Dep. Agric. **8**, 9 (1900).
4. E. Culotta, *Science* **254**, 1445 (1991).
5. T. M. Perring, A. Cooper, D. J. Kazmer, *J. Econ. Entomol.* **85**, 1278 (1992).
6. H. S. Costa and J. K. Brown, *Entomol. Exp. Appl.* **61**, 211 (1991).
7. Type A first was recorded in Florida in 1894 (*3*) and in California in 1928 [L. M. Russel, *Coop. Econ. Insect Rep.* **25**, 229 (1975)]. Type B was found in Florida in ornamental greenhouses, particularly of poinsettias, in 1986 [J. F. Price, D. J. Schuster, D. E. Short, *Greenh. Grow.* **1987**, 55 (December 1987)]. In studies comparing the two types: (i) Type B whiteflies feeding on squash cause squash silverleaf disease, whereas type A whiteflies do not (*1, 6*) [N. Bharathan *et al.*, *Plant Pathol.* **39**, 530 (1990); S. Cohen, J. E. Duffus, H. Y. Liu, *Phytopathology* **82**, 86 (1992)]. (ii) Type B has a different host range than type A and processes more plant phloem sap during feeding, thus excreting a higher volume of honeydew waste product [D. N. Byrne and W. B. Miller, *J. Insect Physiol.* **36**, 433 (1990)]. (iii) Type B lays more eggs than does type A [J. A. Bethke, T. D. Paine, G. S. Nuessly, *Ann. Entomol. Soc. Am.* **84**, 401 (1991)].
8. R. M. Faust, Ed., *Conference Report and 5-Year National Research and Action Plan for Development of Management and Control Methodology for Sweetpotato Whitefly, Houston, Texas, February 18–21, 1992* (U.S. Department of Agriculture, Agricultural Research Service, ARS-107, 1992).
9. P. Gennadius, *Ellenike Georgia* **5**, 1 (1889).
10. L. A. Mound and S. H. Halsey, *Whitefly of the World* (Wiley, New York, 1978).
11. R. J. Gill, *Pan-Pac. Entomol.* **68**, 144 (1992).
12. M. J. W. Cock, *Bemisia tabaci: A Literature Survey on the Cotton Whitefly with an Annotated Bibliography* (Food and Agriculture Organization of the United Nations/CAB International Institute Biocontrol, Sillwood Park, Ascot, United Kingdom, 1986).
13. The name *B. tabaci* currently is synonymous with 22 names in the Aleyrodidae and has been thought to apply to a widespread and morphologically variable species (*10*), with substantial morphological plasticity in certain characters within populations [L. A. Mound, *Proc. R. Entomol. Soc. London Ser. A.* **38**, 171 (1963)]. Among these widely dispersed populations is variability in demographic parameters [D. A. Powell and T. S. Bellows, Jr., *Environ. Entomol.* **21**, 359 (1992)]. Although developmental time varies with host plant, development from egg to adult typically is accomplished in 18 to 30 days at 25°C. Adult longevity may span 10 to 22 days, and total oviposition may range from 30 to 300 eggs per female; averages above 200 eggs per female on favorable host plants are common. *B. tabaci* has been reported from 420 plant species in 74 families worldwide (*12*). Natural enemies of *Bemisia tabaci* include parasitic Hymenoptera (members of the aphelinid genera *Encarsia* and *Eretmocerus*); predaceous Coleoptera, Neuroptera, and Acarina; and parasitic fungi [D. J. Greathead and F. D. Bennett, *Biocontrol News Inf.* **2**, 7 (1981)]. *Bemisia tabaci* transmits 20 viruses [D. N. Byrne, T. S. Bellows, Jr., M. P. Parrella, in *Whiteflies: Their Bionomics, Pest Status and Management*, D. Gerling, Ed. (Intercept, United Kingdom, 1990), p. 227], which cause over 40 diseases of vegetable and fiber crops [J. K. Brown and J. Bird, *Plant Dis.* **76**, 220 (1992)].
14. S. R. Diehl and G. L. Bush, *Annu. Rev. Entomol.* **29**, 471 (1984).
15. T. Y. Li, S. B. Vinson, D. Gerling, *Environ. Entomol.* **18**, 800 (1989).
16. D. N. Byrne and T. S. Bellows, *Annu. Rev. Entomol.* **36**, 431 (1991); L. A. Mound, in *Plant Virus Epidemiology*, R. T. Plumb and J. M. Thresh, Eds. (Blackwell, Boston, 1983), p. 305.
