Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 6368–6371, July 1991
Neurobiology

Nitric oxide mediates glutamate neurotoxicity in primary cortical cultures

(endothelium-derived relaxing factor/*N*-methyl-D-aspartate)

VALINA L. DAWSON*, TED M. DAWSON†, EDYTHE D. LONDON*, DAVID S. BREDT†, AND SOLOMON H. SNYDER†‡

*Neuropharmacology Laboratory, National Institute on Drug Abuse Addiction Research Center, P.O. Box 5180, Baltimore, MD 21224; and †Departments of Neuroscience, Pharmacology and Molecular Sciences, and of Psychiatry, The Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205

Contributed by Solomon H. Snyder, April 22, 1991

ABSTRACT **Nitric oxide (NO) mediates several biological actions, including relaxation of blood vessels, cytotoxicity of activated macrophages, and formation of cGMP by activation of glutamate receptors in cerebellar slices. Nitric oxide synthase (EC 1.14.23.-) immunoreactivity is colocalized with nicotinamide adenine di-nucleotide phosphate diaphorase in neurons that are uniquely resistant to toxic insults. We show that the nitric oxide synthase inhibitors, N^{ω}-nitro-L-arginine (EC_{50} = 20 μM) and N^{ω}-monomethyl-L-arginine (EC_{50} = 170 μM), prevent neurotoxicity elicited by *N*-methyl-D-aspartate and related excitatory amino acids. This effect is competitively reversed by L-arginine. Depletion of the culture medium of arginine by arginase or arginine-free growth medium completely attenuates *N*-methyl-D-aspartate toxicity. Sodium nitroprusside, which spontaneously releases NO, produces dose-dependent cell death that parallels cGMP formation. Hemoglobin, which complexes NO, prevents neurotoxic effects of both *N*-methyl-D-aspartate and sodium nitroprusside. These data establish that NO mediates the neurotoxicity of glutamate.**

Nitric oxide (NO), first identified as endothelium-derived relaxing factor (1–3), is also an important neuronal messenger molecule (4, 5). A physiological role for NO in the nervous system has been established by demonstrations that arginine derivatives, which are potent and selective inhibitors of nitric oxide synthase (NOS; EC 1.14.23.-), block neuronally mediated relaxation of the intestine (6–8) and stimulation of cGMP formation by glutamate in the cerebellum (9, 10). NO is formed from arginine in brain and endothelial cells by NOS, which has been purified to homogeneity (11) and molecularly cloned (12) from rat brain. Macrophages and other blood cells also produce NO, which mediates their bactericidal and tumoricidal effects (13). However, the NOS of macrophages is a distinct protein from NOS in brain and endothelial tissues, differing in cofactor requirements. Immunocytochemical studies have localized NOS to select neuronal populations in the brain, as well as to neurons in the retina, adrenal medulla, and intestine, and to nerve fibers in the posterior pituitary (14, 15).

Glutamate may mediate the neurotoxicity observed in hypoxic-ischemic brain injury, as selective antagonists of the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor prevent neuronal cell death in animal models of hypoxic-ischemic brain injury (16). Glutamate neurotoxicity has also been implicated in neurodegenerative disorders such as Alzheimer and Huntington diseases (16, 17). Glutamate neurotoxicity mediated by NMDA receptors involves calcium entry into cells via ligand-gated ion channels (17). Interestingly, the enhancement of NOS activity by NMDA stimulation of cerebellar slices also derives from channel-associated entry of calcium, which binds to calmodulin associated with NOS (9–11). To ascertain the relationship of NO to glutamate neurotoxicity, we have employed rat primary cortical cultures and demonstrate that glutamate neurotoxicity is prevented selectively by inhibitors of NOS.

MATERIALS AND METHODS

Cell Culture. Primary dissociated cell cultures were prepared from fetal rats (13- to 14-day gestation for cortex and caudate–putamen cultures and 19- to 20-day gestation for hippocampal cultures). The tissue of interest was dissected, incubated for 15 min in 0.027% trypsin in Brooks–Logan solution (5% phosphate-buffered saline/0.04 M sucrose/10 mM Hepes/0.03 M glucose, pH 7.4), and then transferred to modified Eagle's medium (MEM)/10% horse serum/10% fetal bovine serum/2 mM glutamine for trituration. Dissociated cells were plated at a density of $3–4 \times 10^5$ cells per well in polyornithine-coated 15-mm multiwell plates. After 4 days the cells were treated with 10 μg of 5-fluoro-2′-deoxyuridine to prevent proliferation of nonneuronal cells. Cells were maintained in MEM/5% horse serum/2 mM glutamine in 8% CO_2/humidified atmosphere at 37°C. The medium was changed twice weekly. In the present study, mature neurons (3–4 weeks) were used.

