PART V

Substance P and the Inflammatory and Immune Response

PATRICK W. MANTYH

Molecular Neurobiology Lab
Veterans Administration Medical Center
Minneapolis, Minnesota 55417

Several lines of evidence indicate that tachykinin neuropeptides [substance P (SP), neurokinin A (NKA), and neurokinin B (NKB)] play a role in regulating the inflammatory and immune response both in peripheral tissues (such as the lung, joint, and colon) and in the central nervous system. To explore the possible involvement of tachykinins in these processes, we have used quantitative receptor autoradiography to examine alterations in the location and level of tachykinin binding sites in surgical specimens from patients with inflammatory bowel disease and in the rabbit optic nerve after neuronal injury. Results from these experiments demonstrate that binding sites for SP, but not for NKA or NKB, are ectopically expressed in high concentrations (1000–2000 times normal) by small arterioles and lymph nodules in human surgical samples of colon from patients with inflammatory bowel disease, but not from colon tissue obtained from normals. In the central nervous system, section of the rabbit optic nerve, which results in a proliferation of type-1 astrocytes that form the glial scar and that have been hypothesized to block neuronal regeneration, is also accompanied by a dramatic up-regulation of SP (but not NKA or NKB) binding sites. These data suggest that SP may be involved in the response to tissue injury both in peripheral tissues such as the colon and in the CNS after neuronal injury.

INFLAMMATORY BOWEL DISEASE

Neurons with cell bodies located in dorsal root ganglia (DRG) are known to convey specific features of somatic sensory information from peripheral tissues to the central nervous system. Recently, several neuropeptides have been identified within a subpopulation of these sensory neurons.[1] The most extensively characterized of these sensory neuropeptides is SP, a member of the mammalian tachykinin family that also includes NKA and NKB. Although it is clear that SP and NKA are expressed by sensory neurons,[2] it appears that NKB is not expressed in detectable levels in DRG or in most peripheral tissues.

Several of these sensory neuropeptides, most notably SP, have been associated with neurons specifically implicated in the conduction of nociceptive information. Therefore, intrathecal injection of SP produces biting and scratching behavior, consistent with a role for SP as a peptide neurotransmitter associated with primary afferent nociceptors;[3] SP release in the spinal cord is inhibited by opiate analgesics;[4] depletion of SP by capsaicin (a neurotoxin that is relatively selective for unmyelinated sensory neurons,[5] including those containing SP) is associated with a loss of specific nociceptive response; and release of SP in the spinal dorsal horn in response to normally innocuous stimuli is enhanced in polyarthritic rats.[6]

It has become increasingly evident in the last decade that a specific class of DRG

neurons conveying *afferent* somatosensory information from peripheral tissues to the spinal cord are involved in the *efferent* regulation of the peripheral tissues they innervate. Thus, SP-containing DRG neurons have been implicated both in the *afferent* central transmission of nociceptive information and in the *efferent* regulation of inflammation and sensitization of joint sensory endings in a chronic pain state, for example, arthritis.[7,8] Support for this concept includes the following observations: the bulk of the SP synthesized by DRG neurons is transported to the peripheral terminals rather than to the spinal cord;[9] SP is a potent vasodilator in several peripheral tissues;[10] terminals of SP-containing sensory neurons are observed in association with blood vessels;[11] electrical stimulation of these peripheral nerves at intensities that release SP in peripheral tissues reproduces many of the physiological changes seen in acute inflammation,[12] including plasma extravasation;[7] and SP binding sites in experimental animals are expressed by several tissues involved in the inflammatory and immune response.[13]

What emerges from these observations is the hypothesis that sensory neurons containing SP and/or other neuropeptides are involved both in conveying nociceptive information to the spinal cord and in regulating the inflammatory and immune responses in the peripheral tissues they innervate. Hence, the same sensory neuropeptide released by a sensory neuron may signal tissue damage in the spinal cord and may participate in regulating the inflammation, immune response, and (ultimately) wound healing in the affected peripheral tissue.

