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Nerve growth factor rapidly suppresses basal, NMDA-evoked, and AMPA-evoked nitric oxide synthase activity in rat hippocampus *in vivo*

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ABSTRACT **In adult forebrain, nerve growth factor (NGF) influences neuronal maintenance and axon sprouting and is neuroprotective in several injury models through mechanisms that are incompletely understood. Most NGF signaling is thought to occur after internalization and retrograde transport of trkA receptor and be mediated through the nucleus. However, NGF expression in hippocampus is rapidly and sensitively regulated by synaptic activity, suggesting that NGF exerts local effects more dynamically than possible through signaling requiring retrograde transport to distant afferent neurons. Interactions have been reported between NGF and nitric oxide (NO). Because NO affects both neural plasticity and degeneration, and trk receptors can mediate signaling within minutes, we hypothesized that NGF might rapidly modulate NO production. Using *in vivo* microdialysis we measured conversion of L-[^{14}C]arginine to L-[^{14}C]citrulline as an accurate reflection of NO synthase (NOS) activity in adult rat hippocampus. NGF significantly reduced NOS activity to 61% of basal levels within 20 min of onset of delivery and maintained NOS activity at less than 50% of baseline throughout 3 hr of delivery. This effect did not occur with control protein (cytochrome *c*) and was not mediated by an effect of NGF on glutamate levels. In addition, simultaneous delivery of NGF prevented significant increases in NOS activity triggered by the glutamate receptor agonists *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA). Rapid suppression by NGF of basal and glutamate-stimulated NOS activity may regulate neuromodulatory functions of NO or protect neurons from NO toxicity and suggests a novel mechanism for rapidly mediating functions of NGF and other neurotrophins.**

Nerve growth factor (NGF) was discovered as a molecule that regulates the survival and maturation of developing neurons in the peripheral nervous system (1). NGF is also a signaling molecule for neurons in the central nervous system (CNS) and many non-neuronal cell types in the immune and endocrine systems, in adults as well as during development (1, 2). In the adult CNS, NGF has been implicated in a variety of effects ranging from neuronal maintenance and regulation of axon sprouting, to pharmacological protection of neurons from various insults (3–10). Particularly as regards neural plasticity and protection, the mechanisms underlying the diverse effects of NGF are poorly understood.

Classically, NGF signaling in neurons is thought to occur after binding to, internalization, and retrograde transport of receptor, and be mediated through the nucleus (11–13). In the adult forebrain, this type of signaling is in keeping with observations that receptor-bearing basal forebrain cholinergic neurons retrogradely transport radioactively labeled NGF from target regions where NGF is produced, and that exogenous NGF induces gene expression and hypertrophy, whereas depriving cells of target-derived NGF leads to neuronal atrophy (3, 4, 14, 15). Nevertheless, NGF expression in the hippocampus is rapidly and sensitively regulated by synaptic mechanisms (16–18), suggesting that NGF may exert local effects more rapidly than could be mediated by retrograde transport to distant afferent neurons. Rapid effects of other neurotrophins that signal through trk receptors have been reported recently on long-term potentiation and synaptic mechanisms (19), and NGF has been shown to modulate within minutes the functional organization of adult neocortex, the activity of sodium channels, the generation of reactive oxygen species, and the turning of axons *in vitro* (20–23).

Nitric oxide (NO) plays a role in the forebrain as a neuromodulator and regulator of cerebral blood flow, and NO can exert neurotoxic effects at high concentrations (24–29). Because activation of glutamate receptors can stimulate NO production, and blockade of NO production can attenuate excitotoxicity, NO has been suggested as a potential mediator of glutamate neurotoxicity and ischemia-induced neurodegeneration (27, 29, 30). Multiple interactions between NGF and NO have been reported. Chronic NGF delivery stimulates NOS synthesis *in vitro* and *in vivo,* and NO has been implicated as a mediator of PC12 cell differentiation and NGF-stimulated neurite outgrowth (31–33). Because trk receptors can induce signaling within minutes, we speculated that NGF may have rapid effects on NO production in the hippocampus, where NGF expression is rapidly and sensitively regulated and where NGF has effects on neural plasticity and protection through unknown mechanisms.