17. The genetic distance (D) is an estimate of the number of allelic substitutions per locus in two populations. D values for comparisons among a number of vertebrates and invertebrates are 0.00 to 0.05 between populations or races, 0.02 to 0.20 between subspecies, 0.1 to 2.0 between species, and more than 1.0 between genera [M. Nei, in *Population Genetics and Ecology*, S. Karlin and E. Nevo, Eds. (Academic, New York, 1976), p. 723; F. J. Ayala *Population and Evolutionary Genetics: A Primer* (Benjamin-Cummins, Menlo Park, CA, 1985)]. We determined both interstrain (using pooled data from all populations) and intrastrain D values. Populations within each strain showing the most disparate allelic frequencies were compared in order to show the maximum amount of intrastrain genetic differentiation. We also compared two other congeneric but morphologically distinct species, *Trialeurodes vaporariorum* (Westwood) and *Trialeurodes abutilonea* (Haldeman) to determine if the D value between these two species is consistent with the D values recorded for comparisons between other animal species. After examining 16 loci using 14 enzyme stains, we obtained a D value of 0.83, indicative of separate species.
18. R. J. Rodriguez and O. C. Yoder, *Exp. Mycol.* **15**, 232 (1991).
19. J. Sambrook, E. F. Fritsch, T. Maniatis, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Press, Cold Spring Harbor, NY, 1989).
20. The original protocol (*18*) was modified by replacing the final ammonium acetate and isopropanol precipitations of DNA with a precipitation that used 0.1 M NaCl and 2.0 volumes of 95% ethanol (*19*). In addition, whiteflies were pulverized in microfuge tubes after addition of the lysis buffer.
21. J. L. Owen and C. M. Uyeda, *Anim. Biotechnol.* **2**, 107 (1991).
22. M. Gupta and P. Filner, *The International Society for Plant Molecular Biology*, abstr. 1705 (Tucson, AZ, 1991).
23. Polymerase chain reactions [M. K. Mullis and F. A. Falcona, *Methods Enzymol.* **155**, 335 (1987)] were performed in 20-μl reactions containing 10 mM tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 200 μM each of dATP, dCTP, dGTP, and dTTP (Pharmacia), 0.1 unit of Taq I DNA polymerase (Promega), 2 μM of a single oligonucleotide primer, and 50 to 200 ng of whitefly genomic DNA (*21*). Oligonucleotide primers represented repeated DNA sequence motifs as follows: 5′-dCAGCAGCAGCAGCAG-3′ (*18*), 5′-dGACAGACAGACAGACA-3′ [J. Epplen, *J. Hered.* **79**, 409 (1989)], 5′-dGACACGACACGACAC-3′ (*22*) [K. Weising *et al.*, *Nucleic Acids Res.* **17**, 10128 (1989)], 5′-dTCCTCCTCCTCCTCC-3′ (*22*), 5′-dACTGACTGACTGACTG-3′ (*22*), 5′-dAGGAGGAGGAGGAGG-3′, and 5′-dGTGGTGGTGGTGGTG-3′. In the text, these primers have been designated $(CAG)_5$, $(GACA)_4$, $(GACAC)_3$, $(TCC)_5$, $(ACTG)_4$, $(AGG)_5$, and $(GTG)_5$, respectively. Amplification reactions consisted of 30 cycles in an Ericomp Dual Block system following one cycle at 95°C for 5 min. Each cycle consisted of a temperature regime of 95°C for 0.5 min, 60°C or 52°C for 0.5 min [60°C for the $(CAG)_5$ and $(GTG)_5$ primers and 52°C for the remaining primers], and 70°C for 1.5 min. Electrophoresis of the amplified products was performed for 1.5 hours at 7.0 V/cm in 1.5% agarose. The oligonucleotide primers used in these studies were synthesized in the bioinstrumentation facility at the University of California, Riverside, and purified by gel filtration with the use of a column of Sephadex G-100 (Sigma) equilibrated with tris-EDTA buffer (*19*). The DNA from each whitefly type then was amplified using Taq I DNA polymerase and single oligonucleotide primers (*21*).
24. We thank J. Bethke, R. Creamer, D. Daoud, J. Duffus, and P. Stansley for providing whiteflies; K. Truman, J. de la Cruz, R. D. Plaza, and J. L. Ramirez for assistance in southern Mexico; and G. Gordh, J. Lorang, and J. Pinto for comments on an earlier draft of the manuscript. Supported in part by grants from the California Melon Research Board, the California Cotton Committee, the Imperial Valley Whitefly Management Committee to T.M.P. and T.S.B. and in part by an NSF grant (BSR-9115734) to R.J.R.