To test whether this hypothesis is applicable to human inflammatory diseases, we examined the changes in tachykinin neuropeptide receptor binding sites in chronic inflammatory bowel disease (IBD) patients, where surgical removal of the affected tissue is used to ameliorate the disease in severe cases. IBD is a generic term that refers to chronic inflammatory diseases of the intestine that are of unknown etiology, principally ulcerative colitis and Crohn's disease. Ulcerative colitis is an inflammatory, ulcerating process of the colon; Crohn's disease is an inflammation of the intestine characterized by nodular granulomatous inflammatory lesions throughout the entire wall that may involve any part of the intestine, but that primarily attack the distal small intestine and colon.[14] Because surgical removal of the inflamed colon is used in severe cases of IBD, we used these surgical specimens to ask whether significant alterations in either the location or the levels of SP binding sites occur in these human inflammatory diseases. What we found was that SP binding sites were unique among the binding sites examined in that they were dramatically up-regulated in the inflamed colon tissue (FIGURE 1) and that this dramatic up-regulation appeared to be restricted to cells involved in mediating the inflammatory and immune response (FIGURE 2), that is, small arterioles and lymph nodules.

A key question that remains to be answered, though, was the following: what was the normal function of the neuropeptide-containing sensory neurons, which were the presumed source of the SP that would occupy these SP binding sites? One observation that suggests a normal function for these neuropeptide-containing sensory neurons is that one of the most pronounced deficits in neonatal capsaicin-treated rats (the neurotoxin destroys primarily the neuropeptide-containing C-fibers) is that the skin and fur lose their luster and that these animals lack a normal wound-healing response; that is, these animals have numerous small wounds that do not appear to be healing.[15,16] Such a lack of the normal trophic or wound-healing response after sensory denervation can also be inferred from the ulceration of the cornea, which follows trigeminal deafferentation.[17] Together, these observations suggest that, in a pathological state, SP appears to be involved in regulating a hyperinflammatory and immune response and that, in the normal condition, these sensory neuropeptide-

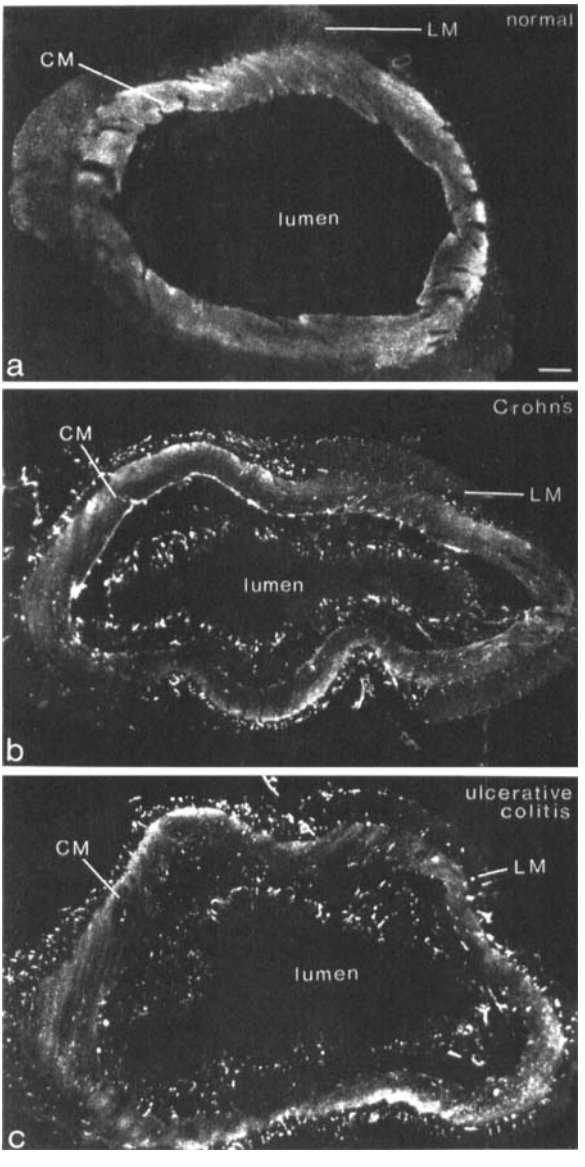

FIGURE 1. Dark-field autoradiograms showing the distribution of substance P (SP) binding sites in transverse sections of colon obtained from the margins of extensive resection for carcinoma (a) and with patients with Crohn's disease (b) and ulcerative colitis (c). In these dark-field autoradiograms, the tritium-sensitive film was used as the negative; white silver grains represent areas of high concentration of SP binding sites. Whereas a moderate concentration of SP receptor binding sites is expressed by the external circular muscle (CM) and the tunica media of a large artery in the serosa in normal colon (a), arterioles, venules, and lymph nodules express very high levels of SP receptor binding sites in both Crohn's disease (b) and ulcerative colitis (c). Abbreviations: CM, external circular muscle; LM, external longitudinal muscle. Line bar = 1.4 mm.

containing neurons may have a trophic action on tissues they innervate and may also regulate wound healing after injury.