NO is formed by NO synthase (NOS), which oxidizes a guanidino nitrogen of arginine to produce NO and citrulline. NOS activity can be measured by the conversion of radiolabeled arginine to citrulline *in vitro* (24). This procedure has been adapted to measure NOS activity *in vivo* by using microdialysis probes to deliver L-[^{14}C]arginine, and measuring the recovery of L-[^{14}C]citrulline in the dialysis effluent (34, 35). In this manner, basal levels of NOS activity, as well as significant increases after stimulation of various glutamate receptor subtypes, can be measured reliably in the hippocampus and other forebrain areas (34, 35). By using this technique, we investigated the effects of NGF on basal and evoked increases in NOS activity in the rat hippocampus. The effects of NGF on basal glutamate levels also were determined.

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Abbreviations: NGF, nerve growth factor; NOS, nitric oxide synthase; NMDA, *N*-methyl-D-aspartate; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CNS, central nervous system; CytC, cytochrome *c*; aCSF, artificial cerebrospinal fluid; nNOS, neuronal NOS.

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EXPERIMENTAL PROCEDURES

Chemicals. L-[^{14}C]arginine (317 mCi/mmol) and ^{125}I-NGF were obtained from Amersham. *N*-methyl-D-aspartic acid (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and cytochrome *c* (CytC, molecular mass 12.3 kDa) were obtained from Sigma. NGF (molecular mass 13 kDa) in the form of human recombinant NGF was a gift from Genentech. Bioactivity of this NGF was confirmed *in vitro* by examining the induction of neurite extension from dorsal root ganglia of embryonic day 16–18 rat embryos.

Animals and Microdialysis Procedure. The experimental protocols used in this study conformed to the guidelines for the care and use of animals in research of the National Institutes of Health (USA) and Home Office (United Kingdom), and were approved by the Institutional Animal Care and Use Committee of Johns Hopkins University. Adult male Wistar rats weighing 300–450 g were anesthetized with halothane (1–2% in oxygen-enriched air). Temperature was monitored with a rectal probe and maintained at 37–38°C with a heating lamp. Under these conditions, rats maintain an arterial pH and partial pressure of blood gases within the normal range (34, 35).

Dialysis probes were constructed with a concentric design by using a single hollow silica glass fiber inside a membrane (50 kDa cut-off, Hospal Dasco, Bologna, Italy) sealed at one end with epoxy, as modified from previous descriptions (34, 35). The functional portion of each probe was 5 mm long.

All experiments were conducted by using bilateral microdialysis and a paired experimental design in which control values obtained on one side were compared directly with test values obtained on the other for each experimental animal. This process was done to reduce the effect of inter-animal variability arising from differences in arterial blood pressure, blood gases, and depth of anesthesia. The number (*n*) of animals per group ranged from six to 11 for all dialysis experiments.

By using Kopf stereotaxic frames, microdialysis probes were inserted bilaterally into the hippocampus (anterior-posterior = 5.4 mm, medio-lateral = 5 mm, dorso-ventral = 7.0 mm) and fixed in the same position throughout the experiment. Starting 1 hr after insertion, the microdialysis probes were perfused at 1 μl/min with artificial cerebrospinal fluid (aCSF) consisting of 131.8 mM NaCl, 24.6 mM $NaHCO_3$, 2.0 mM $CaCl_2$, 3.0 mM KCl, 0.65 mM $MgCl_2$, 6.7 mM urea, and 3.7 mM dextrose. The aCSF was filtered, warmed to 37°C, and bubbled with 95% N_2/5% CO_2 until O_2 and CO_2 tensions were similar to brain tissue. Animals used for measurement of NOS activity were perfused bilaterally with aCSF containing L-[^{14}C]arginine for 3 hr to "load" the cells with the labeled substrate. Animals used for measurement of glutamate were dialyzed with aCSF alone. Every 20 min, 20-μl effluent dialysate samples were collected and assayed for L-[^{14}C]citrulline or glutamate for the duration of the experiment. After the 3-hr equilibration period, microdialysis probes were switched to solutions containing the substances to be tested or continued with aCSF containing L-[^{14}C]arginine or aCSF alone as controls. In all cases the values for the last three samples before this switch did not differ significantly from each other, and the last value was taken as baseline (100%) at time zero (0) on both sides. All other values were expressed as percentages of the baseline values. Rats were randomly assigned to treatment groups to receive perfusates containing NGF (0.01 μg/μl), CytC (0.01 μg/μl), NMDA (1 mM), or AMPA (1 mM), alone or in combination, in aCSF containing L-[^{14}C]arginine for measurement of NOS activity, or in aCSF alone for measurement of glutamate.