15 September 1992; accepted 1 December 1992

Mediation by G Proteins of Signals That Cause Collapse of Growth Cones

Michihiro Igarashi, Stephen M. Strittmatter,* Timothy Vartanian, Mark C. Fishman†

During development, motion of nerve growth cones ceases on contact with particular targets. The signaling mechanism is unknown. In culture, growth cone collapse can be caused by solubilized embryonic brain membranes, central nervous system myelin, a 35-kilodalton protein isolated from myelin, and mastoparan. Collapse induced by each of these is blocked by pertussis toxin. Thus, collapse of growth cones is mediated by G protein–coupled receptors, which may be activated by proteins associated with the cell surface as well as by soluble ligands.

Inhibitory factors expressed on the surface of cellular targets and along migratory pathways regulate the motion of growth cones (*1, 2*). These guidance signals help to direct the assembly of neuronal circuitry (*1–7*) and their presence hinders regeneration of neuronal processes after brain injury (*4*). Several inhibitory factors are associated with the cell surface and have been identified in membrane fractions of embryonic brain, optic tectum, somites, and myelin (*1, 2, 5–7*). Those factors cause collapse of neuronal growth cones in culture (*5–7*). G proteins, which are known to respond to soluble signals through transmembrane receptors (*8*), are enriched in the membrane of the growth cone (*9*). We investigated whether membrane-bound inhibitory ligands function through activation of G proteins.

Developmental Biology Laboratory, Massachusetts General Hospital–East, Charlestown, MA 02129, and Department of Medicine, Harvard Medical School, Boston, MA 02115.

*Present address: Department of Neurology, Massachusetts General Hospital–East, Charlestown, MA 02129.

†To whom correspondence should be addressed.

