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Role of NO Production in NMDA Receptor-Mediated Neurotransmitter Release in Cerebral Cortex

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and 30°C and was independent of the presence of the ligand a-pp (*14*). Thus, the binding-release cycle of DnaK appears not to be stoichiometrically coupled with the hydrolysis of ATP. In the presence of ATP, the binding-release cycle is at least 830 times faster than ATP hydrolysis. Furthermore, nonhydrolyzable ATP analogs also accelerate both binding and dissociation, although less effectively than ATP by one order of magnitude. The following observations suggest that the rate-limiting step for ATP hydrolysis is the release of ADP and inorganic phosphate (P_i) rather than ATP cleavage. The effect of ATP is evident after a delay of only 0.5 s (Fig. 3) although the overall ATPase activity is much slower. However, the effect does not appear to be due to the mere binding of ATP because it is clearly different from the effect of its nonhydrolyzable analogs. In the presence of ATP (Fig. 4), most DnaK molecules may thus be assumed to contain a phosphate group perhaps covalently attached to Thr^{199}, which has been shown to be phosphorylated during incubation of DnaK with ATP in vitro (*15*).

What is the physiological significance of the very weak ATPase activity of DnaK if it is not stoichiometrically coupled to the binding-release cycle? This study shows that ATP and its turnover control the kinetics of the chaperone action. The binding-release cycle is much faster in the presence of ATP—that is, under physiological conditions—than was previously thought and it occurs in the same time range as polypeptide chain elongation and folding. Tuning of the rates of ATP cleavage and release of ADP and P_i by the accessory Hsp's DnaJ and GrpE of *E. coli* (*16*) might thus shift the proportions of the three functional states of DnaK (Fig. 4) and adapt the kinetics of its chaperone action to the changing requirements of protein biogenesis.

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18. The plasmid pTTQ19*dnaK*$^+$ was used to produce DnaK. The expression is under the control of the *tac* promoter, which is inducible by isopropyl-thio-β-D-galactoside. The *E. coli* strain JM83 was used for expression. The collected cells were resuspended in 20 mM tris-HCl (pH 8.0), 50 mM NaCl, 1 mM EDTA, and 1 mM dithiothreitol (DTT) (buffer A), and sonicated. After ultracentrifugation (100,000*g* for 30 min), the supernatant was dialyzed against buffer A and loaded onto an anion-exchange column (Q Sepharose Fast Flow, Pharmacia). DnaK was eluted at the end of a linear gradient of 50 to 500 mM NaCl. The DnaK-containing fractions were pooled and dialyzed against 20 mM sodium acetate (pH 5.5), 0.1 mM EDTA, and 0.1 mM DTT (buffer B), and passed over a 5-ml column of ATP-agarose (C_8 linkage, Sigma). The column was washed with 1 M NaCl in buffer B, and DnaK was then eluted with 5 mM ATP, 7 mM $MgCl_2$, and 10 mM KCl in buffer B. Fractions containing DnaK were concentrated by ultrafiltration and loaded onto a Superose 12 fast protein liquid chromatography column (HR 10/30, Pharmacia) in 25 mM Hepes (pH 8.0), 10 mM 2-mercaptoethanol, and 1 mM EDTA. Fractions with pure DnaK, as analyzed by SDS–polyacrylamide gel electrophoresis were collected and stored in 10% glycerol at −20°C.

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Role of NO Production in NMDA Receptor–Mediated Neurotransmitter Release in Cerebral Cortex

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L-Glutamate and norepinephrine are examples of a major excitatory neurotransmitter and a neuromodulator in the cerebral cortex, respectively. Little is known of how chemical signaling between the anatomically distinct chemical pathways occurs. Specific activation of the *N*-methyl-D-aspartate (NMDA) class of glutamate receptor in synaptosomal preparations from guinea pig cerebral cortex caused release of both of these chemicals, and this release was blocked by agents that inhibit nitric oxide (NO) production or remove NO from the extracellular space. Furthermore, neurotransmitter release correlated with cortical NO production after NMDA receptor stimulation. These results suggest that NO production and its extracellular movement may be links in the pathway from NMDA receptor activation to changes in chemical signaling in surrounding synaptic terminals in the cerebral cortex.