Histology. At the end of experiments, dialysis probes were removed, and the animals were killed by barbiturate overdose. The brains were removed, immersed in fixative, and processed for histological visualization of the probe tracts by routine preparation of frozen sections and staining with cresyl violet. After dialysis with ^{125}I-NGF for 3 hr, brains were collected in a similar manner and some sections were processed for autoradiography (14).

Measurement of NO Production. NO production was determined in 20-μl samples of dialysis fluid effluent as modified from Bredt *et al.* (24) and described in detail elsewhere (34, 35). Briefly, samples were diluted with 200 μl of water and poured over 0.5 ml of resin AG-50WX8 (Na^+ form, pH 7.0) 400 mesh columns. Columns were washed with 2 ml of buffer (30 mM Hepes/3 mM EDTA, pH 5.2), and 1 ml of water. Radioactivity of the flow through the column was quantified by liquid scintillation spectroscopy. To determine resin efficiency of arginine trapping, 20 μl of aCSF containing 3 μM L-[^{14}C]arginine (not used for dialysis) was diluted in 200 μl of water, poured over a column, and washed as above. Specific activity was corrected for counting efficiency and background activity and expressed as fmol/min of perfusion. As an internal control, 100 μl of aCSF not used for dialysis was directly assayed for activity to ensure that consistent concentrations of L-[^{14}C]arginine were added to the aCSF.

Measurement of Glutamate. Glutamate levels in 20-μl samples of dialysate were analyzed by HPLC and fluorescence detection (Anachem, Milton Keynes, United Kingdom). Chromatographic peaks were identified and quantified by reference to known concentrations of standards and materials as supplied for use in the HP AminoQuant amino analyzer system (Hewlett-Packard).

Statistics. Data are presented as mean ± SEM (*n* equaled 6–11 animals per group in all cases). Data were analyzed by two-way ANOVA with independent, posthoc, pairwise comparisons (Sidak's test). $P < 0.05$ was considered statistically significant.

RESULTS

Validation of Microdialysis Probe Placement and NGF Delivery. The locations of the microdialysis probes were verified histologically. In all cases used for analysis, bilateral probes were found well placed in the descending portion of the hippocampal formation (Fig. 1*A*). To minimize damage to hippocampal tissue, NGF, CytC, and glutamate agonists were administered through the same dialysis probes used to deliver

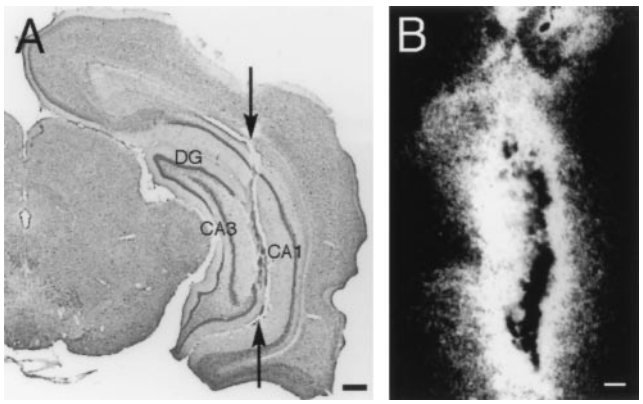

FIG. 1. Histological demonstration of the typical location of a microdialysis probe and delivery of ^{125}I-NGF to hippocampus. (*A*) Cresyl violet-stained, frozen coronal section of rat forebrain showing the typical appearance and location of the tract of a microdialysis probe (arrows) in the hippocampus near CA1, CA3, and the dentate gyrus (DG) after an experiment. (Bar = 500 μm.) (*B*) Dark-field illumination of a frozen coronal section through the rat hippocampus, which shows by autoradiographic detection of silver grains the widespread diffusion of chromatographically purified ^{125}I-NGF in hippocampal tissue after delivery for 3 hr through a dialysis probe. (Bar = 300 μm.)

