observations with considerable success (9). It has also been realized that plumes, because of their relatively high temperature and potential to uplift the surface, may have played a much more important role in the geology of continents than had been hitherto suspected (10).

There has been controversy over whether the transition zone of the mantle, where pressure-induced phase transformations occur near a depth of 650 km, prevents vertical flow and divides the mantle into separately convecting layers (11). It is argued, for example, that the composition of the lower mantle is different from that of the upper mantle or that local buoyancies associated with the phase transformations block lithosphere penetration, though these arguments are disputed (12). It is also argued that structural complications in subduction zones (13) imply that the descent of subducted lithosphere is being blocked there, and that chemical heterogeneities in the mantle sources of basalts require layering in order to explain their isolation in the mantle for up to 2 Ga. Both of these points may be as explicable with a viscosity increase across the transition zone as with a barrier to flow (14).

Two arguments against two-layered convection seem to be particularly robust. First, plumes or other upwellings originating in the transition zone would have to carry more than 70% of the earth's heat budget, instead of less than 10%, because there is not enough radioactivity in the upper mantle to account for the observed surface heat flux. Heat coming from deeper down would have to be conducted through the transition zone interface, above which a thermal boundary layer would develop, giving rise to strong plumes. There is no evidence in sea-floor topography or elsewhere for such strong upwellings (2). Second, the positive gravity and geoid anomalies over subduction zones require a large vertical separation between the dense subducted slabs and the balancing deflections of fluid surfaces required by the laws of mechanics. If there is a viscosity jump rather than a barrier to flow at 650 km, then the main compensating deflection occurs at the core-mantle boundary, so providing the required large separation. Other models do not seem to be capable of quantitatively satisfying the gravity constraints (15).

If the arguments for a single layer of convection are accepted, a relatively simple picture of mantle convection emerges in which plates comprise the top, dominant boundary layer, plumes come from a weaker bottom boundary layer, and viscosity increases by a factor of 100 or so from top to bottom. This picture seems, at this stage, to promise a satisfactory account of the dynamics of the plate-mantle system as it operates at present (16). It implies that plates cool the mantle while plumes cool the core, and it installs "plume tectonics" as an agent complementary to plate tectonics, and possibly nearly as important in the evolution of the continents.

REFERENCES

1. G. F. Davies, *J. Geophys. Res.* **93**, 10451 (1988).
2. _____, *ibid.*, p. 10467.
3. D. T. Sandwell and M. L. Renkin, *ibid.*, p. 2775 (1988); G. F. Davies, *ibid.*, p. 10481; _____ and F. Pribac, *J. Geophys. Res.*, in press.
4. W. J. Morgan, *Nature* **230**, 42 (1971); *Mem. Geol. Soc. Am.* **132**, 7 (1972).
5. S. T. Crough, *Annu. Rev. Earth Planet. Sci.* **11**, 165 (1983); R. W. Griffiths and M. A. Richards, *Geophys. Res. Lett.* **16**, 437 (1989).
6. N. H. Sleep, *J. Geophys. Res.* **95**, 6715 (1990).
7. J. A. Whitehead and D. S. Luther, *ibid.* **80**, 705 (1975).
8. W. J. Morgan, in *The Sea*, C. Emiliani, Ed. (Wiley, New York, 1981), vol. 7, p. 443.
9. M. A. Richards, R. A. Duncan, V. E. Courtillot, *Science* **246**, 103 (1989); R. W. Griffiths and I. H. Campbell, *Earth Planet. Sci. Lett.* **99**, 66 (1990); I. H. Campbell and R. W. Griffiths, *ibid.*, p. 79; M. A. Richards, D. L. Jones, R. A. Duncan, D. J. DePaolo, *Science* **254**, 263 (1991).
10. R. I. Hill, I. H. Campbell, G. F. Davies, R. W. Griffiths, *Science* **256**, 186 (1992).
11. C. J. Allegre and D. L. Turcotte, *Geophys. Res. Lett.* **12**, 207 (1985); P. G. Silver, R. W. Carlson, P. Olson, *Annu. Rev. Earth Planet. Sci.* **16**, 477 (1988); A. E. Ringwood and T. Irifune, *Nature* **331**, 131 (1988); R. Jeanloz and E. Knittle, *Philos. Trans. R. Soc. London Ser. A* **328**, 377 (1989).
12. S. E. Kesson and J. D. Fitz Gerald, *Earth Planet. Sci. Lett.*, in press; Y. Wang *et al.*, *Science* **251**, 410 (1991); U. Christensen, *Nature* **336**, 462 (1989); M. A. Richards and G. F. Davies, *Geophys. Res. Lett.* **16**, 831 (1989).
13. Y. Fukao, M. Nenbai, M. Obayashi, H. Inoue, *J. Geophys. Res.* **97**, 4809 (1992).
14. M. Gurnis and B. Hager, *Nature* **335**, 317 (1988); G. F. Davies, *Earth Planet. Sci. Lett.* **99**, 94 (1990).
15. M. A. Richards and B. H. Hager, *J. Geophys. Res.* **89**, 5987 (1984); B. H. Hager, *ibid.*, p. 6003.
16. G. F. Davies and M. A. Richards, *J. Geol.* **100**, 151 (1992).
17. G. F. Davies, *Geophys. J. Int.* **98**, 461 (1989).