Activation of the NMDA glutamate receptor on neurons in the vertebrate central nervous system is important for excitatory synaptic transmission (*1*, *2*), developmental and synaptic plasticity (*2*, *3*), and neurotoxicity (*4*) and can lead to the production of the membrane-permeant gas NO (*5*). Production of NO may contribute to neurotoxicity (*6*), possibly by means of the formation of peroxynitrite (ONOO) from NO and the superoxide ion (*7*). Further, NO may participate in synaptic plasticity at glutamatergic synapses in the hippocampus (*8*) [carbon monoxide (CO), another membrane-permeant gas made in the brain, also may play a role in this plasticity (*9*, *10*)]. However, the mechanisms underlying these actions remain to be elucidated.

In the cerebral cortex, the role of NO in synaptic transmission and plasticity is murkier. Nitric oxide and other potential neuromodulators such as norepinephrine (*11*) are likely to be important in both transient (*12*) and more long-lasting (*9*, *13*) alterations in functional forebrain circuitry. Pharmacologi-

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cal blockade of the synthetic enzyme for NO, NO synthase (NOS) (*14*), suggests that NO produced in the cortex influences both general cerebrovascular tone and local coupling of neural activity to blood flow changes (*15*). Because NO is a nonpolar membrane-permeant gas, it could influence synapses throughout a local, diffusion-defined domain in the brain (*9, 13*). This could effectively couple neural activity in a local volume of cerebral cortex, operating beyond the boundaries of conventional anatomically defined synapses.

To evaluate whether excitatory synaptic transmission at NMDA receptors is linked to the release of other neurotransmitters such as norepinephrine or subsequent glutamate release through NO production, we used a synaptosomal preparation from guinea pig cerebral cortex. We measured the effect of NMDA on the release of tritiated norepinephrine ([^{3}H]NE) and endogenous L-glutamate (L-Glu) (*16*); NMDA caused a dose-dependent release of both of these neurotransmitters (Fig. 1, A and B). The active *S* isomer (*S*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) (100 μM) did not cause release of either neurotransmitter. The release of both L-Glu and [^{3}H]NE was blocked in a dose-dependent fashion by the competitive NMDA receptor blocker 2-amino-5-phosphono-valerate (D-APV) (Fig. 1, C and D), augmented by removal of extracellular Mg^{2+}, and blocked by removal of extracellular Ca^{2+} (Fig. 1, A and B, arrows). The half-maximal effective inhibition constant (IC_{50}) for D-APV was <1 μM for the NMDA receptor–mediated release of both L-Glu and [^{3}H]NE. Depolarization of the synaptosomes with elevated KCl (50 mM) resulted in a modest release of both neurotransmitters, which was also dependent on extrasynaptosomal calcium (Fig. 1, C and D). For both L-Glu and [^{3}H]NE, the K^+-induced depolarization caused 4 to 6% of the available neurotransmitter to be released (*16, 17*), and this release was independent of D-APV concentration (Fig. 1, C and D). With 100 μM NMDA as the stimulus, 21 ± 3% of the [^{3}H]NE and 35 ± 4% of the available L-Glu was released (*18*). Taken together, these observations suggest that specific activation of the NMDA receptor and subsequent Ca^{2+} fluxes are required for the release of both L-Glu and norepinephrine.

The NOS inhibitors *N*-nitro-L-arginine (L-NOARG) and *N*-monomethyl-L-arginine (L-NMMA) blocked the NMDA-mediated release of both neurotransmitters in a dose-dependent fashion (Fig. 2). A similar effect was obtained with 7-nitro-indazole (7-NI), a selective inhibitor of brain NOS (*19*). The effect was stereospecific, with the inactive optical isomers having no effect (Fig. 2). The release induced by K^+ depolarization was not influenced by either the D or L forms of the NOS inhibitors. The L-NMMA blockade of NMDA-stimulated release could be overcome by washing for 15 min in 1 mM L-arginine, the normal substrate for NOS. The effects of L-NOARG could not be similarly reversed, consistent with its reported high affinity for NOS (*20*). The IC_{50} of 100 μM NMDA-mediated neurotransmitter release occurred at 0.7 μM L-NOARG for L-Glu and 1.0 μM for [^{3}H]NE (Fig. 2, C and D). The stereospecificity of the block and the reversibility of the weaker inhibitor suggest that the inhibitors do not act by a nonspecific removal of the ability of the synaptosomes to release transmitter. Rather, they act by a specific blockade of NOS. This point is further supported by the observation that K^+-induced release of neurotransmitter was unaffected by the presence of the blockers (Fig. 2). Thus, activation of the NMDA receptor alone is not sufficient to mediate neurotransmitter release, but release also requires NOS activity.