Cytoxicity. Cells were exposed to excitatory amino acids according to the method of Koh and Choi (18). Before exposure, the cells were washed three times with Tris-buffered control salt solution (CSS) (18), containing 120 mM NaCl/5.4 mM KCl/1.8 mM $CaCl_2$/25 mM Tris hydrochloride, pH 7.4 at room temperature/15 mM glucose). Brief exposures to glutamate, NMDA (plus 10 μM glycine), quisqualate, and sodium nitroprusside (SNP) were performed for 5 min in CSS. The exposure solution was then washed away and replaced by MEM with 21 mM glucose; then the cells were placed in an incubator for 20–24 hr. Long exposures to kainate were performed in MEM/21 mM glucose for 20–24 hr in the incubator. After exposure to the excitatory amino acids, the medium was replaced by CSS/0.4% trypan blue, which stains nonviable cells. Two to four photographs (10–20×) were made of each well, and viable versus nonviable cells were counted. The cytotoxicity data represent 6–24 separate wells assayed per data point, with ≈500–1500 cells counted per well. In some experiments, overall neuronal cell injury was also assessed by the measurement of lactate

Abbreviations: CSS, control salt solution; Hb, hemoglobin; L-Arg, L-arginine; D-Arg, D-arginine; MEM, modified Eagle's medium; NADPH, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; NOS, nitric oxide synthase; N-Arg, N^{ω}-nitro-L-arginine; NMDA, *N*-methyl-D-aspartate; Me-Arg, N^{ω}-monomethyl-L-arginine; SNP, sodium nitroprusside.
‡To whom reprint requests should be addressed.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

dehydrogenase released by damaged or destroyed cells into the extracellular fluid after drug exposure (18).

Biochemical Assays. For determination of cGMP the cells were washed three times with CSS. After a 1-min exposure to drug solutions the cells were inactivated with 15% trichloroacetic acid. After ether extraction, cGMP levels were determined by RIA. [^{3}H]MK-801 binding to the NMDA receptor/channel complex was assayed, as described (19). NO synthase activity was assayed by the conversion of [^{3}H]arginine to [^{3}H]citrulline (9, 11). For immunoblots, 200–400 μg of crude tissue protein was separated on a 7.5% SDS/polyacrylamide gel and transferred to nitrocellulose. Lanes were incubated with affinity-purified antibody (1:1000) (14).

Electrophysiology. Cortical neurons in sister cultures were voltage clamped by using the whole-cell version of the patch-clamp technique (20).

Materials. [^{3}H]Arginine (53 Ci/mmol; 1 Ci = 37 GBq) was obtained from DuPont/NEN. cGMP RIA kits were obtained from Amersham. N^{ω}-monomethyl-L-arginine (Me-Arg) was obtained from Calbiochem. All other chemicals were purchased from Sigma.

RESULTS

Before initiating cytotoxicity experiments with NOS inhibitors, we evaluated the potency of NMDA applied for 5 min in inducing cell death by examining exclusion of 0.4% trypan blue by viable cells or measuring lactate dehydrogenase released by damaged or destroyed cells into the extracellular fluid. As reported earlier (18), NMDA displays a toxic LD_{50} of 280 μM (data not shown), and 300 μM NMDA consistently yields 60% cell death (Fig. 1). Simultaneous application of 100 μM N^{ω}-nitro-L-arginine (N-Arg), a potent NOS inhibitor (21, 22), with NMDA reduces cell death by 70% (Fig. 1). Addition of 1 mM L-arginine (L-Arg) to the exposure solution completely reverses the effect of N-Arg (Fig. 1). To further ascertain whether NO is involved in NMDA neurotoxicity we added 500 μM of reduced hemoglobin (Hb), which binds NO, simultaneously with 300 μM NMDA (Fig. 1). Hb completely prevents NMDA-induced cell death at 500 μM, similar to the concentration required for reduced myoglobin to prevent macrophage-mediated cell death (23).

