Brain Peptides: Is Substance P a Transmitter of Pain Signals?

Numerous peptides are turning up in the brain. Some of them may be chemical transmitters of nerve impulses.

The brain is often compared to a computer, a comparison that does not do justice to the brain. One of the major differences between brains and computers is the reliance of the latter on but one kind of signal—electrical impulses—for communicating among their components. Nerve cells in the brain and elsewhere also use electrical signals, but in addition they communicate chemically with neurotransmitters.

No one knows how many neurotransmitters there are, but extensive efforts to identify the chemicals and map the neurons producing them are currently under way. The goal is a better understanding of how the brain receives information from the body and the environment, integrates it into a picture of what is happening, and then tells the body how to react.

The first agents to be identified as neurotransmitters were all small molecules, single amino acids or their derivatives or such simple compounds as acetylcholine. The idea that peptides may also act as neurotransmitters is a relatively recent one in neurobiology, only attaining respectability within the past 5 years—but with a vengeance. At present, about 20 peptides, which is roughly double the number of firmly established neurotransmitters, are under consideration as transmitters of nerve signals.

Since the identities of the chemical transmitters used by most neurons—90 percent or more of them—are unknown, gaping holes exist in our understanding of how the brain works. The peptides are thus providing a new and possibly very large category of potential neurotransmitters that may fill some of these holes.

One peptide causing a great deal of excitement is substance P, which has a very long history. Now—2 years short of the 50th anniversary of its discovery—there is growing acceptance of the possibility that substance P may be a transmitter of the brain signals carried by sensory nerves into the spinal cord and then relayed to the brain. In fact, the endorphins, the brain's built-in opiates (*Science*, 24 September 1976, p. 1227), may produce their analgesic effects by suppressing substance P release in the spinal cord.

The endorphins are themselves peptides that are considered good candidates to be neurotransmitters, particularly for nerve pathways suppressing pain perception (*Science*, 4 February 1977, p. 471). The discovery a few years ago of the endogenous opiates is another development in brain peptide research that has been attracting much attention from both the scientific community and the public.

This is the second of a series of occasional articles on recent developments in neurobiology. The first story dealt with the search for new psychoactive drugs (Science, *24 August, p. 774). Future articles will discuss sex hormones and sexual behavior and the development of the nervous system.*

In 1931, Ulf S. von Euler of the Karolinska Institute and the late John H. Gaddum were looking for acetylcholine in extracts of brain and intestine when they stumbled on a substance that causes intestinal contractions and lowers blood pressure by producing blood vessel dilation. The material did not prove to be acetylcholine, however, and they simply designated it as substance P because it was contained in a certain preparation (in powder form) of their tissue extracts. The choice of the letter P turned out to be an appropriate one for a peptide that appears to be a transmitter of pain impulses.

Von Euler and Gaddum got as far as determining that substance P is a peptide, but they did not purify it and determine its structure. In fact, its amino acid sequence was not worked out until 1970, when Susan Leeman and Michael Chang, who were then at Brandeis University, pulled off the feat.

Leeman, incidentally, was not looking for substance P either, but was trying to isolate corticotropin-releasing factor from the hypothalamus. Leeman and Chang did not find the elusive releasing factor—nor has anyone else—but they did identify a material that stimulates salivation in laboratory animals. They went on to show that the material was none other than substance P and determined the sequence of the 11 amino acids in the peptide.

Arg–Pro–Lys–Pro–Gln–Gln–Phe–Phe–Gly–Leu–Met

Amino acid sequence of substance P

The structure determination sparked a new wave of interest in substance P. The research had not exactly languished, especially during the 1950's and 1960's, but results obtained with impure preparations of a material of unknown structure are always suspect. With the structure in hand, however, large quantities of pure substance P could be synthesized and specific antibodies could be prepared for use in radioimmunoassays. Radioimmunoassay allows investigators to identify minute quantities of a particular chemical in tissue. The development of the technique has been one of the factors contributing to the great surge of interest in peptides in the nervous system.