These results do not distinguish between intrasynaptosomal NO action and the need for an extrasynaptosomal signal that depends on NO production and travels between distinct synaptosomal elements. We addressed these alternative possibilities by incubating the synaptosomes with hemoglobin (Hgb), a molecule that remains outside the synaptosomes and chelates NO (*21*). While Hgb strongly chelates NO, it may also react with superoxide anion or peroxynitrite. It prevented NMDA-mediated release of L-Glu and [^{3}H]NE (Fig. 2, A and B). The IC_{50} for block of the NMDA-mediated release of neurotransmitters by Hgb was 0.2 μM for both L-Glu and [^{3}H]NE release. Because Hgb remains outside the synaptosomes, either synaptosomes must cooperate in the production of a threshold concentration of NO sufficient for neurotransmitter release or only a subset of the synaptosomes make the NO, which then mediates release from the surrounding synap-

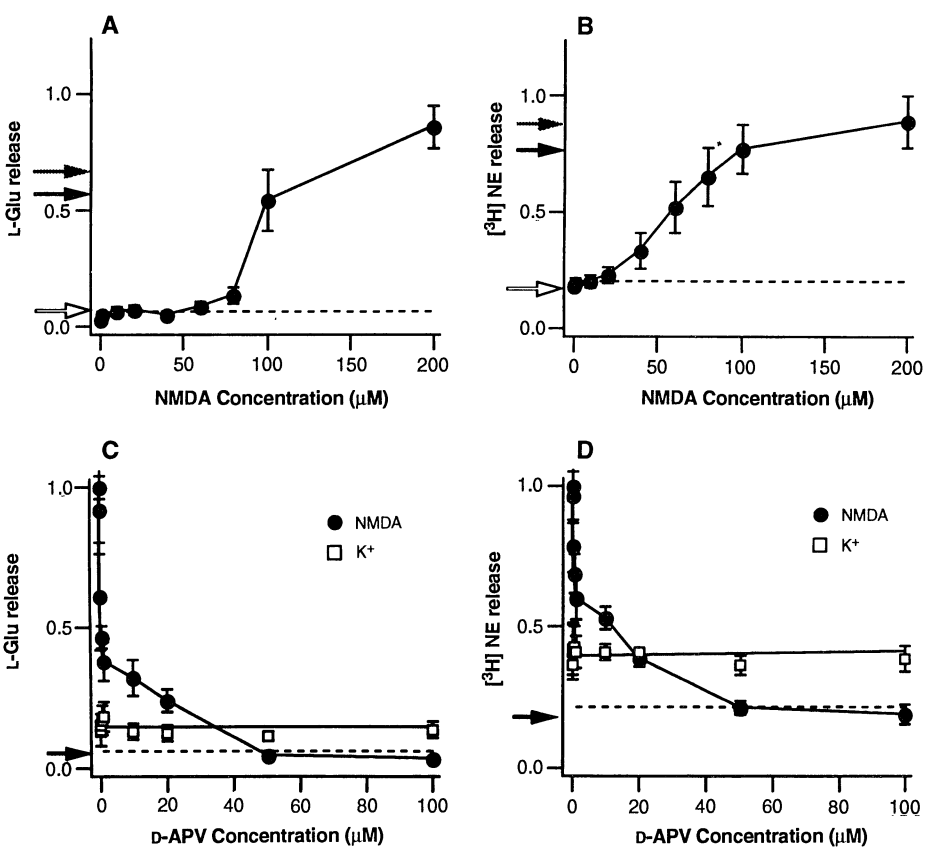

Fig. 1. The NMDA receptor–mediated release of neurotransmitter. Dose-response curves for NMDA-mediated (**A**) L-Glu release or (**B**) [^{3}H]NE release. With 100 μM NMDA as a stimulus, release was completely blocked in Ca^{2+}-free media (open arrow), augmented by 18% (for L-Glu) or 15% (for [^{3}H]NE) in low concentrations (zero) of Mg^{2+} (top solid arrow), and inhibited by 35% (for L-Glu) or 38% (for [^{3}H]NE) in high concentrations (10 mM) of Mg^{2+} (not shown). The lower solid arrow indicates the normalized release in response to 100 μM NMDA in control levels of divalent cations (1.2 mM Ca^{2+} and 1.2 mM Mg^{2+}). Effect of D-APV:dose-response curves for (**C**) L-Glu release and (**D**) [^{3}H]NE release with 100 μM NMDA as a stimulus (●). The K^+ depolarization–induced release was not influenced by the presence of D-APV (□); however, depolarization-induced release was completely blocked in Ca^{2+}-free medium (arrows). The dotted lines indicate baseline, unstimulated release of neurotransmitter. All points represent normalized mean percentage neurotransmitter release (±SEM) (*16*). Each point is the mean of four to seven experiments. Antagonists were applied for 30 min before and in conjunction with stimulation with NMDA or K^+.

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tosomes. This latter possibility would be consistent with the reported localization of NOS primarily in the cell bodies of smooth inhibitory neurons that contain γ-aminobutyric acid (GABA) and somatostatin (*21*). These cells are a small fraction of the neurons in the cerebral cortex, although there is considerable NOS activity in the neuropil of the cortex (*22*). In the presence of Hgb, NO made by these synaptosomes permits this subset of synaptosomes to release their neurotransmitters (GABA and somatostatin), but our assays would not have detected these substances.

To directly evaluate the NMDA receptor–mediated production of NO in the cerebral cortex, we used a biological assay of NO production (Fig. 3). This assay tests whether cortical NOS activation produces NO that travels outside the synaptosomes. Rodent aortic rings, smooth muscle with endothelial cells removed, were exposed to the effluent from synaptosomes in a closed system (*23*). Vasculature prepared in this way changed its tension in response to various vasoactive compounds and relaxed in the presence of NO (*24*). The rings were physically separated from the synaptosomes and were connected to sensitive force transducers that measure tension (*23*). Application of 100 μM NMDA to the synaptosomes caused a reduction in tension in the aortic rings (Fig. 3, A and C), indicating the production of a vasorelaxing compound. This same stimulus caused the release of both [^{3}H]NE and L-Glu (Figs. 1 and 2). When NOS was blocked by 10 μM L-NOARG (which abolishes the NMDA-mediated release of transmitter), the same 100 μM NMDA stimulus caused no change