Nitric Oxide: First in a New Class of Neurotransmitters?

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Nitric oxide (NO) is a simple gas with free radical chemical properties, but it is often confused with the chemically distinct nitrous oxide, N_2O, which is used as an anesthetic and is chemically stable. In bacteria NO participates in nitrogen fixation and has recently been shown to function in mammals as well.

NO serves as the messenger whereby macrophages exert their tumoricidal and bactericidal effects (1). When macrophages are activated by endotoxin, a bacterial cell wall lipopolysaccharide that elicits inflammatory responses, the enzyme that makes NO, NO synthase (NOS), which transforms arginine into NO and citrulline, is markedly activated. Inhibition of NO formation by removal of arginine or by *N*-methyl-arginine, an NOS inhibitor, blocks the tumoricidal and bactericidal actions of macrophages.

NO is also a physiologic mediator of blood vessel relaxation. Stimuli that dilate blood vessels, such as acetylcholine, bradykinin, and adenosine triphosphate (ATP), lose their vasodilating activity in blood vessels stripped of endothelium (2). These mediators act upon receptors on endothelial cells to trigger the release of an "endothelial-derived relaxing factor" (EDRF), which diffuses to adjacent smooth muscle cells to elicit relaxation. EDRF has been definitively identified as NO or a close derivative that releases NO (3). Although normal release of NO mediates physiologic vasodilation, excessive release may play a role in septic shock, the symptoms of which can be relieved in animals (4) and in humans (5) by treatment with NOS inhibitors.

The existence of NO in the brain was first suggested by demonstrations that cerebellar neuronal cultures release a factor with properties resembling NO (6), as well as by observations of NO-forming activity in brain extracts (7) and slices (8). NO acts as a messenger in the brain, where it can influence guanosine 3′,5′-monophosphate (cGMP) formation. In blood vessels NO relaxes smooth muscle by stimulating the formation of cGMP through activation of guanylyl cyclase. NO binds with very high affinity to iron in the heme of guanylyl cyclase, eliciting a conformational change that enhances the enzyme's catalytic activity. Cyclic GMP stimulates protein phosphorylation by cGMP-dependent protein kinase, leading to muscle relaxation, although the exact mechanisms are unclear. In the brain, the highest concentrations of cGMP occur in the cerebellum where glutamate, the major excitatory neurotransmitter in the brain, rapidly increases cGMP levels ten times via the *N*-methyl-D-aspartate (NMDA) subtype of the glutamate receptor (9). In these slices glutamate or NMDA triples NOS activity with concentration-response relations identical to those for the increases in cGMP (8). Moreover, selective inhibitors of NOS block the

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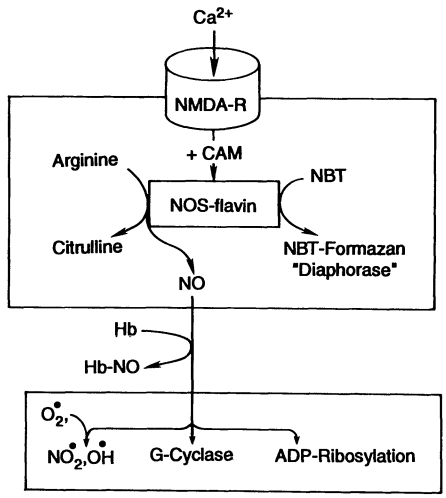
NMDA-induced elevation of cGMP (*8*, *10*).