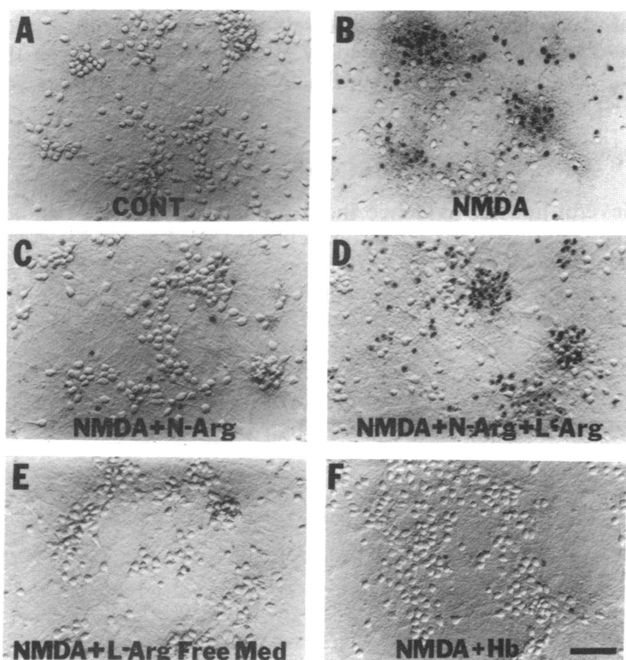

FIG. 1. Bright-field photomicrographs of cortical cell cultures 24 hr after treatment and after 0.4% trypan blue dye exclusion to stain nonviable cells. Dead cells appear as black dots, and live cells appear as raised cells against the gray background. (*A*) Control (Cont). (*B*) NMDA (300 μM plus 10 μM glycine). (*C*) NMDA (300 μM plus 10 μM glycine) plus 100 N-Arg demonstrates inhibition of NMDA cytotoxicity. (*D*) L-Arg (1 mM) reverses the inhibition by 100 μM N-Arg of 300 μM NMDA (plus 10 μM glycine) cytotoxicity. (*E*) Treatment of cell cultures for 24 hr in L-Arg-free medium abolishes NMDA toxicity. (*F*) Reduced Hb plus 300 μM NMDA (with 10 μM glycine) reverses cell death. Photomicrographs were taken randomly from culture wells and are representative of 6–24 determinations.

The cortical cultures possess substantial NOS catalytic activity and protein, ≈10% of cerebellar levels (data not shown). Furthermore, NMDA can stimulate the formation of NO in cortical cultures. Formation of cGMP can be used as an indirect measure of NO formation (9, 10), as NO binds to the heme moiety of guanylate cyclase and subsequently activates the enzyme. As has been shown in cerebellar slices, NMDA stimulation of cGMP levels in cultures is antagonized by inhibiting NOS with N-Arg or complexing NO with Hb (Fig. 2). N-Arg inhibition of cGMP formation by NMDA is competitively reversible by L-Arg. Superoxide dismutase, which removes superoxide that would degrade NO, potentiates the formation of cGMP by NMDA (Fig. 2). Thus, as in cerebellar slices, in cortical cell cultures NMDA stimulation of cGMP formation involves NO.

N-Arg (100 μM and 500 μM) has no effect on [^{3}H]MK-801 binding to NMDA receptor channels in rat cerebral cortical membranes (data not shown). Similarly, 100 μM N-Arg has no effect on NMDA-induced currents measured in the cortical cultures by whole-cell patch-clamp analysis (data not shown). Therefore, N-Arg does not directly act on NMDA receptors.

To ascertain the potency of N-Arg in preventing cell death, we varied the concentration of N-Arg (Fig. 3). Approximately 50% prevention of NMDA-induced cell death is evident at 20 μM N-Arg. The potency of N-Arg is similar whether applied simultaneously with NMDA or 5 min earlier. Me-Arg, another NOS inhibitor less potent than N-Arg (21, 22), also prevents NMDA-induced cell death with 50% prevention of death at a concentration of 170 μM (Fig. 3).