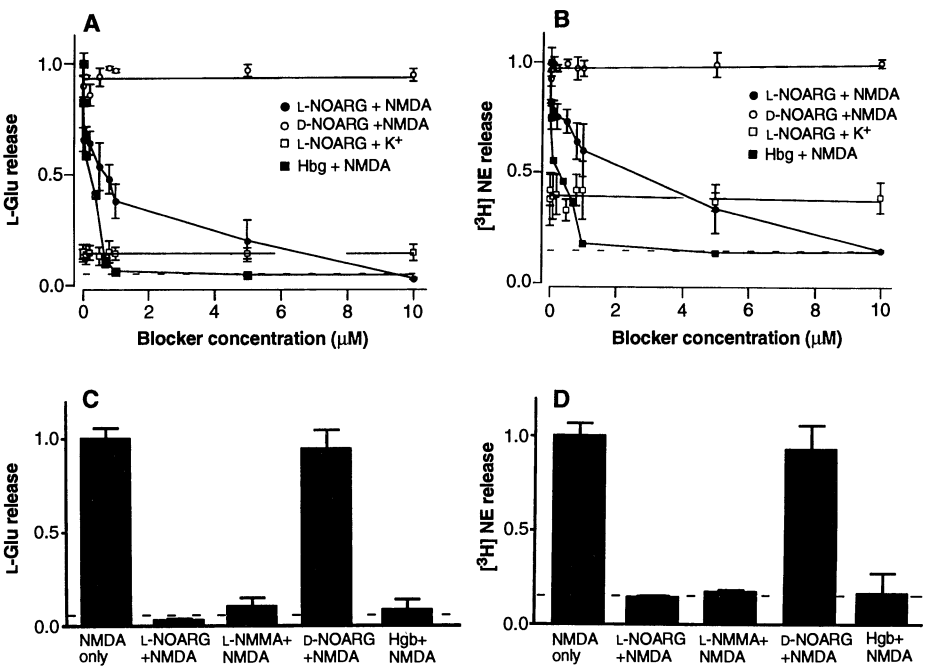

Fig. 2. Stereospecific blockade of NOS or chelation of NO prevents NMDA-mediated release. (**A**) The NOS inhibitor L-NOARG (●) blocked NMDA-simulated release (100 μM) of both (**A**) L-Glu and (**B**) [^{3}H]NE in a dose-dependent fashion, but D-NOARG (○) had no effect. Depolarization-induced neurotransmitter release (50 mM KCl) was unaffected by L-NOARG (□). Hemoglobin (Hgb) (■) also blocked NMDA-simulated release (100 μM) of L-Glu and [^{3}H]NE in a dose-dependent fashion. Similar results were obtained with L-NMMA and 7-NI (not shown). Control data points for effects of L-NOARG on K^+-induced release (□) and effects of D-NOARG (○) are fit by linear regression. Pooled data for the various conditions for (**C**) L-Glu release and (**D**) [^{3}H]NE release, respectively: NMDA only (n = 26 and 45), L-NOARG + NMDA (n = 23 and 38), L-NMMA (n = 6 and 8), D-NOARG + NMDA (n = 7 for both), Hgb + NMDA (n = 6 and 7). The L-NOARG, L-NMMA, and Hgb results are significantly different (χ^2 test; $P < 0.001$) from the NMDA responses for both L-Glu and [^{3}H]NE release. Baseline release is indicated by dashed lines in all panels. All values are normalized neurotransmitter release.

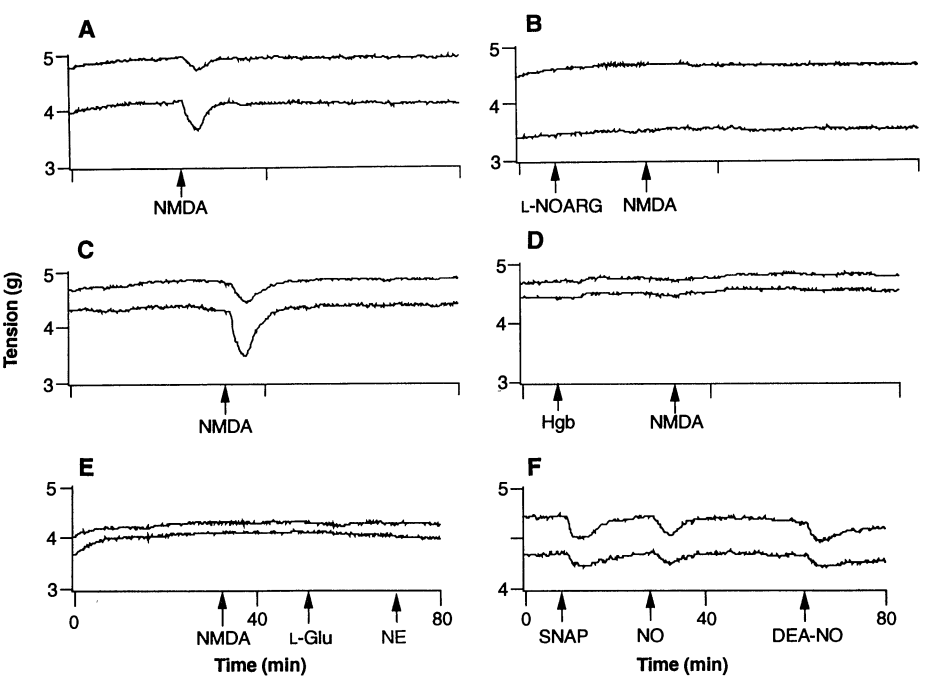

Fig. 3. Independent bioassay of stimulated NO production. (**A**) Application of 100 μM NMDA (arrow) caused a vasodilatory response in the aortic rings. (**B**) Preincubating the synaptosomes with 10 μM L-NOARG blocked the NMDA-mediated vasodilatory response. As a control, the synaptosome aliquots used in (A) and (B) were derived from the same preparation. (**C**) Application of 100 μM NMDA (arrow) in another experiment again caused a vasodilatory response in the aortic rings. (**D**) Preincubating these synaptosomes with 5 μM Hgb (Fig. 2) blocked the NMDA-mediated vasodilatory response. As a control, the synaptosome aliquots used in (C) and (D) were derived from the same preparation. (**E**) NMDA (100 μM), L-Glu (50 μM), and [^{3}H]NE (20 μM) were applied to the aortic rings in the absence of the synaptosomes. (**F**) Various sources of NO (SNAP, DEA-NO, or NO gas) were applied directly to the preparation containing just the aortic rings to verify that these same compounds used in the experiment outlined in Fig. 4 were indeed generating an NO-type response. In all panels, the vertical axis is the mass in grams needed to create the tension exerted by aortic rings. This experiment was repeated four times with identical results.