L-[^{14}C]arginine and recover L-[^{14}C]citrulline. Probes therefore were constructed with dialysis membrane having a pore size allowing passage of these molecules. The efficacy of NGF delivery was tested *in vitro* and *in vivo* by using chromatographically purified ^{125}I-NGF, which we previously have shown to be taken up and retrogradely transported by trkA-receptor bearing septal cholinergic neurons after injection into the hippocampus (14). Dialysis with ^{125}I-NGF for 3 hr *in vitro* showed a mean transfer of radioactive counts into 20 μl of external buffer equivalent to 24% of the ^{125}I-NGF passing through the probe. Dialysis for 3 hr *in vivo* showed a mean transfer of radioactive counts into hippocampal tissue equivalent to 28% (range = 21–35%, $n = 3$) of the ^{125}I-NGF passing through the probe, and was further confirmed by autoradiography (Fig. 1*B*). For the NGF delivery studies described below, NGF was administered through the dialysis tubing at 10 ng/μl per min, which (based on the ^{125}I-NGF diffusion values) was estimated to give a rate of delivery of 2–3 ng/min of NGF into the hippocampal tissue. CytC was delivered as a control protein at the same concentration and flow rate. Concentrations of 0.1 ng/μl of NGF previously have been shown to stimulate the maturation of septal cholinergic neurons *in vitro* (36).

Effects of NGF on Baseline NOS Activity in Hippocampus. In agreement with previous studies, continuous perfusion of the hippocampus with L-[^{14}C]arginine in aCSF after bilateral placement of the microdialysis probes resulted in a gradual, time-dependent increase in L-[^{14}C]citrulline in the effluent (Fig. 2) caused by the diffusion times and gradual accumulation of L-[^{14}C]arginine and L-[^{14}C]citrulline in the tissue (34, 35). In the first sample collected at 20 min after initiation of unilateral infusion of NGF, L-[^{14}C]citrulline levels on the NGF-infused side had fallen significantly to 62% of those on the control side (Fig. 2). The effect of NGF was maximal within 40 min, by which time L-[^{14}C]citrulline levels on the NGF-infused side had fallen to 15% of those on the control side. After 120 min, L-[^{14}C]citrulline levels on the NGF-infused side had recovered to 41% and remained significantly reduced at between 40% and 50% of those on the control side for the 3-hr duration of the experiment (Fig. 2). CytC, a protein of similar size to NGF that is used regularly as a control protein in NGF studies (6, 8), showed no significant effect on NOS levels under the same delivery regimen (Fig. 2). These findings demonstrate a rapid and pronounced suppression of basal NOS activity by NGF.

Effects of NGF on Hippocampal Glutamate Levels. Because NOS activity in the hippocampus can be modulated by glutamate receptor activation (35), and neurotrophins other than NGF have been reported to influence hippocampal synaptic mechanisms over a time course of minutes (19), we examined the effect of NGF on hippocampal glutamate levels by using the same basic microdialysis procedure and measurement of glutamate levels in the effluent by using HPLC. Glutamate levels fell steadily during the equilibration period immediately after probe placement (data not shown), probably reflecting a gradual stabilization after a large initial release caused by tissue damage. After stable levels were achieved, continuous unilateral infusion of either NGF or CytC for 3 hr showed no significant effects on glutamate levels as compared with the noninfused side (Fig. 3). These findings indicate that the