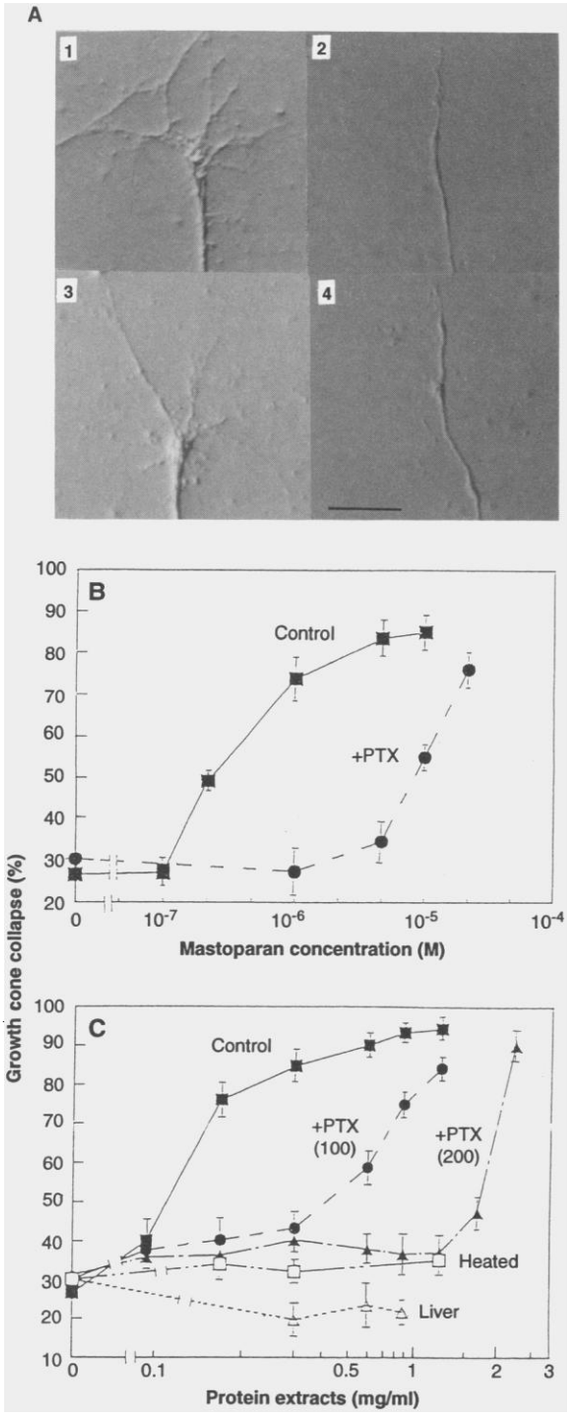

Fig. 1. Collapse of dorsal root ganglion growth cones caused by brain membranes and mastoparan and blocked by PTX. (**A**) Typical Nomarski optical views of growth cones of dorsal-root ganglia. Growth cones extending on laminin-coated glass after a 30-min incubation with (1) F12 medium only, where the growth cones are spread, (2) chick E10 brain-derived collapsing activity (0.3 mg/ml), (3) the same concentration of collapsing activity as in (2) but after PTX pretreatment for 2 hours, and (4) 10 μM mastoparan. Bar represents 10 μm. (**B**) Mastoparan causes collapse of dorsal root ganglion growth cones, an effect that is blocked by PTX. Control (filled squares) or PTX-pretreated (100 ng/ml, filled circles) growth cones of chick E7 dorsal root ganglia were measured in the presence of the indicated final concentrations of mastoparan. Data shown are the average and SEM of four to six independent experiments. (**C**) Collapse of growth cones of dorsal root ganglia induced by brain membrane extracts is blocked by PTX. Explants of E7 dorsal root ganglia were treated for 2 hours with no PTX (control, filled squares), 100 ng/ml PTX (filled circles), or PTX (200 ng/ml, filled triangles), and then CHAPS-solubilized embryonic brain extracts were added. Liver membranes of E10 chicks (open triangles) and heat-treated (90°C, 10 min) brain membranes (open squares) were also examined. Neither extracts of liver membrane nor extracts of heated brain membrane showed significant collapsing activity (*6*). The percentage of collapsed growth cones is shown as a function of the protein concentration of the extract. Data shown are the average and SEM of four to six independent experiments (*10*).

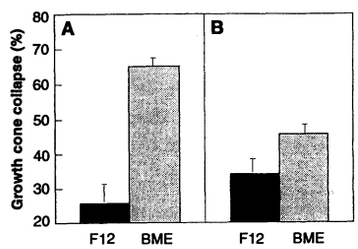

Fig. 2. Pertussis toxin partially blocks collapse of retinal growth cones caused by brain-membrane extracts. The effects of CHAPS-solubilized membranes from chick embryonic brain on retinal growth cones (**A**) without and (**B**) with PTX pretreatment. The temporal halves of chick E7 retinae were isolated and cut into small fragments. Each fragment was explanted onto laminin-coated plastic chamber slides. The collapse assay with brain-membrane extracts (BME; protein concentration: 1.2 mg/ml) was performed as in Fig. 1 (*10*). In control samples (F12), only F12 medium was added to the retinal explants. For PTX pretreatment, retinal explants were incubated with F12 medium containing PTX (100 ng/ml) for 2 hours before addition of the membrane extracts. Data shown are the average and SEM of four to six independent experiments. The difference between the group exposed to brain-membrane extracts with and without PTX was significant ($P < 0.05$). With PTX, there was no significant difference between the control (F12) and the exposed groups (at the 5% significance level).