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in ring tension (Fig. 3B). Similarly, the presence of 5 μM Hgb abolished the relaxation subsequent to stimulation with 100 μM NMDA (Fig. 3D).

Direct application of 100 μM NMDA applied to the aortic rings in the absence of the synaptosomes caused no change in tension (Fig. 3E). To rule out a direct effect of the released neurotransmitters on ring tension, we also applied L-Glu and [^{3}H]NE directly to the isolated aortic rings in quantities computed from the release data (*25*) (Fig. 3). This manipulation also caused no change in ring tension. Acetylcholine (Ach) caused no changes in tension. If endothelial cells were still attached to the aortic rings, this concentration of Ach would cause a relaxation (*24, 26*).

These results demonstrate that NMDA receptor activation results in L-Glu and [^{3}H]NE release, possibly through a link to NOS activation. Production of NO and its movement through extracellular space may be a necessary step in this link because (i) Hgb causes block of NMDA-mediated release (Fig. 2); (ii) L-NOARG, L-NMMA (Figs. 2 and 3), and 7-NI cause a concentration-dependent stereospecific block of NMDA-mediated release; (iii) stimuli that cause neurotransmitter release also cause a potent vasodilatory response in isolated aortic rings (Figs. 2 and 3); and (iv) agents that block NO production or chelate NO in the extrasynaptosomal space prevent the NMDA-mediated vasodilatory response in aortic rings (Figs. 2 and 3).

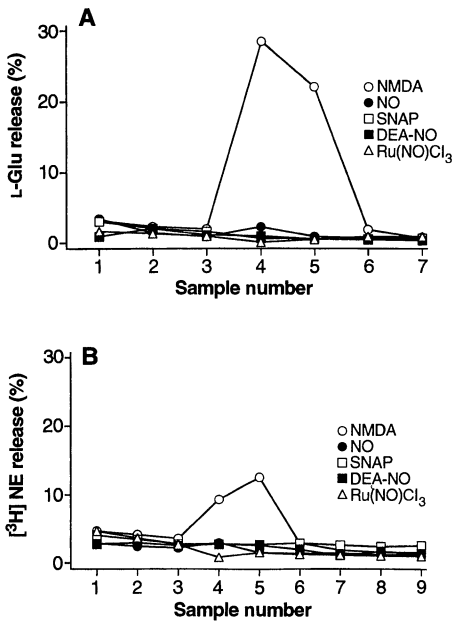

Fig. 4. Effects of NO application to synaptosomes. Individual runs from one series of experiments where synaptosomes were exposed to NO or NO-generating agents. Stimulus [40 nM NO (*27*), 100 μM SNAP, 100 μM DEA-NO, or photoactivated $Ru(NO)Cl_3$] was applied at sample number 4. For comparison, responses to 100 μM NMDA are indicated. (**A**) L-Glu release. (**B**) [^{3}H]NE release. Other experiments were run with similar results with NO concentrations varying from 1 nM to 1 mM.

In the hippocampus, the conjunction of NO or CO and presynaptic activity is sufficient to induce a long-term enhancement in synaptic transmission (*9, 10*). To assess the role of NO in release of neurotransmitter in the cerebral cortex, we added NO directly to the synaptosomes in the presence and absence of NOS blockade by 10 μM L-NOARG (*27*). For both L-Glu and [^{3}H]NE, NO alone had no effect on the baseline neurotransmitter release (Fig. 4). Moreover, a variety of NO generating compounds—including diethylamine-NO (DEA-NO), (±*S*)-nitroso-*N*-acetylpenicillamine (SNAP), and ruthenium nitrosyl chloride [$Ru(NO)Cl_3$], which releases caged NO when activated by near-ultraviolet light (*28*)—did not cause release of either neurotransmitter. However, when applied directly to the aortic rings, these same compounds caused a reduction in tension (Fig. 3F). Thus, some other event triggered by NMDA receptor activation, in addition to NO production, may be required for neurotransmitter release.