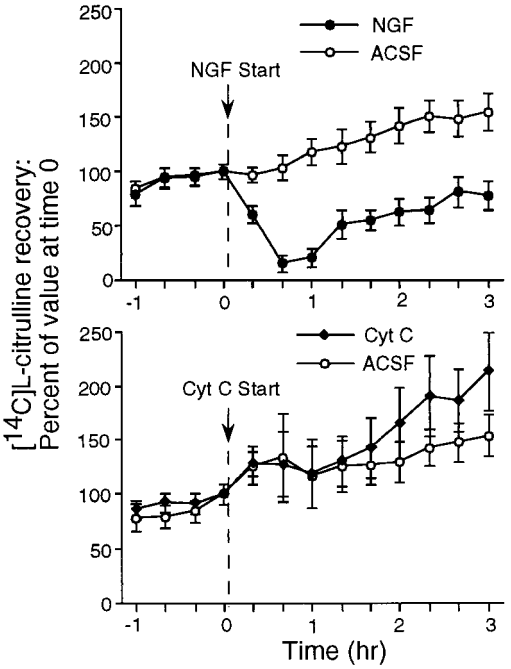

FIG. 2. Graphs showing the effects of NGF or CytC on basal NOS activity in the rat hippocampus. Samples were collected bilaterally every 20 min, and L-[^{14}C]citrulline was measured in the effluent. The values of the last samples after equilibration were designated as baseline (100%) at time zero (0) on both sides, and all other values were expressed as percentages of these values. Expressed in this manner, the baseline values did not differ significantly from the values of the previous three samples. Immediately after the samples were collected at time 0, the dialysis perfusates on one side were switched to either NGF or CytC (0.01 μg/μl). NGF induced a rapid and statistically significant ($P < 0.05$) drop in L-[^{14}C]citrulline recovery at all time points after initiation of delivery ($n = 11$). CytC had no statistically significant effect ($n = 6$). (ANOVA, with Sidak's posthoc pairwise comparison.)

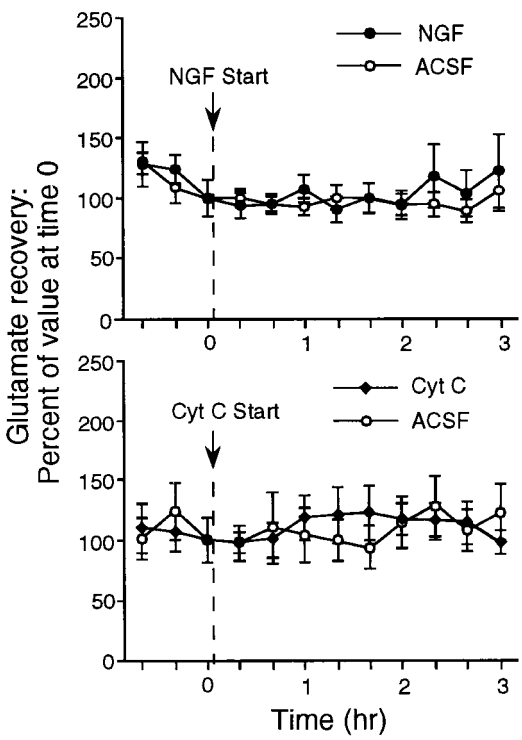

FIG. 3. Graphs showing the effects of NGF or CytC on glutamate levels in the rat hippocampus. Samples were collected bilaterally every 20 min, and glutamate in the effluent was measured by HPLC. The values of the last samples after equilibration were designated as baseline (100%) at time zero (0) on both sides, and all other values were expressed as percentages of these values. Expressed in this manner, the baseline values did not differ significantly from the values of the previous two samples. Immediately after the samples were collected at time 0, the dialysis perfusates on one side were switched to either NGF or Cyt (0.01 μg/μl). Neither NGF ($n = 8$) nor CytC ($n = 6$) had a statistically significant effect on glutamate levels (ANOVA).

effects of NGF on hippocampal NOS activity are not mediated by altering glutamate levels and show that NGF is not having a generalized and nonspecific disruptive effect on hippocampal function or the microdialysis procedure.

Effects of NGF on Glutamate Receptor-Stimulated NOS Activity in Hippocampus. Because NO production is markedly up-regulated by glutamate and NO may play a role in glutamate-mediated neurotoxic events (27, 35), and because NGF protects neural tissue against ischemia and glutamate-receptor mediated injury by unknown mechanisms (8–10, 37), we tested the effects of NGF on glutamate-stimulated increases in NOS activity. Microdialysis probes were placed bilaterally in the hippocampus and perfused with L-[^{14}C]arginine in aCSF. Because recovery across the dialysis probe is approximately 15–20% *in vitro* and because radial diffusion is expected to rapidly reduce the concentration of administered drugs in surrounding tissue, NMDA and AMPA were infused at 1 mM to attain approximately 20–200 μM interstitial concentrations.