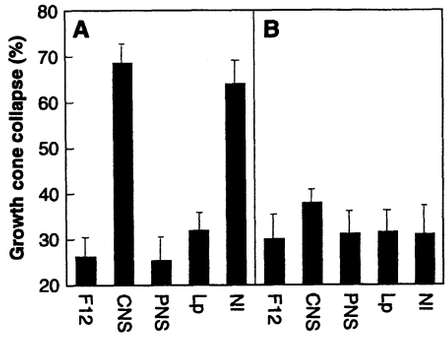

Fig. 3. Collapse of growth cones caused by rat central nervous system myelin is blocked by PTX. The collapsing activities of the CNS and PNS myelin proteins solubilized by octylglucoside (protein concentration: 1.4 mg/ml) and of NI-35 reconstituted into liposomes (NI) were examined. As controls, F12 medium (F12) or unreconstituted liposomes (Lp) were added. The percentage of collapsed growth cones of E7 dorsal root ganglia was scored in the (**A**) absence or (**B**) presence of PTX (100 ng/ml). The collapse assay was performed as in Fig. 1 (*14*). Only the CNS myelin and NI-35 have collapsing activity, and both are blocked by PTX. Data shown are the average and SEM of six to ten independent experiments.

In cell culture, about 75% of growth cones from chick dorsal-root ganglia were fan-shaped and the remainder were collapsed (Fig. 1A) (*10*). Continuous observation indicates that individual growth cones alternate between the two states (6) and that the collapsed form is associated with cessation of growth (*5*). The identity and function of the G protein–linked receptors on these growth cones is unknown. Therefore, we tested the involvement of several G proteins by the use of the wasp venom peptide mastoparan, which stimulates G proteins directly by a receptor-like mechanism (*11*), and with pertussis toxin (PTX), which blocks receptor- and mastoparan-stimulated activity of G_o and G_i (respectively, the unknown and inhibitory subtypes of G protein) (*12*). When compared to control levels, PTX alone did not change the percentage of collapsed growth cones. This result indicated that, under standard culture conditions, components of the medium or serum did not regulate the shape of growth cones by PTX-sensitive G proteins. On the other hand, direct stimulation of G proteins by mastoparan caused many growth cones to collapse in a dose-dependent fashion, with a median effective concentration (EC_{50}) of 0.25 μM (Fig. 1B). The activity of mastoparan was blocked by PTX, which indicates that its action in this regard is through G proteins (Fig. 1B). Thus, stimulation of PTX-sensitive G proteins caused the collapse of growth cones.

Exposure to extracts of embryonic brain membranes increased the percentage of collapsed growth cones (6), with an EC_{50} of about 0.15 mg of protein per milliliter (Fig. 1, A and C). Preincubation of the explants with PTX blocked collapse induced by the brain membrane (Fig. 1C). At the highest concentrations of brain-membrane extracts, some collapse still occurred after preincubation with PTX, perhaps because of incomplete adenosine diphosphate ribosylation by PTX or a low-affinity, PTX-insensitive mechanism.

Extracts of embryonic brain membrane also cause collapse of chick retinal-neuron growth cones (6), which are a component of the central nervous system (CNS). In our experiments, retinal growth cones did collapse upon exposure to extracts of brain membrane, although they appeared less sensitive than those of dorsal root ganglion neurons (Fig. 2). This collapse was greatly reduced by pretreatment with PTX (Fig. 2).

Myelin from the CNS but not from the peripheral nervous system (PNS) also contains an inhibitory activity (*13*) that has been fractionated into two predominant components, with respective atomic masses of 35 kD and 250 kD (*7*). Central nervous system myelin proteins solubilized by octylglucoside (*14*) collapsed growth cones of dorsal-root ganglion neurons (Fig. 3). Myelin extracted from the PNS did not alter the percentage of collapsed growth cones (Fig. 3). Pertussis toxin reduced the percentage of growth cones that collapse in response to central myelin from 68 to 38%.

Protein NI-35, a 35-kD protein with growth-inhibiting properties, has been partially isolated from CNS myelin (*7*, *13*). Neutralization of NI-35 by the monoclonal antibody IN-1 permits neurites to grow over myelin in vitro (*13*) and enhances growth of regenerating axons of the spinal cord in vivo (*15*). Partially purified NI-35 caused growth cones of dorsal root ganglia to collapse (*2*, *16*), a response blocked by antibody IN-1 (*17*) and PTX (Fig. 3). Antibody IN-1 did not affect the activity of embryonic extracts of brain membrane (*17*). Recently, NI-35 has been shown to cause release of Ca^{2+} from intracellular stores (*18*) with consequent increase in Ca^{2+} in growth cones (*16*). These results are compatible with NI-35 acting through activation of G proteins.