Since NO itself is labile, with a half-life of about 5 s, most insights into NO in neuronal systems have come from studies of the synthetic enzyme, NOS. Brain NOS was stabilized and purified to homogeneity on the basis of its requirement for calmodulin (*11*). Molecularly cloned brain NOS (*12*) shows 50 to 60% sequence identity to subsequently cloned macrophage NOS (*13*) and endothelial NOS (*14*). This explains how glutamate, acting at NMDA receptors, can markedly activate enzyme activity in a matter of seconds. Stimulation of NMDA receptors opens channels that admit calcium, which enters the cell and binds to calmodulin, thereby activating NOS. Antisera to purified NOS have permitted its immunohistochemical localization in brain and endothelium (*15*). Macrophage NOS is sufficiently different in protein structure (*13*) that it does not react with these antisera so that its localization has not yet been established.

NOS occurs in the endothelial lining of blood vessels, confirming the role of NO as EDRF. Aside from endothelial cells, immunoreactivity for brain NOS occurs exclusively in neurons both in the brain and peripheral tissues. In all regions of the gastrointestinal tract, NOS occurs in the myenteric plexus of neurons (*15*). These neurons mediate the physiologic relaxation of the gut that participates in the normal peristaltic activities of digestion. For many years the neurotransmitter of this nonadrenergic, noncholinergic (NANC), neuronally evoked relaxation has been elusive (*16*). Agents that generate NO mimic NANC relaxation (*17*), and an NO-like substance is released by NANC nerve stimulation (*18*). Moreover, potent, selective inhibitors of NOS, such as *N*-nitroarginine or *N*-methyl-arginine, fully prevent nerve-evoked relaxation of the gut (*17*, *19*). These lines of evidence suggest that NO is participating as a neurotransmitter in this system.

Equally convincing are studies of the neural innervation of blood vessels. In most peripheral tissues, NOS in blood vessels is restricted to the endothelial layer. However, in cerebral arteries (*15*, *20*) and arteries to the penis (*21*), NOS is localized to the nerve plexus in the outer, adventitial layer of vessels. In cerebral arteries these neurons are parasympathetic with their cell bodies in the sphenopalatine ganglia in the neck (*22*). Relaxation of the cerebral arteries by stimulation of these nerves is blocked by *N*-nitroarginine (*23*) as is neurally mediated relaxation of the smooth muscle of the penis (*21*, *24*). In the penis, NOS-containing neurons surround the deep cavernous artery as well as its helicine branches with NOS nerve fibers extending out into the walls of the sinusoids in the erectile corpora cavernosae. In intact rats physiologic stimulation of the pelvic nerves leads to erection, which is completely blocked by low doses of *N*-nitroarginine and *N*-methyl-arginine (*21*). This establishes that NO is the major, if not the sole, mediator of penile erection and is evidently the neurotransmitter of the nerves subserving erection.



A model for nitric oxide actions in neurons. NBT, nitroblue tetrazolium; CAM, calmodulin; NMDA, *N*-methyl-D-aspartate; R, receptor; O_2^-, superoxide free radical; OH^-, hydroxide free radical; Hb, hemoglobin.

In all of these systems NO satisfies several criteria for a neurotransmitter—including occurrence of the synthetic enzyme in the relevant neurons, mimicking by NO of the effects of physiologic nerve stimulation, and blockade of the effects of nerve stimulation after inhibition of NO synthesis. Nevertheless, NO is an unconventional neurotransmitter candidate. Most neurotransmitters are stored in synaptic vesicles, released by fusion with the synaptic membrane, a process called exocytosis, and act at receptor proteins on adjacent neuronal membranes. By contrast, electron microscopic immunohistochemistry establishes that in myenteric plexus nerve terminals NOS does not occur in synaptic vesicles or in association with plasma membrane, but appears to be largely cytoplasmic (*25*). It thus is synthesized on demand and diffuses out of neurons, and in postsynaptic cells its only known "receptor" is iron in guanylyl cyclase (see figure). NO also potently enhances adenosine diphosphate (ADP) ribosylation of glyceraldehyde-3-phosphate dehydrogenase (*26*). Because of its chemical reactivity, there may be multiple receptor targets for NO. It is not yet clear if NO acts alone or together with peptides such as vasoactive intestinal polypeptide (VIP), which is stored together with NOS in myenteric plexus (*27*) and cerebral artery neurons (*22*).