Our data demonstrate that glutamatergic synaptic transmission at the NMDA receptor in the cerebral cortex can further enhance subsequent L-Glu release at neighboring synapses and can communicate with catecholamine systems through a signal that moves between cells in neural tissue. This signal requires the production of NO and its extrasynaptic diffusion; however, it does not preclude a role for other diffusible compounds for which synthesis or activation first requires NMDA receptor activation and the production of NO. The neurotransmitter release may actually result from production of other chemical species, such as superoxide and peroxynitrite, subsequent to the initial NO production and movement into extracellular space (*7, 29, 31*). The observation that NMDA receptor activation, but not exogenous NO application alone, was sufficient to mediate L-Glu and [^{3}H]NE release in the synaptosome preparation suggests that the binding of ligand to the NMDA receptor mediates several interactive processes.

One possibility is that an NMDA receptor–mediated NO signal (from post- or presynaptic elements, or both) and a calcium signal mediated by presynaptic NMDA receptors (*30*) provide the required conjunction to induce neurotransmitter release. However, in experiments where NOS blockade was induced with L-NOARG and NMDA receptor activation was combined with concomitant exogenous NO application, neither L-Glu nor [^{3}H]NE were released. In control experiments on synaptosomes with intact NOS simultaneous, application of exogenous NO (either as NO gas or through the application of SNAP) reduced the NMDA-mediated neurotransmitter release significantly in accord with previous reports that NO inhibits the NMDA receptor (*31*). While the evidence presented suggests that nitrogen oxide metabolism facilitates neurotransmitter release and effectively couples chemical pathways in cerebral cortex, the exact species of NO (either redox state or chemical compound) that manifests the effects remains unknown.

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16. Male guinea pigs (n = 64) weighing 300 to 900 g were anesthetized with ether and decapitated. Their brains were rapidly removed and placed in chilled, aerated Krebs-bicarbonate buffer. Synaptosomes were prepared as described [R. A. Nichols *et al.*, *J.*

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Neurochem. **52**, 521 (1989)]. After preparation, the synaptosomes were suspended in Hepes-buffered saline containing 142 mM NaCl, 2.4 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM $CaCl_2$, 1.2 mM K_2HPO_4, 10 mM glucose, and 10 mM Hepes, pH 7.2, with a final protein concentration of 1 mg per milliliter of suspension. The synaptosomes were loaded with radiolabeled neurotransmitter by incubation in 2 ml of 0.2 μM [^{3}H]NE (11 Ci/mmol) for 10 min at 37°C in Hepes-buffered saline. Then, 1 ml of synaptosome suspension was placed on filters (Whatman glass-microfiber filters; 0.7-μm pore size) in 10 parallel wells over a vacuum manifold, with each well being used for a single release condition. Hepes-buffered saline was the carrier solution for all agents; each wash was 2 ml and required about 1 s. Sequential washes were applied at one per minute. After seven initial washes, all washes were collected and the tritium quantitated. At the end of each experiment, the filter paper was also collected and counted. The percent of radioactivity released at each wash was calculated as a fraction of the total amount of radioactivity recovered during an experiment (after baseline had been established). This total included residual counts on the filter paper and unreleased counts in the synaptosomes (after lysis). Total release percentages from the stimulus sample plus the two following samples were normalized to the maximum amount released in a given experiment. In the experiments with Hgb, corrections for quenching were made. The L-Glu release was measured with a bioluminescence assay [V. M. Fosse *et al.*, *J. Neurochem.* **47**, 340 (1986)]. The procedure for computing the percentage of L-Glu released during each wash was analogous to that used for [^{3}H]NE release. The bioluminescence measurements for endogenous L-Glu release were performed concurrently with a Monolight (Analytical Luminescence Laboratory, San Diego, CA) 2010 luminometer on a 50-μl aliquot of each wash. Measurements were linear over the range of 10^{-7} to 10^{-6} M. After an experiment, the synaptosomes were osmotically ruptured, and the residual L-Glu remaining on the filters was measured and added to the total amount of L-Glu recovered during the washes. The percentage released was expressed as a fraction of this total.