As noted here, SP and other sensory neurotransmitters appear to be released centrally in the spinal cord to signal pain and peripherally to produce vasodilatation, plasma extravasation, and homing of leukocytes to the area of injury. Because there cannot be tissue repair until there has been an appropriate inflammatory and immune response,[18] the initial action of SP may be to promote and direct the inflammatory and immune responses in the damaged tissue. After the infection and

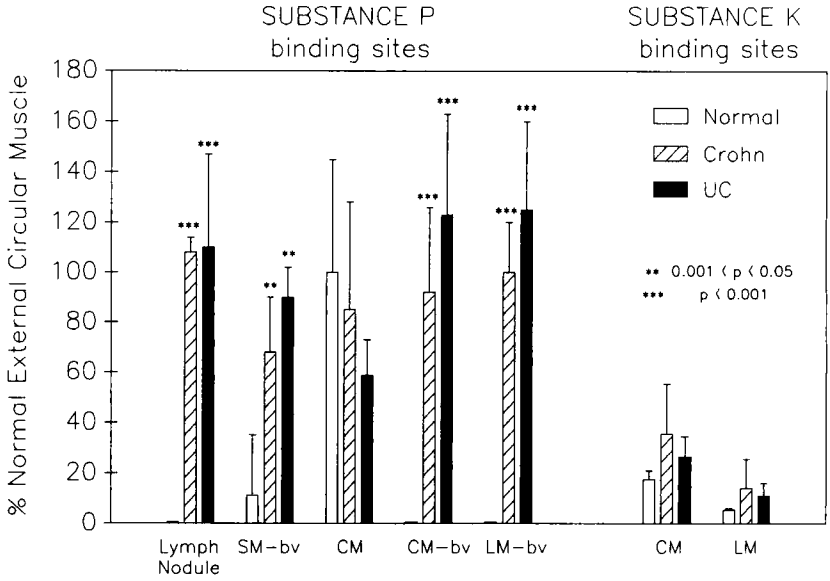

FIGURE 2. Histogram showing the changes in the location and concentration of SP and NKA (also known as substance K, SK) binding sites in the surgical specimens of normal colon obtained from carcinoma resection (open bars), Crohn's disease (hatched bars), and ulcerative colitis (dark bars). In this histogram, 100% specific binding is that concentration of specific SP binding sites expressed by the smooth muscle of the normal external circular muscle (CM). Whereas SP receptor binding sites are undetectable in blood vessels (bv) vascularizing the circular (CM) and longitudinal muscle (LM) and in the lymph nodules of normal patients, they reach levels in the Crohn's and ulcerative colitis patients that are the highest observed in the human GI tract. Distinct binding sites for NKB were not detected in any area of the human GI tract. Abbreviations: bv, blood vessel (arteriole or venule); CM, external circular muscle; LM, external longitudinal muscle; SM, submucosa.

damaged tissue have been cleared, SP and other neuropeptide growth factors continue to be released; however, their effects would now be directed towards mitogenesis and tissue remodeling. Two sensory neuropeptides, SP and NKA, have been shown to be potent mitogens for myocytes and fibroblasts in culture.[19] The key to this hypothesis is that SP should be both stimulatory and inhibitory towards the same cells, depending on the context of other chemical signals present. This multifunctional role for SP is not unprecedented for other peptide growth factors as it has been shown that another peptide, transforming growth factor-β, stimulates the

growth of certain fibroblasts *in vitro* in the presence of platelet-derived growth factor, but inhibits the growth of the same cells if epidermal growth factor is present.[20,21] In human inflammatory diseases, it may be that ectopic expression of SP or its binding site is not the primary pathology, but rather that the pathology may involve whatever other factors are needed to switch SP action from the "catabolic" mode, where inflammatory and immune responses are promoted, to the "anabolic" process of tissue growth. What remains to be determined is what factors regulate SP binding site expression and what the physiological consequences of SP release and SP binding site activation are in both normal and inflamed tissues.