Possible functions for NOS as a neurotransmitter are suggested by the localizations of NOS in the brain. NOS occurs in discrete neuronal populations throughout the brain (*15*, *20*). In some areas it resides in all neurons of a class, such as basket and granule cells of the cerebellum. In other areas, such as the cerebral cortex, hippocampus, and corpus striatum, it is localized to isolated aspiny neurons, comprising only 1 to 2% of the neuronal population. The distribution of NOS neurons appears identical to that of neurons staining for NADPH-diaphorase (NDP) (*20*, *28*), and purified brain NOS has NDP activity (*29*). Molecular cloning of NOS permitted co-staining for NOS and NDP in cells transfected with varying amounts of NOS and the demonstration that the ratio of NOS to NDP staining in transfected cells is identical to the corresponding ratio in neurons (*20*, *28*). This establishes definitively that NOS catalytic activity, which requires NADPH, is responsible for NDP histochemical activity.

NDP is a histochemical stain obtained when incubating brain slices with the dye nitroblue tetrazolium in the presence of NADPH, a known NOS cofactor. NDP-positive neurons are notable for their resistance to destruction in neurodegenerative conditions (*30*) after the ischemic damage of strokes (*31*) and after NMDA neurotoxicity in primary brain cultures that mimic stroke damage (*32*). In primary cerebral cortical cultures, NO released by NOS neurons in response to NMDA kills adjacent neurons (see figure). Thus, treatment with *N*-nitroarginine or *N*-methyl-arginine completely prevents neurotoxicity, as does removal of arginine from the incubation medium (*33*). Hemoglobin, which binds extracellular NO, also prevents neurotoxicity, indicating that NO must pass between cells to elicit neurotoxicity (*33*). Toxicity may involve NO itself or its combination with superoxide free radical (O_2) to form peroxynitrite that decomposes to hydroxide free radical (OH) and NO_2 free radical (NO_2), which are substantially more reactive and toxic than NO (*34*) (see figure). How can NOS neurons kill the majority of other neurons if the NOS neurons are only 2% of the neuronal population? NOS neurons are extensively branched with their fibers contacting almost all other neurons in brain sections or primary cultures. After NMDA application to cultures, dead neurons invariably lie in clumps next to processes of NOS neurons. The demonstration that NO is responsible for NMDA-type glutamate neurotoxicity in primary cultures predicts a role for NO in neurotoxic stroke damage. Initial studies in a model of stroke in which the middle cerebral artery is ligated in mice support this conclusion (*35*). As little as 1 mg per kilogram of body weight of nitroargi-

nine administered in several doses after ligating the middle cerebral artery provides up to 73% protection against stroke damage, whereas MK-801, the most potent known NMDA antagonist, provides only 50 to 60% protection.

Neurotoxicity may not be the only messenger function of NO in the brain. NO has been proposed as a mediator in two models of synaptic plasticity, long-term depression (LTD) and long-term potentiation (LTP). LTD in the cerebellum involves coincident stimulation of climbing fibers and parallel fibers that contact Purkinje cells, resulting in LTD of transmission from parallel fibers to Purkinje cells (*36*). NOS inhibitors as well as hemoglobin block LTD, and nitroprusside, which generates NO, can substitute for the stimulation of climbing fibers in eliciting LTD. Similarly, in the hippocampus NOS inhibitors, added in the incubation bath or directly injected into hippocampal pyramidal cells, block LTP (*37*). NO is postulated to be a retrograde mediator of hippocampal LTP, and so should be formed in pyramidal cells. However, these cells do not stain for NOS (*20*).