The nervous system includes more than just the brain and spinal cord, and substance P's distribution is widespread in nervous tissue. "One of the amazing things," says Thomas Jessell of Harvard Medical School, "is that it is in almost every division of the nervous system you look at." Substance P is in neurons of the autonomic nervous system, which controls more or less involuntary activities such as breathing and the beating of the heart. It is present in the myenteric plexus, a nerve network innervating the intestinal tract that may regulate the undulating movements that propel food through the intestines. It is even in some endocrine-like cells of the intestinal lining, where its function is totally unknown.

One of the commonest findings in neurobiology today is the presence of many peptides in both the brain and peripheral tissues, especially the gastrointestinal tract (see box). According to Leslie Iversen of Cambridge University, "It is almost the rule, rather than the ex-

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ception, that you find them in both places." Right now, more is known about the role of substance P in the spinal cord than elsewhere in the nervous system or in the intestinal tract.

The suggestion that the peptide might be a neurotransmitter dates back to the mid-1950's. Fred Lembeck of the University of Graz in Austria found that the dorsal root, a nerve tract carrying sensory impulses from the body periphery to the spinal cord, contains ten times more substance P than the ventral root, which carries nerve fibers leaving the spinal cord (Fig. 1). He suggested that the substance P in the dorsal root might be a neurotransmitter by which the incoming sensory fibers communicate with neurons in the dorsal horn of the spinal cord. The nerve fibers of the dorsal horn then carry the impulses to the brain.

Lembeck, who says that his laboratory at that time was "fairly primitive," was quite pleased when his finding of high substance P concentrations in the dorsal root was confirmed about 5 years ago by Masanori Otsuka and his colleagues at the Tokyo Medical and Dental University. Otsuka's group also showed that substance P is concentrated in the dorsal horn and that it excites spinal cord nerve cells—the first demonstration of such an effect of the peptide.

Proving that a chemical is a neurotransmitter is not easy. Its mere presence in the right portion of the brain or spinal cord is not adequate, nor is any other single criterion sufficient. Although neurobiologists do not always agree on how to go about proving that a particular chemical is a neurotransmitter, there are nine or ten criteria which, if met, go a long way toward gaining admission of the chemical to the rather exclusive neurotransmitter club. Substance P has fulfilled about half the criteria in support of its postulated role as a transmitter of pain impulses.

Several lines of evidence suggest that substance P is present in nerve fibers that carry pain signals. Tomas Hökfelt of the Karolinska Institute has shown that many of the small-diameter nerve fibers of the dorsal horn, which have traditionally been considered to be pain fibers, contain substance P. Also, the nerves of the tooth pulp, which carry many pain fibers, contain the peptide.

Moreover, experiments with capsaicin, a chemical isolated from Hungarian red peppers, suggest that the substance P neurons are pain nerves. Administration of capsaicin causes an immediate sensation of intense pain in humans and experimental animals; after prolonged administration of the chemical, however, the animals become insensitive to painful stimuli. Lembeck, with Rainer Gamse, who went to Harvard Medical School after leaving Graz, and Jessell have independently shown that capsaicin causes the release of substance P from the dorsal horn, which could account for the initial pain sensations. Eventually the peptide becomes depleted, a circumstance that may explain the decrease in pain sensitivity caused by prolonged capsaicin treatment. (One may wonder if a person contemplating a trip to the dentist should first indulge in a prolonged chicken paprikash binge.)