GLIAL EXPRESSION OF SUBSTANCE P RECEPTORS AFTER NEURONAL INJURY

A major question in neurobiology is why damaged mammalian central nervous system (CNS) neurons do not regenerate *in vivo*. In recent years, the focus of attention has shifted from CNS neurons themselves, which appear to have the capacity to regenerate, to CNS glia, which apparently inhibit the regrowth of axons in the CNS. Thus, it has been demonstrated that, after injury, regenerating axons grow a short distance until they reach the glial scar, at which time they appear to stop growing and degenerate.[22-27]

The major cellular constituent of a CNS glial scar is the reactive astrocyte.[25] Unlike fibroblasts, which form scars in nonneural tissue by secreting large amounts of collagenous extracellular matrix, astrocytes form scars by extending numerous processes that become packed with intracellular glial filaments.[28] Astrocytes proliferate in response to injury[29] and it appears that these "reactive astrocytes" are biochemically different from the major class of astrocytes that are present in the normal, nonlesioned brain.[25] Recently, several neuropeptides, including bombesin, NKA, and substance P, have been shown to be mitogenic[30,31] for several cell types that may be involved in the inflammatory and wound-healing responses in peripheral tissues.[32] *In vitro* studies suggest that glia are potential targets for a variety of neurotransmitters,[33] including SP,[34-37] somatostatin,[38,39] and vasoactive intestinal peptide.[36,38,39] By analogy with the inflammatory and immune responses that occur in response to injury in peripheral tissues, neuropeptides may also regulate glial mitogenesis and glial response to injury of the CNS. However, because glial cells exhibit different functional properties depending on their biochemical environment,[33] it is imperative to demonstrate that these receptor binding sites, which have been shown to be expressed by glia *in vitro*, are also expressed by glia *in vivo*.

To determine if similar neurotransmitter receptors are also expressed by glia *in vivo*, we examined the glial scar in the transected optic nerve of the albino rabbit using quantitative receptor autoradiography. Receptor binding sites for radiolabeled calcitonin gene–related peptide, cholecystokinin, galanin, somatostatin, SP, and vasoactive intestinal peptide were examined. Specific receptor binding sites for each of these neurotransmitters were identified in the rabbit forebrain, but were not detected in the normal optic nerve or tract. In the transected optic nerve and tract, only receptor binding sites for SP were expressed at detectable levels.[40] The density of SP receptor binding sites observed in this glial scar is among the highest observed in the rabbit forebrain (FIGURE 3). Ligand displacement and saturation experiments indicate that the SP receptor binding site expressed by the glial scar has pharmacological characteristics similar to those of NK-1 receptors in the rabbit striatum (FIGURE 4), the rat brain, and the rat and canine gut. These results demonstrate that glial cells

in vivo express high concentrations of SP receptor binding sites after transection of retinal ganglion cell axons.

Because SP has been shown to regulate inflammatory and immune responses in peripheral tissues, SP may also, by analogy, be involved in regulating the glial response to injury in the central nervous system. This is consistent with previous *in vitro* studies that demonstrated that SP receptors are present in (2–3)-week-old primary cultures of cortical astrocytes from newborn mice and that addition of substance P to these astrocyte cultures stimulates phosphatidylinositol turnover.[37] SP has also been shown to stimulate the cyclooxygenase pathway of arachidonic acid metabolism in (2–3)-week-old cultures of rat astrocytes and to evoke the formation of prostaglandin E and thromboxane B_2 in a dose-dependent manner.[34] These studies together with the present results are consistent with the suggestion that "reactive astrocytes" that proliferate after neuronal injury—and that are hypothesized to play