Might NO be only the first of a class of decidedly atypical neurotransmitter-like neuronal messengers? One other candidate is carbon monoxide (CO). CO is generated by heme oxygenase (HO), which cleaves the heme ring to form biliverdin, the major breakdown product of heme from red blood cells, and CO. Two forms of HO exist (*38*). Type 1 (HO1) is most abundant in the spleen and other peripheral tissues associated with destruction of red blood cells. Type 2 (HO2) occurs in high concentrations in the brain. During hyperthermia, HO1 levels in the brain increase and are localized to neuronal as well as non-neuronal cells (*39*). HO2 mRNA is highly concentrated in discrete neuronal populations in the brain (*40*). In various neuronal populations, including olfactory neurons of the brain and PC12 cells, endogenous cGMP levels are physiologically maintained by CO, as zinc protoporphyrin IX, an extremely potent and selective inhibitor of HO, depletes endogenous cGMP levels (*40*). A major role for CO in regulating brain cGMP fits with the virtually identical in situ hybridization localizations for mRNA of HO2 and soluble guanylyl cyclase (*40*). Electrons for HO activity are donated by cytochrome P-450 reductase (CPR), the only mammalian protein with close sequence similarity to NOS (*12*). NOS itself can transfer electrons by using the CPR-resembling portion of NOS, while HO requires the "partnership" of CPR. In the liver CPR donates electrons for the P-450 drug metabolizing enzymes, while the substantial neuronal CPR activity of the brain (*38*, *41*) might be associated with CO formation.

The possibility that CO and other hitherto unidentified substances are neurotransmitter-like messengers is speculative. NO clearly satisfies the major criteria for a neurotransmitter, but markedly alters our conceptualizations as to the types of substances that can be transmitters and how they go about fulfilling such a role.

