Excitatory amino acid release from rat hippocampal slices as a consequence of free-radical formation. J Neurochem 51:1960–1963
29. Faraci FM, Brian JE (1994) Nitric oxide and the cerebral circulation. Stroke 25:692–703
30. Ashwal S, Cole DJ, Osborne TN, Pearce WJ (1993) Low dose L-NAME reduces infarct volume in the rat MCAO/reperfusion model. J Neurosurg Anesthesiol 5:241–249
31. Kahn RA, Panah M, Kiffel S, Weinberger J (1997) Modulation of ischemic excitatory neurotransmitter and γ-aminobutyric acid release during global temporary cerebral ischemia by local nitric oxide synthase inhibition. Anesth Analg 84:1004–1010