Thus, PTX blocks growth cone–collapsing activities from two different sources, both of which are associated with membranes (*6*, *7*). Dorsal root ganglion and retinal neurons respond similarly, suggesting that the intracellular cascade that regulates induced collapse may be widespread. Both G_o and, to a lesser extent, G_i are among the most common proteins of growth cone membrane (*9*), and we propose that the collapse is triggered by an inhibitory ligand binding to a receptor coupled to a G protein. Such receptors might be present at low quantities in the growth cone membrane and their signals would be amplified by the G protein transduction cascade (*9*, *19*).

This work indicates that cell-associated ligands act through G protein–coupled systems, transduction cascades that are well studied with regard to soluble ligands. Some soluble factors are known to cause collapse of growth cones (*20*). There is also some evidence that G proteins may mediate cell contact interactions in that cell adhesion molecules can alter PTX-sensitive G protein transduction. For example, the morphoregulatory action of a neural cell adhesion molecule (N-CAM) and N-cadherin is blocked by PTX (*21*, *22*), and antibodies to a Ca^{2+}-independent neural cell adhesion molecule, L1, and to N-CAM alter amounts of intracellular Ca^{2+} and phosphoinositides in a PTX-sensitive fashion (*22*).

Victims of neurological trauma or stroke often recover only partial function because inhibitory factors block axonal regeneration in the CNS (*4*, *15*). Therefore, agents that modify the PTX-sensitive activity of the growth cone might have salutary effects on regeneration.