In inhibiting NOS activity of purified enzyme preparations, N-Arg and Me-Arg have K_i values of 200 nM and 2 μM, respectively (22). The higher concentrations of these inhibitors required to prevent NMDA neurotoxicity probably reflects competition by the high 1 mM L-Arg concentration in the culture medium. Therefore, we added arginase in amounts that should completely degrade L-Arg. Additionally, sister cultures were grown for 24 hr in medium without L-Arg, but containing glutamine, a procedure that fully depletes cells of

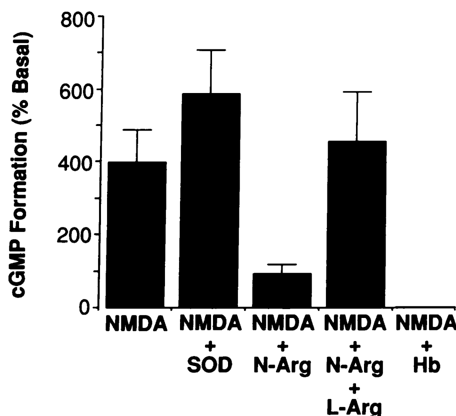

FIG. 2. cGMP formation after 1-min application of 300 μM NMDA (plus 10 μM glycine) to cortical cultures with or without 100 μM N-Arg, 100 μM N-Arg plus 1 mM L-Arg, 100 units of superoxide dismutase (SOD), or 500 μM reduced Hb. Basal level of cGMP is 1.5 pmol/mg of protein. Data are means ± SEM (n = 6–8).

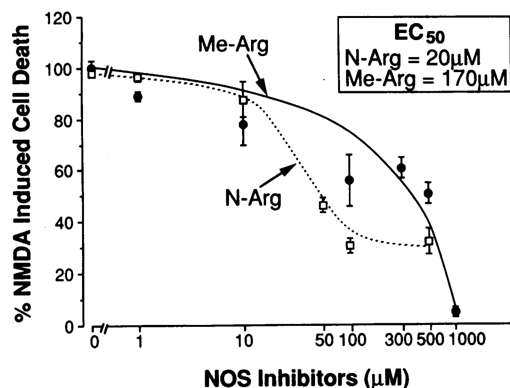

FIG. 3. Concentration–response relationship of NOS inhibitors in inhibiting NMDA neurotoxicity. Data are means ± SEM (n = 6).

L-Arg (24). In both arginase-treated preparations and in experiments with L-Arg-free medium, 300 μM NMDA no longer causes cell death (Fig. 1), and the 50% lethal concentration of NMDA is increased >20-fold to 7.5 mM (data not shown). Adding graded concentrations of L-Arg to L-Arg-free medium reveals a requirement for 100 μM L-Arg to obtain maximal NMDA effects (data not shown).

Glutamate neurotoxicity in primary cortical cultures can be elicited by NMDA, quisqualate, and kainate, although the NMDA receptor presumably accounts for most neurotoxicity associated with synaptically released glutamate in various pathologic conditions (16, 17). Quisqualate and kainate act more slowly and less potently than NMDA and via somewhat different mechanisms (25). To examine the role of NO in other forms of glutamate neurotoxicity, we compared the effects of N-Arg on cytotoxicity induced by quisqualate, kainate, and NMDA (Table 1). A portion of cell death elicited by quisqualate is prevented by N-Arg with reversal by L-Arg, but higher concentrations of N-Arg are required than with NMDA toxicity. N-Arg provides no protection against kainate-induced cell death. The slight protection afforded by N-Arg with quisqualate neurotoxicity may relate to the portion of quisqualate cell death occurring via NMDA receptor activation (25). The relative effects of the glutamate analogs and N-Arg are similar in cultures from caudate–putamen, hippocampus, and cerebral cortex (data not shown).

To ascertain the specificity of L-Arg in reversing N-Arg effects by arginine, we compared L-Arg with homoarginine

Table 1. Inhibition of glutamate neurotoxicity by N-Arg and reversal by L-Arg in cortical culture

	Cell death, % ± SEM
500 μM glutamate	48.4 ± 4.4
+ 100 μM N-Arg	28.9 ± 6.0*
+ 100 μM N-Arg + 1 mM L-Arg	48.2 ± 3.2
300 μM NMDA + 10 μM glycine	57.8 ± 2.6
+ 100 μM N-Arg	17.7 ± 3.0**
+ 100 μM N-Arg + 1 mM L-Arg	63.4 ± 5.5
500 μM quisqualate	64.2 ± 3.7
+ 500 μM N-Arg	51.1 ± 2.4***
+ 500 μM N-Arg + 5 mM L-Arg	66.6 ± 5.3
100 μM kainate	81.4 ± 7.1
+ 500 μM N-Arg	87.0 ± 3.2

Data are means ± SEM (n = 6–24). Cell death was determined by 0.4% trypan blue exclusion by viable cells (see text). Significant overall F values were obtained by using a one-way, between-groups analysis of variance. Specific comparisons on all possible pairwise combinations were made with the Student's t test for independent means, $P < 0.05$. *F, 2, 16, 29.3, $P < 0.001$; **F, 2, 33, 59.7, $P < 0.001$; ***F, 2, 46, 6.1, $P < 0.005$.