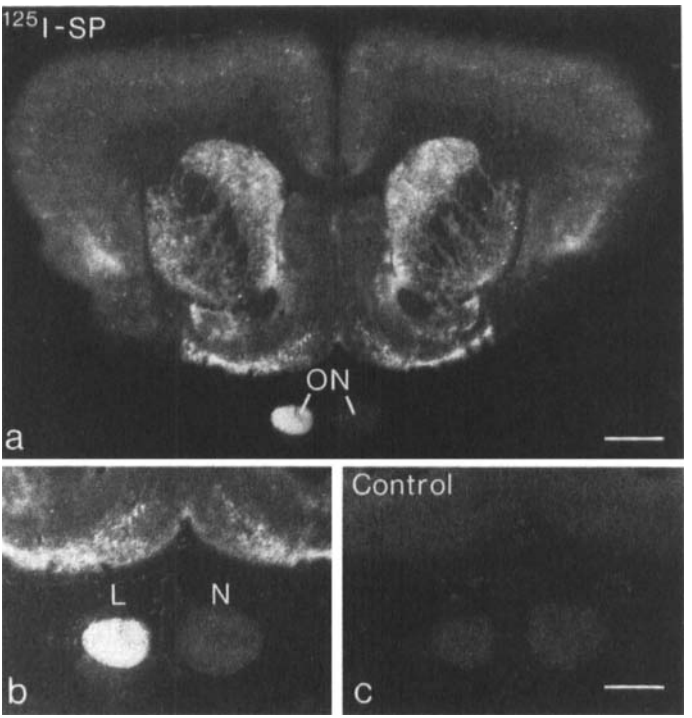

FIGURE 3. A series of dark-field photomicrographs showing the autoradiographic localization of SP receptor binding sites in a coronal section of the rabbit brain (animal #05) 99 days after unilateral transection of the optic nerve (ON). Autoradiograms a and b show the total binding, whereas c is the nonspecific binding. The control section (c) illustrating the nonspecific binding was treated identically to the adjacent section, which shows the total binding (b) except that nonradioactive SP (1 μM) was added to the incubation medium. In all the dark-field autoradiograms, the highest density of white silver grains represents the highest concentration of binding sites. The specific binding is obtained by subtracting the binding in c from b. Whereas the lesioned (L) optic nerve is reduced in size, it expresses a high density of specific receptor binding sites relative to the larger normal (N) optic nerve, in which specific binding sites are not detectable. Line bar (a) = 1.9 mm; (b & c) = 1.2 mm.

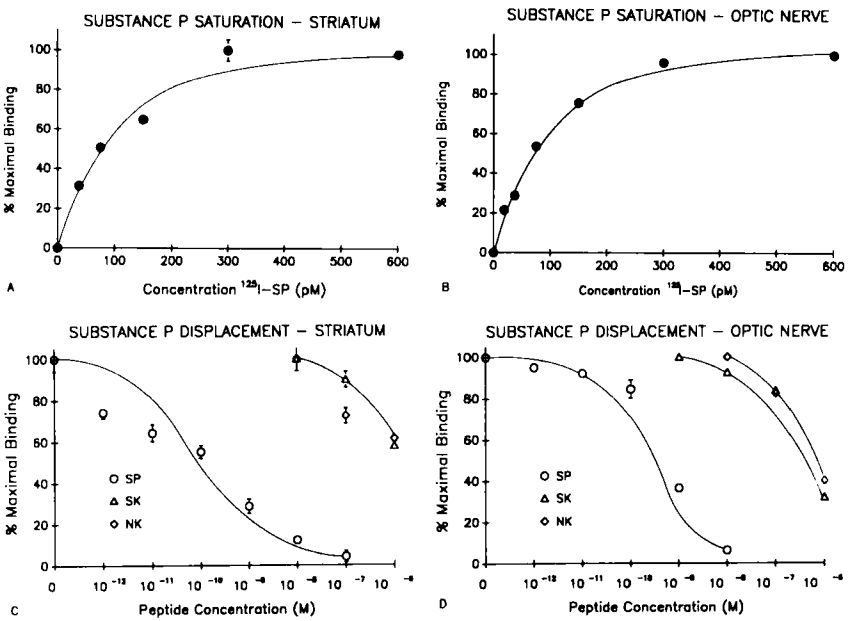

FIGURE 4. Saturation (A,B) and displacement (C,D) curves for SP receptor binding sites expressed by the striatum (A,C) and the lesioned optic nerve (B,D) 99 days after transection (rabbit #04). Note that both the saturation and displacement curves for SP receptor binding sites in the normal striatum (A,C) and the transected optic tract (B,D) are similar, suggesting that the SP receptor binding site expressed by the lesioned optic nerve is similar to the SP receptor binding site expressed by the normal striatum. The data shown are from a representative experiment. Each point represents the mean of triplicate determinations.

a role in inhibiting neuronal regeneration[25]—express functional SP receptors. These data suggest that, when glia are separated from neurons either *in vitro* or *in vivo* after the transection and degeneration of retinal ganglion cell axons, the glia that survive and proliferate express SP receptors.