In agreement with previous studies (35), unilateral infusion of either NMDA or AMPA significantly and substantially increased recovery of L-[^{14}C]citrulline. This increase was seen within 20 min and increased in magnitude for the duration of the 3-hr experiment as compared with the control side (data not shown). Combined perfusion of NGF together with either NMDA or AMPA completely prevented the increase in L-[^{14}C]citrulline seen with NMDA or AMPA alone (Fig. 4), demonstrating that NGF can prevent glutamate-receptor-mediated increases in NOS activity.

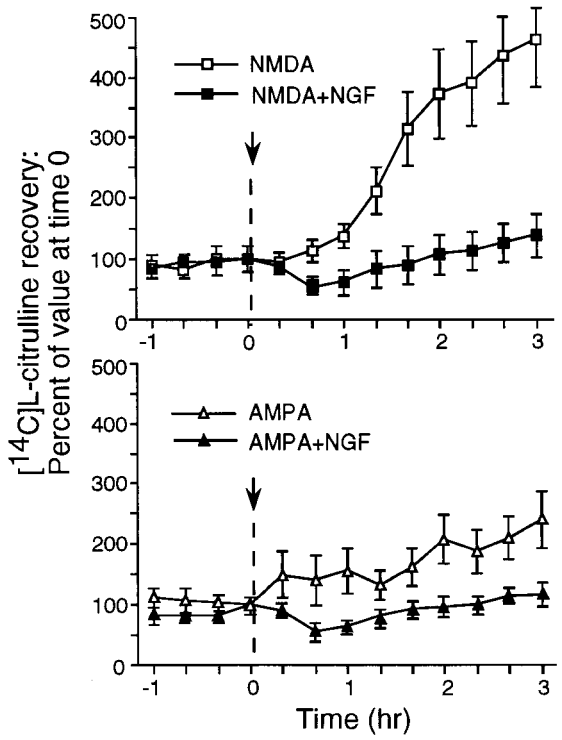

FIG. 4. Graphs showing the effects of NGF or CytC on NMDA- and AMPA-evoked NOS activity in the rat hippocampus. Samples were collected bilaterally every 20 min, and L-[^{14}C]citrulline was measured in the effluent. The values of the last samples after equilibration were designated as baseline (100%) at time zero (0) on both sides, and all other values were expressed as percentages of these values. Expressed in this manner, the baseline values did not differ significantly from the values of the previous three samples. Immediately after the samples were collected at time 0, the dialysis perfusates were switched to either NMDA or AMPA (1 mM) alone on one side, and to either NMDA+NGF or AMPA+NGF (0.01 μg/μl) on the other side. Both NMDA ($n = 7$) and AMPA ($n = 7$) induced sustained and statistically significant increases in L-[^{14}C]citrulline recovery as compared with baseline values ($P < 0.05$). In contrast, simultaneous delivery of NGF with NMDA ($n = 7$) or AMPA ($n = 6$) did not significantly alter L-[^{14}C]citrulline recovery as compared with baseline values. (ANOVA, with Sidak's posthoc pairwise comparison)

DISCUSSION

In this study we found that NGF rapidly suppressed basal levels of NO production and prevented the marked increase in NO production evoked by activation of NMDA and AMPA glutamate receptors in the rat hippocampus. These findings suggest a novel mechanism for rapidly mediating neuromodulatory or neuroprotective functions of NGF.