17. The K^+ depolarization did not nonspecifically damage the synaptosomes because the NMDA-induced release remained intact after this depolarization and the depolarization-induced release was Ca^{2+} dependent.
18. For a given experiment, multiple aliquots from the same batch of synaptosomes would release a relatively constant fraction of the available transmitter (*15*). The variability originated primarily from batch to batch variation.
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23. The effluent from each batch of synaptosomes was delivered to two separate rat aortic rings connected by surgical suture to separate Statham (Gould Instruments Division, Atlanta, GA) force transducers. The endothelium of these aortic rings was removed so that their NO would not be available as a relaxant in this system. In each experiment, the aortic rings were first contracted with 1×10^{-8} to 3×10^{-8} M U46619 (a thromboxane analog) to give a broad working range for measurable changes in tension, and the rings were constantly exposed to the U46619 for the duration of the experiment [M. J. Winn *et al.*, *J. Pharmacol. Toxicol. Methods* **28**, 49 (1992)].
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25. It is possible that the baseline release of the neurotransmitters combined with the pulsatile release in response to the 100 μM NMDA stimulus directly caused the vasodilatory response. To control for this possibility, we computed the amount of L-Glu and [^{3}H]NE released during the NMDA stimulus wash and the succeeding two washes and exposed the aortic rings to these concentrations over a 3-min period. Neither L-Glu (5 to 50 μM), [^{3}H]NE (1 to 20 μM), nor Ach (1 μM) caused any change in ring tension.
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32. We thank F. Hester for luminometer measurements, J. Beckman for providing NO and help with its application and measurement, T. Sejnowski, J. Hablitz, S. Nowlan, and P. Dayan for helpful comments on an earlier draft of the manuscript, C. Stevens, M. Nusbaum, and J. Beckman for helpful discussions, J. Neville and L. A. Faulkner for word processing, K. Ramer for figure preparation, and N. Veyna for synaptosome and luminometry protocols. Supported by NIH EY05116 (M.J.F.) and NIH EY06714 (R.B.M.).

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Paralysis and Early Death in Cysteine String Protein Mutants of *Drosophila*

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Multimeric complexes of synaptic vesicle and terminal membrane proteins are important components of the neurotransmitter release mechanism. The *csp* gene of *Drosophila* encodes proteins homologous to synaptic vesicle proteins in *Torpedo.* Monoclonal antibodies demonstrate different distributions of isoforms at distinct subsets of terminals. Deletion of the *csp* gene in *Drosophila* causes a temperature-sensitive block of synaptic transmission, followed by paralysis and premature death.

Fast, excitation-coupled neurotransmitter release is mediated by the fusion of synaptic vesicles with the nerve terminal. Proteins of both vesicles and other synaptic compartments, including presynaptic Ca^{2+} channels, participate in this process by forming a multimeric protein complex to ensure rapid and regulated release (*1*). Although the molecular mechanisms are not understood, considerable progress has been made in identifying some of the components (*2*). However, only synaptotagmin has thus far been genetically shown to be involved (*3*).

The *Drosophila melanogaster* cysteine string proteins (Dcsp's) (*4*) are membrane proteins containing an unusual cysteine motif ($C_2X_5\ C_{11}\ X_2\ C_2$; C is cysteine and X is any amino acid) and the "J" domain found in dnaJ proteins, which mediate the regulation of heat shock protein (hsp) 70 protein activity and thereby the assembly of multimeric protein complexes (*5*). A *csp* homolog of *Torpedo californica* (Tcsp) has been identified (*6*) that copurifies with synaptic vesicles (*7*). The inhibition of N-type Ca^{2+} channel activity by coinjection of *Tcsp* antisense mRNA into *Xenopus* oocytes suggests that Tcsp may be a regulatory subunit of Ca^{2+} channels (*6*). We show that, in *Drosophila*, the Dcsp proteins are localized to synaptic terminals and that deletion of the *csp* gene interferes with synaptic transmission, causing paralysis and early death.

To localize the gene product, we generated monoclonal antibodies (mAbs) from fusion protein (*8*). The DCSP-2 mAb demonstrates the presence of Dcsp protein presynaptically at larval and adult neuromuscular junctions; synaptic boutons are strongly stained (Fig. 1, A to C). In the central nervous system, synapse-rich regions of the neuropil are positive, whereas the surrounding cell bodies are negative, as exemplified

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