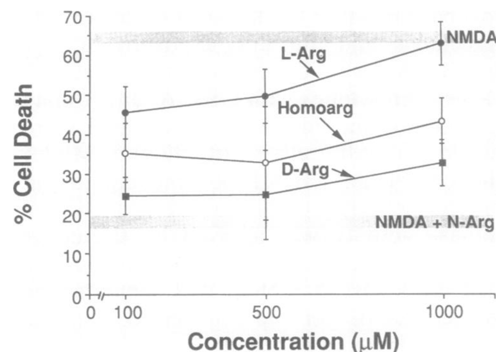

FIG. 4. Selectivity for reversal of N-Arg protection from NMDA cytotoxicity by L-Arg, homoarginine (Homoarg), and D-Arg. Solid horizontal lines represent the percent of cell death after exposure to 300 μM NMDA (plus 10 μM glycine) or 300 μM NMDA (with 10 μM glycine) plus 100 μM N-Arg. Shading represents the mean and SEM for NMDA or NMDA plus N-Arg. Data are means ± SEM (n = 6).

and D-arginine (D-Arg) (Fig. 4). Homoarginine can serve as a precursor of NO but has less affinity for NOS than L-Arg (26). Homoarginine reverses effects of N-Arg but is less potent than L-Arg. The stereoisomer D-Arg is much weaker than L-Arg, possibly reflecting a 1–2% contamination with L-Arg, as the extent of N-Arg reversal by 1 mM D-Arg is comparable to that obtained with 10 μM L-Arg (data not shown).

To determine the direct effect of NO upon the cortical cells, we exposed the cells for 5 min to SNP, which spontaneously releases NO, and assessed cell death 24 hr later (Fig. 5). SNP elicits cell death in a concentration-dependent fashion with a 50% maximal response at 50 μM. SNP enhances cGMP levels in the cortical cells with similar potency. The influence of SNP on cGMP levels in these cells appears to involve NO, as it is blocked by Hb, which complexes with NO. Furthermore, reduced Hb (500 μM) completely prevents SNP-induced cell death.

DISCUSSION

Several lines of evidence indicate that NO mediates glutamate neurotoxicity in neuronal cultures. N-Arg and Me-Arg, selective inhibitors of NOS, prevent glutamate receptor-mediated cell death in proportion to their potencies as inhibitors of NOS. These agents are competitive inhibitors of NOS, and their prevention of cell death is reversed in a concentration-dependent fashion by L-Arg. Moreover, the effect of L-Arg is exerted by various arginine derivatives in proportion to their activity as substrates for NOS. Removal

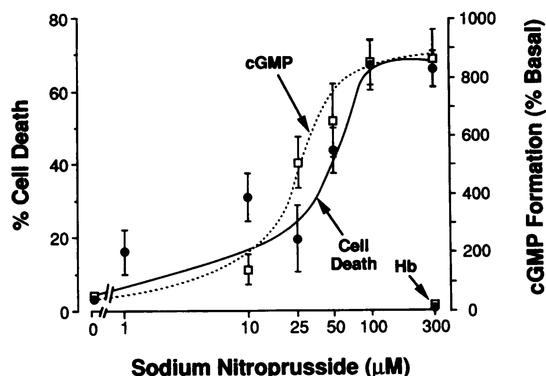

FIG. 5. Comparison of cytotoxicity after 5-min exposure to SNP and cGMP formation after a 1-min exposure. Hb (500 μM) completely reverses SNP-mediated cytotoxicity and cGMP formation (arrow). Basal level of cGMP is 1.5 pmol/mg of protein. Data are means ± SEM (n = 8–12).

of arginine from the culture medium by the enzyme arginase or the use of arginine-free medium also prevents NMDA-induced cell death. In addition, generation of NO by exposure to SNP elicits cell death with a concentration–response relationship corresponding to the ability of SNP to stimulate cGMP formation, an effect that is mediated by NO. Finally, reduced Hb, which binds NO, prevents both SNP- and NMDA-induced neurotoxicity.