REFERENCES AND NOTES

1. J. B. Hibbs, Jr., R. R. Taintor, Z. Vavrin, E. M. Rachlin, *Biochem. Biophys. Res. Commun.* **157**, 87 (1988); J. B. Hibbs, Jr., Z. Vavrin, R. R. Taintor, *J. Immunol.* **138**, 550 (1987); M. A. Marletta, P. S. Yoon, R. Iyengar, C. D. Leaf, J. S. Wishnok, *Biochemistry* **27**, 8706 (1988); C. F. Nathan and J. B. Hibbs, Jr., *Curr. Opin. Immunol.* **3**, 65 (1991); D. J. Stuehr and C. F. Nathan, *J. Exp. Med.* **169**, 1543 (1989).
2. R. F. Furchgott and J. V. Zawadzki, *Nature* **288**, 373 (1980).
3. L. J. Ignarro, *Annu. Rev. Pharmacol. Toxicol.* **30**, 535 (1990); R. F. Furchgott and P. M. Vanhoutte, *FASEB J.* **3**, 2007 (1989); S. Moncada, R. M. J. Palmer, E. A. Higgs, *Pharmacol. Rev.* **43**, 109 (1991); R. M. J. Palmer, A. G. Ferrige, S. Moncada, *Nature* **327**, 524 (1987).
4. E. Nava, R. M. J. Palmer, S. Moncada, *Lancet* **338**, 1555 (1991).
5. A. Petros, D. Bennett, P. Vallance, *ibid.*, p. 1557.
6. J. Garthwaite, S. L. Charles, R. Chess-Williams, *Nature* **336**, 385 (1988).
7. M. W. Radomski, R. M. J. Palmer, S. Moncada, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 5193 (1990).
8. D. S. Bredt and S H. Snyder, *ibid.* **86**, 9030 (1989).
9. J. A. Ferrendelli, M. M. Chang, D. A. Kinscherf, *J. Neurochem.* **22**, 535 (1974).
10. J. Garthwaite, G. Garthwaite, R. M. J. Palmer, S. Moncada, *Eur. J. Pharmacol.* **172**, 413 (1989).
11. D. S. Bredt and S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 682 (1990).
12. D. S. Bredt *et al.*, *Nature* **351**, 714 (1991).
13. C. R. Lyons, G. J. Orloff, J. M. Cunningham, *J. Biol. Chem.* **267**, 6370 (1992); Q.-w. Xie *et al.*, *Science* **256**, 225 (1992); C. J. Lowenstein, C. S. Glatt, D. S. Bredt, S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
14. S. Lamas, P. A. Marsden, G. K. Li, P. Tempst, T. Michel, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
15. D. S. Bredt, P. M. Hwang, S. H. Snyder, *Nature* **347**, 768 (1990).
16. G. Burnstock, D. G Satchell, A. Smythe, *Br. J. Pharmacol.* **46**, 234 (1972).
17. H. Bult *et al.*, *Nature* **345**, 346 (1990); G. E. Boeckxstaens, P. A. Pelckmans, H. Butt, J. G. DeMan, A. G. Herman, *Eur. J. Pharmacol.* **190**, 239 (1990).
18. G. E. Boeckxstaens *et al.*, *Br. J. Pharmacol.* **103**, 1085 (1991).
19. K. M. Desai, W. C. Sessa, J. R. Vane, *Nature* **351**, 477 (1991); J. S. Gillespie, X. Liu, W. Martin, *Br. J. Pharmacol.* **98**, 1080 (1989); A. Gibson, S. Mirzazadeh, A. J. Hobbs, P. K. Moore, *ibid.* **99**, 602 (1990); M. V. Ramagopal and H. J. Leighton, *Eur. J. Pharmacol.* **174**, 297 (1989).
20. D. S. Bredt *et al.*, *Neuron* **7**, 615 (1991).
21. A. L. Burnett, C. J. Lowenstein, D. S. Bredt, T. S. K. Chang, S. H. Snyder, *Science* **257**, 401 (1992).
22. K. Nozaki *et al.*, *J. Neurosci.*, in press.
23. N. Toda and T. Okamura, *J. Pharmacol. Exp. Ther.* **258**, 1027 (1991).
24. J. Rajfer, W. J. Aronson, P. A. Bush, F. J. Dorey, L. J. Ignarro, *N. Engl. J. Med.* **326**, 90 (1992), L. J. Ignarro *et al.*, *Biochem. Biophys. Res. Commun.* **170**, 843 (1990); F. Holmquist, H. Hedlund, K.-E. Andersson, *Acta Physiol. Scand.* **141**, 441 (1991); F. Holmquist, C. G. Stief, V. Jonas, K.-E. Andersson, *ibid.* **143**, 299 (1991); R. S. Pickard, P. H. Powell, M. A. Zar, *Br. J. Pharmacol.* **104**, 755 (1991).
25. I. J. Llewellyn-Smith, Z.-M. Song, M. Costa, D. S. Bredt, S. H. Snyder, *Brain Res.*, in press.
26. B. Brune and E. G. Lapetina, *J. Biol. Chem.* **264**, 8455 (1989); J. Zhang and S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.*, in press; A. Y. Kots, A. V. Skurat, E. A. Sergienko, T. V. Bulargina, E. S. Severin, *FEBS Lett.* **300**, 9 (1992).
27. M. Costa, J. B. Furness, S. J. H. Brookes, D. S. Bredt, S. H. Snyder, *Proc. Aust. Physiol. Pharmacol. Soc.* **22**, 97P (1991).
28. T. M. Dawson, D. S. Bredt, M. Fotuhi, P. M. Hwang, S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 7797 (1991).
29. B. T. Hope, G. J. Michael, K. M. Knigge, S. R. Vincent, *ibid.*, p. 2811.
30. R. J. Ferrante *et al.*, *Science* **230**, 561 (1985).
31. Y. Uemura, N. W. Kowall, M. F. Beal, *Ann. Neurol.* **27**, 620 (1990).
32. D. W. Choi, *Neuron* **1**, 623 (1991).
33. V. L. Dawson, T. M. Dawson, E. D. London, D. S. Bredt, S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 6368 (1991); V. L. Dawson, T. M. Dawson, G. R. Uhl, S. H. Snyder, *Soc. Neurosci. Abstr.*, in press.
34. J. S. Beckman, T. W. Beckman, J. Chen, P. A. Marshall, B. A. Freeman, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 1620 (1990).
35. J. P. Nowicki, D. Duval, H. Poignet, B. Scatton, *Eur. J. Pharmacol.* **204**, 339 (1991).
36. M. Ito, *Annu. Rev. Neurosci.* **12**, 85 (1989).
37. T. J. O'Dell, R. D. Hawkins, E. R. Kandel, O. Arancio, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 11285 (1991); G. A. Bohme, C. Bon, J.-M. Stutzmann, A. Doble, J.-C. Blanchard, *Eur. J. Pharmacol.* **199**, 379 (1991); E. M. Schuman and D. V. Madison, *Science* **254**, 1503 (1991); J. E. Haley, G. L. Wilcox, P. F Chapman, *Neuron* **8**, 211 (1992).
38. M. D. Maines, *FASEB J.* **2**, 2257 (1988).
39. J. F. Ewing and M. D. Maines, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 5364 (1991).
40. D. Hirsch, A. Verma, C. Glatt, G. Ronnett, S. H. Snyder, *Soc. Neurosci. Abstr.*, in press.
41. L. Haglund, C. Köhler, T. Haaparanta, M. Goldstein, J.-Å. Gustafsson, *Nature* **307**, 259 (1984).

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