Potential Sources of NOS Activity Measured by Microdialysis of Hippocampus *in Vivo*. The microdialysis probes used in this study were located in the descending hippocampal formation, such that large portions of CA1, CA3, and dentate gyrus were within the 3-mm diffusion distance previously demonstrated for L-[^{14}C]arginine in this system (34). The compartmental kinetics of (*i*) diffusion of labeled arginine across the dialysis membrane and through the interstitial space, (*ii*) the cellular uptake of labeled arginine and efflux of labeled citrulline, and (*iii*) the diffusion of labeled citrulline back to and across the dialysis membrane have been discussed previously, and the measurement of L-[^{14}C]citrulline recovered in this manner has been verified to be an accurate marker of NOS activity *in vivo* (34, 35). It is these compartmental kinetics that are likely to give rise to the gradual time-dependent increase in recovery of L-[^{14}C]citrulline under all experimental conditions. As the tissue gradually becomes loaded with increasing amounts of labeled substrate (arginine), gradually increasing amounts of labeled product (citrulline) are generated and become available for recovery (34, 35). An underlying assumption of the technique is that the statistically significant changes in L-[^{14}C]citrulline recovery induced by specific test substances under experimental conditions reflect changes in NOS activity rather than changes in arginine and citrulline transport kinetics. In agreement with this assumption, the increased recovery of L-[^{14}C]citrulline seen after infusion of NMDA or AMPA is inhibited by L-nitroarginine methyl ester (L-NAME), a specific blocker of NOS activity, but not by D-nitroarginine methyl ester (D-NAME) (34, 35). Although it cannot be ruled out that the effects we observed of NGF on L-[^{14}C]citrulline recovery might be caused by effects on the diffusion, metabolism, or transport of labeled arginine and citrulline, there is to our knowledge no evidence for any effects on these or related processes being caused by any neurotrophin *in vivo* or *in vitro*.

There are a number of possible cellular sources for the NOS activity measured in the hippocampus in our study: (*i*) neuronal NOS (nNOS) deriving from local hippocampal interneurons as well as from fibers and terminals of afferent basal forebrain cholinergic neurons (31, 38); (*ii*) endothelial NOS (eNOS) deriving from both endothelial cells in blood vessels and pyramidal neurons in CA1-CA3 (38, 39); (*iii*) a constitutive NOS activity deriving from astrocytes (40), and (*iv*) inducible or immunologic NOS (iNOS) deriving from various non-neuronal cells, including microglia, astrocytes, and endothelia, and blood-borne inflammatory and immune cells (29, 40). The procedures we used will not differentiate these types of NOS activity or their cellular sources, but they will accurately reflect total NO production in the hippocampus at any given time. An important observation of our study is that the effects of NGF were subtotal, raising the possibility that NGF attenuated some, but not other, forms of NOS activity. Given the multiple potential cellular sources of NOS activity, it is important to consider which of these is likely to have been affected by NGF.

NGF-Responsive Cell Types with NOS Activity. NGF signaling is thought to occur through high and low affinity receptors, trkA and p75^{NTR}, respectively, present in the cell membrane (41). In the hippocampal region we studied, both of

these receptors are located most prominently to the afferent fibers and terminals of septal cholinergic neurons (41), many of which also contain nNOS (31). Large doses of NGF administered chronically for 10 days to the lateral cerebral ventricle increases expression of nNOS mRNA by these septal cholinergic neurons (31). Together, these findings suggest that some of the NGF-mediated inhibition of NOS activity we observed may have occurred in the fibers of these NGF-responsive neurons. However, the time frame of our study is not compatible with a signaling mechanism that requires retrograde transport from hippocampus to septum and acts through the nucleus to regulate nNOS synthesis. The relationship between the rapid down-regulation of NOS activity we observed and the up-regulation of NOS synthesis after chronic NGF is uncertain. A possible explanation is that after chronic delivery of NGF, nNOS synthesis is stimulated in these neurons by a regulatory feedback loop responding to prolonged inhibition of NOS activity.

In addition to the fibers of septal cholinergic neurons, there are several other neuronal and non-neuronal sources of NOS activity in hippocampus that may have been affected directly through NGF receptors in our study. Hippocampal pyramidal neurons express endothelial NOS (38, 39) and also may express trkA (42), although evidence to the contrary exists (43). Although it is not known how NGF signaling might alter NOS activity in neurons, NGF stabilizes neuronal Ca^{2+} levels (44) and activation of NOS is Ca^{2+} dependent (27), but further work will be required to demonstrate a causal interaction. It deserves emphasis that many non-neuronal cell types that have NOS activity also have functioning NGF receptors, including glial, inflammatory, and immune cells (2, 29, 40), raising the possibility that NGF may have protective effects by altering NO production by non-neuronal cells. Lastly, it is important to consider that NGF-responsive non-neuronal cells have the capacity to produce many cytokines and other signaling molecules (45), which in turn may alter NO production by neighboring neuronal and non-neuronal cells. Such interactions may be particularly important after ischemia, glutamate excitotoxicity, and other forms of CNS insult.