Our demonstration that NO mediates the killing of neuronal cells is reminiscent of the actions of NO in killing tumor cells and bacteria when NOS activity is induced in macrophages and neutrophils (13, 23). NOS of macrophages differs from the brain-endothelial enzyme in its requirement for tetrahydrobiopterin and its independence from calmodulin (27). In adult rat cerebellum, virtually all NOS activity requires calcium, suggesting that there is a negligible contribution from a macrophage-like enzyme (11). NOS in primary brain cultures has not been sufficiently characterized to rule out the presence of a macrophage-like enzyme. However, in macrophages only negligible NOS activity can be demonstrated in cells that have not been stimulated with endotoxin, γ-interferon, and lipopolysaccharides (27, 28). Conceivably, macrophage-like NOS, perhaps in microglia of neuronal cultures, could be induced by glutamate stimulation.

If neuronal NOS mediates glutamate neurotoxicity, it is important to determine which central neurons possess NOS. In the cerebral cortex NOS immunoreactivity is confined to a discrete population of medium-to-large aspiny neurons comprising $\approx$1–2% of the total population (14, 15). Recently, we have shown that NOS neurons in the brain correspond to those that stain selectively for nicotinamide adenine dinucleotide phosphate (NADPH) diaphorase (5, 15). NADPH diaphorase-containing neurons are selectively resistant to degeneration in Huntington disease (29), in Alzheimer disease (30), in hypoxic-ischemic brain injury (31), and in response to neurotoxins (18, 32). Thus, some feature of NOS-containing neurons renders them resistant to various forms of glutamate neurotoxicity. If neuronal NOS provides the NO that mediates glutamate neurotoxicity, then these NOS/NADPH diaphorase neurons are its source. Choi and coworkers (18, 32) have shown that NADPH diaphorase neurons in primary cortical cultures resist NMDA toxicity but are more susceptible than other neurons to kainate or quisqualate toxicity. Low-dose quisqualate treatment of the cultures, which selectively kills NOS/NADPH diaphorase neurons, reduces the toxicity of subsequently administered NMDA (unpublished work), suggesting that these neurons mediate NMDA killing of other neurons.

The mechanism by which NO kills cells is unknown. Free radical formation has been implicated in various forms of neurotoxicity (16, 17), and NO is a reactive free radical. NO can also react with superoxide to yield perioxynitrate, which is extremely reactive (33). In models of macrophage-mediated cytotoxicity NO can complex with the iron–sulfur center of enzymes to inactivate them (23, 34). Because several of these enzymes are in the mitochondrial electron-transport complex, NO can inhibit mitochondrial respiration, diminishing the ability of the cells to deal with oxidative stress. Interestingly, glutamate transiently decreases activity of NAD(P)H:(quinone-acceptor)oxidoreductase (EC 1.6.99.2), an iron–sulfur-containing enzyme, whereas its induction prevents glutamate toxicity in a neuronal cell line (35).

NO mediation of glutamate neurotoxicity may have clinical implications. Centrally selective NOS inhibitors should have therapeutic utility in conditions involving neurotoxicity mediated through glutamate receptors.

We thank Dr. James Bell for consulting on the electrophysiology and Carol Beglan for technical assistance. This research was conducted while T.M.D. was a Pfizer Postdoctoral Fellow. T.M.D. was also supported by the Dana Foundation, the French Foundation for Alzheimer Research, and the American Academy of Neurology. V.L.D. is a recipient of a Pharmacology Research Associate Training Program Award (National Institute of General Medical Sciences). This work was supported by U.S. Public Health Service Grant DA-00266, Contract DA-271-90-7408, Research Scientist Award DA-00074 to S.H.S., Training Grant GM-07309 to D.S.B., a grant of the International Life Sciences Institute, and a gift from Bristol-Myers Squibb.