REFERENCES AND NOTES

1. J. Walter, T. E. Allsopp, F. Bonhoeffer, *Trends Neurosci.* **13**, 447 (1990); J. A. Davies and G. M. W. Cook, *BioEssays* **13**, 11 (1991); R. J. Keynes and G. M. W. Cook, *Curr. Opin. Neurobiol.* **2**, 55 (1992).
2. M. E. Schwab, *Trends Neurosci.* **13**, 452 (1990).
3. S. M. Strittmatter and M. C. Fishman, *BioEssays* **13**, 127 (1991); J. L. Bixby and W. A. Harris, *Annu. Rev. Cell Biol.* **7**, 117 (1991).
4. S. David and A. J. Aguayo, *Science* **214**, 931 (1981); L. M. Mendell and G. R. Lewin, *Curr. Biol.* **2**, 259 (1992).
5. J. A. Davies, G. M. W. Cook, C. D. Stern, R. J. Keynes, *Neuron* **4**, 11 (1990); E. C. Cox, B. Müller, F. Bonhoeffer, *ibid.*, p. 31.
6. J. A. Raper and J. P. Kapfhammer, *ibid.*, p. 21.
7. P. Caroni and M. E. Schwab, *J. Cell Biol.* **106**, 1281 (1988).
8. H. G. Dohlman, J. Thorner, M. G. Caron, R. J. Lefkowitz, *Annu. Rev. Biochem.* **60**, 653 (1991).
9. S. M. Strittmatter, D. Valenzuela, T. E. Kennedy, E. J. Neer, M. C. Fishman, *Nature* **344**, 836 (1990); E. T. Edmonds, C. R. Moomaw, J. T. Hsu, C. Slaughter, L. Ellis, *Dev. Brain Res.* **56**, 131 (1990).
10. Collapse of growth cones was determined as described (*5*, *6*). Dorsal root ganglia were removed from embryonic day (E7) chick embryos were explanted onto laminin-coated glass chamber slides in F12 medium containing nerve growth factor (5 ng/ml) and 10% fetal calf serum. Embryonic brain membranes were prepared from E10 chick brain as described (*6*). Such membrane fractions (10 mg of protein) were suspended in 1 ml of 2% CHAPS in phosphate-buffered saline (PBS) and centrifuged at 100,000*g* for 1 hour. The supernatant was dialyzed against PBS and F12 medium subsequently to remove the detergent. This dialysate (50 μl), or mastoparan, was added to the explants cultured in 0.5 ml of medium, incubated at 37°C for 30 min, and then fixed with 4% paraformaldehyde in PBS. All individual growth cones from each explant (50 to 100 per explant) were scored. Extracts of liver membrane from E10 chicks were prepared and assayed by the same methods as those used on brain membranes. In some experiments, the explants were preincubated with PTX (100 or 200 ng/ml; Calbiochem) for 2 to 4 hours.
11. T. Higashijima, J. Burnier, E. M. Ross, *J. Biol. Chem.* **265**, 14176 (1990).
12. Y. Kaziro, H. Itoh, T. Kozasa, M. Nakafuku, T. Satoh, *Annu. Rev. Biochem.* **60**, 349 (1991).
13. C. Bandtlow, T. Zachleder, M. E. Schwab, *J. Neurosci.* **10**, 3837 (1990).
14. Myelin from the CNS and the PNS was prepared from adult rat cortex and sciatic nerves, respectively [W. T. Norton and S. E. Poduslo, *J. Neurochem.* **21**, 749 (1973)]. Myelin proteins (10 mg) were solubilized by 60 mM octylglucoside (1 ml) at 4°C for 1 hour [M. I. Aveldaño, R. S. Díaz, P. Regueiro, J. Monreal, *ibid.* **57**, 250 (1991)]. The supernatant was collected and dialyzed with the same method as that for chick brain membranes (*10*). The 35-kD protein was reconstituted into liposomes as described (*7*). After ethanol-ether extraction of central myelin, the insoluble fraction was solubilized by 2% SDS and separated by electrophoresis. The gel region corresponding to a 35-kD protein molecular weight was excised, and protein was electroeluted and reconstituted into phosphatidylcholine liposomes formed on Bio-Beads SM-2 (Bio-Rad). The solubilized myelin fraction or the reconstituted liposomes were diluted and added to explants of chick dorsal-root ganglia (see legend to Fig. 1). The incubation time was 1 hour with total myelin fractions and 2 hours with the liposomes.
15. L. Schnell and M. E. Schwab, *Nature* **343**, 269 (1990).
16. M. E. Schmidt *et al.*, *Soc. Neurosci. Abstr.* **17**, 927 (1991).
17. The liposome solution (80 μg of protein per milliliter) containing NI-35 or the extracts of embryonic brain membrane (6 mg of protein per milliliter) were diluted (1:1, v/v) with IN-1 hybridoma supernatant and added to the culture medium. Collapse assay was as above (*15*). Most of the collapse induced by NI-35 (82.7 ± 5.3%; four independent experiments) was blocked by IN-1 antibody. A control antibody, O_1, directed against galactocerebroside did not block the collapsing activity of NI-35.
18. C. E. Bandtlow, M. F. Schmidt, T. D. Hassinger, S. B. Kater, M. E. Schwab, *Science* **259** (1993).
19. L. Birnbaumer *et al.*, *CRC Crit. Rev. Biochem. Mol. Biol.* **25**, 225 (1990).
20. P. G. Haydon, D. P. McCobb, S. B. Kater, *Science* **226**, 561 (1984); K. L. Lankford, F. G. De Mello, W. L. Klein, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 4567 (1988); H. S. Suidan, S. R. Stone, B. A. Hemmings, D. Monard, *Neuron* **8**, 363 (1992).
21. P. Doherty, S. V. Ashton, S. E. Moore, F. S. Walsh, *Cell* **67**, 21 (1991); P. Doherty, S. D. Skaper, S. E. Moore, A. Leon, F. S. Walsh, *Development* **115**, 892 (1992).
22. U. Schuch, M. Lohse, M. Schachner, *Neuron* **3**, 13 (1989).

23. We thank C. E. Bandtlow, M. E. Schwab (who also provided the IN-1 and the O_1 antibodies), M. F. Schmidt, T. D. Hassinger, and S. B. Kater for their unpublished results on intracellular Ca^{2+} effects of NI-35 and D. Y. Stainier and Y. Sudo for advice. M.I. is a recipient of a Long-term Overseas Research Fellowship from the Ministry of Education, Sciences, and Culture of Japan. S.M.S. is a recipient of a Clinical Investigator Development Award from the National Institutes of Health. Supported in part by a grant from Sumitomo Chemical and Sumitomo Pharmaceutical Companies (M.C.F.).

23 June 1992; accepted 1 September 1992