Rapid Signaling Through NGF Receptors. As mentioned above, the time course of NGF-mediated effects on NOS activity observed in this study is not compatible with retrograde transport to distant afferent neurons and signaling through the nucleus. Although the precise cellular and molecular signaling cascade by which NGF regulates NO levels requires further elucidation, the rapid effect that we observed is compatible with, and extends, several reports that signaling through trkA receptors is possible within minutes and can regulate a variety of functions, including the organization of adult neocortex, the activity of sodium channels, the generation of reactive oxygen species, or the turning of axons *in vitro* (20–23). In addition, the low-affinity NGF receptor, $p75^{NTR}$, is a member of the tumor necrosis factor/$p75^{NTR}$/CD95 receptor superfamily, which also can rapidly activate second messenger systems (46). Thus, our findings add to a growing body of evidence that indicates that NGF is able to mediate rapid local signaling not only on neuronal cell bodies and terminals, but also on non-neuronal cells and in a wide range of contexts (2). Moreover, our findings provide a potential mechanism for transduction of some of the effects of this signaling.

Potential Roles of NGF Regulation of NO Production. The functions of NGF in the adult CNS are incompletely understood. Available evidence indicates that NGF acts, at least in part, as a retrograde signal derived from target cells to regulate certain aspects of neuronal maintenance and gene expression in receptor-bearing neurons in the adult forebrain (3, 4, 14, 15). However, NGF synthesis by mature hippocampal neurons is rapidly and sensitively regulated by neural activity and transmitter agonists (16–18), and NGF rapidly induces various effects *in vitro* and after injury *in vivo* (8, 9, 20–23, 37). Such rapid modulation of synthesis or induction of effects seem unlikely to be required, or transduced, by a signaling mechanism based on retrograde transport to distant afferent neurons. The acute regulation of NOS activity by NGF that we report here offers a rapid signaling mechanism for NGF that may be important in neural plasticity and protection. NO has been implicated in several forms of neural plasticity including synaptic plasticity and axonal sprouting, on which NGF and other neurotrophins have well-documented effects through as yet unknown means (5, 19, 27, 32, 47–49). Interestingly, NO inhibits neurite sprouting (47), and NGF stimulates neurite sprouting in several types of neurons (5, 50). In addition, considerable evidence now indicates that acutely administered NGF affords widespread protection to neurons against damage caused by ischemia or excitotoxic injury in the forebrain (8, 9, 37) (unpublished observations). This widespread protective effect of NGF is puzzling because it affects more neurons than just those with NGF receptors (37). Our findings offer a potential mechanism whereby NGF could be globally neuroprotective. Much of the damage induced by ischemia and excitotoxicity is delayed and generated in a poorly understood manner through a cascade of events that includes sequential and synergistic interactions between glutamate-responsive neurons, in which NO may play a role (27, 29, 30, 35). Glutamate receptor activation stimulates NO production, which in turn may facilitate downstream glutamate release and activate other NOS-containing cells (27, 29, 34, 35). NGF may interrupt this cascade by suppressing NOS activity in receptor-bearing neuronal and non-neuronal cells, which in turn could reduce the spread of excitotoxic damage and protect cells without NGF receptors. An important aspect of the effects of NGF may be the suppression of NO production in some, but not other, cell types according to the distribution of NGF receptors. NO is a principle regulator of vascular hemodynamics, and considerable evidence indicates that endothelial NOS activity in endothelial cells is neuroprotective during ischemia and CNS injury by sustaining vascular perfusion of the tissue (29). Our findings indicate that NGF prevented glutamate-receptor activation of NOS but did not suppress NOS activity entirely, and that NGF only partially lowered basal NOS activity levels. We speculate that NGF may be a particularly potent neuroprotective agent because it inhibits NOS activity in cellular compartments that precipitate damage, including neurons and non-neuronal cells such as microglia, astrocytes, inflammatory, and immune cells that become active in CNS tissue after ischemia and glutamate excitotoxicity (2, 29), but preserves the NOS activity required to maintain tissue perfusion.

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