SUMMARY

These findings suggest that SP may have proinflammatory actions in both the peripheral tissue and the central nervous system after tissue injury. Although the possibility that the same neuropeptide could have actions in both the brain and the peripheral tissues is certainly not without precedent, there is a key difference in the source of the ligand in these tissues. Unlike peripheral tissues such as the gastrointestinal tract or skin, where there is a dense innervation by SP-containing dorsal root ganglion neurons, the brain lacks such a sensory innervation. This important difference raises the question as to the possible origin of the SP that could occupy the SP receptors expressed by the CNS glia after neuronal injury. Whereas the answer to this question is currently unknown, an important clue may be the findings that circulating leukocytes have been reported to synthesize neuropeptides such as ACTH, opiates,[41] and SP.[42]

To begin to fully understand the role that SP may play in coordinating the inflammatory and immune response to tissue injury, we must first understand where SP fits into the cascade of events that occur after tissue injury, what events lead to nociceptor sensitization (which may lead to an increase in SP release), and what regulates SP receptor expression (which may be involved in the direction of leukocytes to the site of injury, plasma extravasation, or the proliferation/hypertrophy of reactive astrocytes). Although this may seem like a daunting task, several recent advances including the cloning of the three mammalian tachykinin receptors and the introduction of highly potent and specific SP receptor antagonists[43] should make this a highly fruitful field of investigation.

REFERENCES

1. HUNT, S. P. 1983. *In* Chemical Neuroanatomy. P. C. Emson, Ed.: 53–84. Raven Press. New York.
2. MAGGIO, J. E. & J. C. HUNTER. 1984. Brain Res. **307:** 370–373.
3. HYLDEN, J. L. K. & G. WILCOX. 1981. Brain Res. **217:** 212–215.
4. JESSEL, T. J. & L. L. IVERSEN. 1977. Nature **268:** 549–551.
5. NAGY, J. I., S. P. HUNT, L. L. IVERSEN & P. C. EMSON. 1981. Neuroscience **6:** 1923–1934.
6. OKU, R., M. SATOH & H. TAKAGI. 1987. Neurosci. Lett. **74:** 315–319.
7. LEMBECK, F. & P. HOLZER. 1979. Naunyn-Schmiedeberg's Arch. Pharmacol. **310:** 175–183.
8. LEVINE, J. D., S. J. DARDICK, M. F. ROEZEN, C. HELMS & A. I. BASBAUM. 1986. J. Neurosci. **6:** 3423–3429.
9. BRIMIJOIN, S., J. M. LUNDBERG, E. BRODIN, T. HÖKFELT & G. NILSSON. 1980. Brain Res. **191:** 443–457.
10. LUNDBERG, J. M., E. BRODIN, X. HUA & A. SARIA. 1984. Acta Physiol. Scand. **120:** 217–227.
11. FURNESS, J., R. E. PAPKA, N. G. DELLA, M. COSTA & R. L. ESKAY. 1982. Neuroscience **7:** 447–459.
12. CHAHL, L. A. & R. J. LADD. 1976. Pain **2:** 25–34.
13. MANTYH, P. W., C. R. MANTYH, T. GATES, S. R. VIGNA & J. E. MAGGIO. 1988. Neuroscience **25:** 817–838.
14. ROSENBERG, I. H. 1985. *In* Cecil Textbook of Medicine. Wyngaarden & Smith, Eds.: 740–756. Saunders. Philadelphia.
15. BUCK, S. H. & T. F. BURKS. 1983. Trends Pharmacol. Sci. **4:** 84–87.
16. NAGY, J. I., S. P. HUNT, L. L. IVERSEN & P. C. EMSON. 1981. Neuroscience **6:** 1923–1934.
17. BEUERMAN, R. W. & B. SCHIMMELPFENNIG. 1980. Exp. Neurol. **69:** 196–201.
18. GALLIN, J. I. & A. S. FAUCI. 1982. Advances in Host Defense Mechanisms. Vol. 1—Phagocytic Cells. Raven Press. New York.
19. NILSSON, J., A. M. VON EULER & C. J. DALSGAARD. 1985. Nature **315:** 61–63.
20. ROBERTS, A. B., M. A. ANZANO, L. M. WAKEFIELD, N. ROCHE, D. F. STERN & M. B. SPORN. 1985. Proc. Natl. Acad. Sci. U.S.A. **82:** 119–123.
21. SPORN, M. B. & A. B. ROBERTS. 1988. Nature **332:** 217–219.
22. AGUAYO, A. J., S. DAVID & G. M. BRAY. 1981. J. Exp. Biol. **95:** 231–240.
23. EYSEL, U. T. & L. PEICHL. 1985. Exp. Neurol. **88:** 757–766.
24. LIUZZI, F. J. & R. J. LASEK. 1987. Science **237:** 642–645.
25. MILLER, R. H., E. R. ABNEY, S. DAVID, C. FRENCH-CONSTANT, R. LINDSAY, R. PATEL, J. STONE & M. RAFF. 1986. J. Neurosci. **6:** 22–29.
26. RAMON Y CAJAL, S. 1928. Degeneration and Regeneration of the Nervous System. Vol. II, p. 583–596. Oxford University Press. London/New York.
27. VILLEGAS-PEREZ, M. P., M. VIDAL-SANZ, G. M. BRAY & A. J. AGUAYO. 1988. J. Neurosci. **8:** 265–280.
28. MAXWELL, D. S. & L. KRUGER. 1965. J. Cell Biol. **25:** 141–157.
29. LATOV, N., N. GAJANAN, E. A. ZIMMERMAN, W. G. JOHNSON, A. SILVERMAN, R. DEFENDINI & L. COTE. 1979. Dev. Biol. **72:** 381–384.