1. Moncada, S., Palmer, R. M. J. & Higgs, E. A. (1989) *Biochem. Pharmacol.* **38**, 1709–1715.
2. Furchgott, R. F. (1990) *Acta Physiol. Scand.* **139**, 257–270.
3. Ignarro, L. J. (1990) *Annu. Rev. Pharmacol. Toxicol.* **30**, 535–560.
4. Garthwaite, J. (1991) *Trends Neurosci.* **14**, 60–67.
5. Snyder, S. H. & Bredt, D. S. (1991) *Trends Pharmacol. Sci.* **12**, 125–128.
6. Bult, H., Boeckxstaens, G. E., Peickmans, P. A., Jordaens, F. H., Van Maercke, Y. M. & Herman, A. G. (1990) *Nature (London)* **345**, 346–347.
7. Gillespie, J. S., Liu, X. & Martin, W. (1989) *Br. J. Pharmacol.* **98**, 1080–1082.
8. Ramagopal, M. W. & Leighton, H. J. (1989) *Eur. J. Pharmacol.* **174**, 297–299.
9. Bredt, D. S. & Snyder, S. H. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 9030–9033.
10. Garthwaite, J., Garthwaite, G., Palmer, R. M. J. & Moncada, S. (1989) *Eur. J. Pharmacol.* **172**, 413–416.
11. Bredt, D. S. & Snyder, S. H. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 682–685.
12. Bredt, D. S., Hwang, P. M., Glatt, C. S., Lowenstein, C., Reed, R. R. & Snyder, S. H. (1991) *Nature (London)*, in press.
13. Hibbs, J. B., Jr., Vavrin, Z. & Taintor, R. R. (1987) *J. Immunol.* **138**, 550–565.
14. Bredt, D. S., Hwang, P. M. & Snyder, S. H. (1990) *Nature (London)* **347**, 768–770.
15. Dawson, T. M., Bredt, D. S., Fotuhi, M., Hwang, P. M. & Snyder, S. H. (1991) *Proc. Natl. Acad. Sci. USA*, in press.
16. Choi, D. W. (1990) *J. Neurosci.* **10**, 2493–2501.
17. Meldrum, B. & Garthwaite, J. (1990) *Trends Pharmacol. Sci.* **11**, 379–387.
18. Koh, J.-Y. & Choi, D. W. (1988) *J. Neurosci.* **8**, 2153–2163.
19. Javitt, D. C. & Zukin, S. R. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 740–744.
20. Hamill, O. P., Neher, M. E., Sakmann, B. & Sigworth, F. J. (1981) *Pflügers Arch.* **391**, 85–100.
21. Hecker, M., Mitchell, J. A., Harris, H. J., Katsura, M., Thiemermann, C. & Vane, J. (1990) *Biochem. Biophys. Res. Commun.* **167**, 1037–1043.
22. Dwyer, M., Bredt, D. S. & Snyder, S. H. (1991) *Biochem. Biophys. Res. Commun.* **176**, 1136–1141.
23. Stuehr, D. J. & Nathan, C. J. (1989) *J. Exp. Med.* **169**, 1543–1555.
24. Sessa, W. C., Hecker, M., Mitchell, J. A. & Vane, J. R. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 8607–8611.
25. Koh, J.-Y., Goldberg, M. P., Hartley, D. M. & Choi, D. W. (1990) *J. Neurosci.* **10**, 693–705.
26. Knowles, R. G., Palacios, M., Palmer, R. M. J. & Moncada, S. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5159–5162.
27. Tayeh, M. A. & Marletta, M. A. (1989) *J. Biol. Chem.* **264**, 19654–19658.
28. Marletta, M. A. (1989) *Trends Biochem. Sci.* **14**, 488–492.
29. Ferrante, R. J. (1985) *Science* **230**, 561–563.
30. Kowall, N. W. & Beal, M. F. (1988) *Ann. Neurol.* **23**, 105–114.
31. Uemura, Y., Kowall, N. W. & Beal, M. F. (1990) *Ann. Neurol.* **27**, 620–625.
32. Koh, J.-Y., Peters, S. & Choi, D. W. (1986) *Science* **234**, 73–76.
33. Beckman, J. S., Beckman, T. W., Chen, J., Marshall, P. A. & Freeman, B. A. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 1620–1624.
34. Hibbs, J. B., Taintor, R. R., Vavrin, Z. & Rechlin, E. M. (1988) *Biochem. Biophys. Res. Commun.* **157**, 87–94.
35. Murphy, T. M., DeLong, M. J. & Coyle, J. T. (1991) *J. Neurochem.* **56**, 990–995.