30. LOPEZ-RIVAS, A., S. A. MENDOZA, E. NANBERG, J. SINNETT-SMITH & E. ROZENGURT. 1987. Proc. Natl. Acad. Sci. U.S.A. **84:** 5768–5772.
31. ZICHE, M., L. MORBIDELLI, M. PACINI, P. DOLARA & C. A. MAGGI. 1990. Br. J. Pharmacol. **100:** 11–14.
32. MANTYH, C. R., T. S. GATES, R. P. ZIMMERMAN, M. L. WELTON, E. P. PASSARO, JR., S. R. VIGNA, J. E. MAGGIO, L. KRUGER & P. W. MANTYH. 1988. Proc. Natl. Acad. Sci. U.S.A. **85:** 3235–3239.
33. MURPHY, S. & B. PEARCE. 1987. Neuroscience **22:** 381–394.
34. HARTUNG, H. P., K. HEININGER, B. SCHAFER & K. V. TOYKA. 1988. FASEB J. **3:** 48–51.
35. PERRONE, M. H., R. D. LEPORE & W. SHAIN. 1986. J. Pharmacol. Exp. Ther. **238:** 389–395.
36. ROUGON, G., M. NOBLE & A. W. MUDGE. 1983. Nature **305:** 715–717.
37. TORRENS, Y., J. C. BEAUJOUAN, M. SAFFROY, M. C. DAGUET DE MONTETY, L. BERGSTROM & J. GLOWINSKI. 1986. Proc. Natl. Acad. Sci. U.S.A. **83:** 9216–9220.
38. CHNEIWEISS, H., J. GLOWINSKI & J. PREMONT. 1985. J. Neurochem. **44:** 779–786.
39. EVANS, T., K. D. MCCARTHY & T. K. HARDEN. 1984. J. Neurochem. **43:** 131–138.
40. MANTYH, P. W., D. JOHNSON, C. BOEHMER, M. CATTON, H. VINTERS, J. MAGGIO, H. TOO & S. VIGNA. 1988. Proc. Natl. Acad. Sci. U.S.A. **86:** 5193–5197.
41. BLALOCK, J. E., K. L. BROST & E. M. SMITH. 1985. J. Neuroimmunol. **10:** 31.
42. WEINSTOCK, J. V., A. BLUM, J. WALDER & R. WALDER. 1988. J. Immunol. **141:** 961–966.
43. SNIDER, R. M., J. W. CONSTANTINE, J. A. LOWE, K. P. LONGO, W. S. LEBEL, H. A. WOODY, S. E. DROZDA, M. C. DESAI, F. J. VINICK, R. W. SPENCER & H. J. HESS. 1991. Science **251:** 435–437.