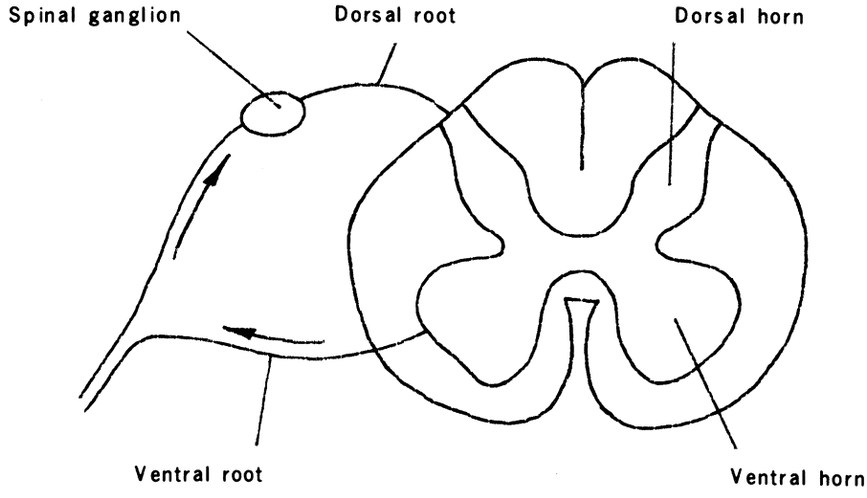

Fig. 1. Cross section of the spinal cord. The dorsal horns of the centrally located gray matter mainly contain nerve fibers that carry sensory information up to the brain; the ventral horns carry the descending nerve fibers. The dorsal root carries the incoming sensory nerve fibers whose cell bodies are located in the spinal ganglia. The ventral root carries the fibers leaving the spinal cord. The cell bodies of the ventral root nerves are inside the cord.

Not only is substance P present in the appropriate neurons, but several investigators have found that it fulfills the second criterion for a neurotransmitter—that of being present in the terminals of those neurons. It is the terminals that are in contact with the next neuron in a pathway and that release the transmitter.

Although neurotransmitters must be present in the nerve terminals, peptides are probably synthesized in the cell body. The cell body, which contains the neuronal nucleus, may be some distance away from the terminals of a long nerve fiber. The third criterion, then, is demonstration of the transport of the putative transmitter to the terminals. A number of investigators have evidence for such transport of substance P. For example, cutting or tying off the sensory nerves between the spinal cord and the nerve cell bodies, which are located in ganglia outside the cord, causes accumulation of substance P on the cell body side of the blockage. It disappears from the dorsal horn, indicating that the supply was interrupted.

A fourth criterion is demonstration of the release of the peptide in response to appropriate stimulation. Otsuka has shown that electrical stimulation of dorsal root nerves provokes substance P release by spinal cords. And Jessell and Iversen have demonstrated release of the peptide in another laboratory preparation thought to mimic nerve stimulation in nature. Moreover, Jessell, with Tony Yaksh of the Mayo Clinic, has shown that electrical stimulation of the sciatic nerve, the large nerve bundle running from the leg to the spinal cord, causes release of substance P in the cords of living animals. Only when the applied stimulus was sufficient to excite the small pain neurons, which require more intensive stimulation than other neurons, did Jessell and Yaksh observe the release.

A fifth criterion to be met by a neurotransmitter is the ability of the pure material or its analogs to mimic the effects supposedly mediated by it. Substance P appears to meet this criterion. When it is applied to spinal neurons, including those that receive pain impulses, it causes their excitation.

There are some problems with these experiments that have not been resolved, however. As R. Alan North of the Loyola University Stritch School of Medicine points out, the stimulation produced by the peptide develops slowly and is long-lasting. In contrast, excitation by accepted neurotransmitters occurs very rapidly and is short-lived.

According to Leeman, who is now at Harvard Medical School, the slow action of substance P is not necessarily an obstacle to its acceptance as a transmitter

of nerve impulses. Sometimes technical problems with the types of experiments in which chemicals are applied to neurons slow the movement of the chemical to the nerve cells.

Although North is not convinced by the evidence that substance P is an excitatory neurotransmitter for pain nerves, his own research tentatively suggests that it may play this role in the myenteric plexus. Even if the peptide is not a neurotransmitter, it may still be a neuromodulator—that is, a chemical that alters nerve cell responsiveness to neurotransmitters but does not itself carry nerve signals.

There is very little information about substance P related to the remaining criteria for a neurotransmitter. Its synthesis in sensory neurons mediating pain transmission has not been directly demonstrated, nor has the manner in which its action may be turned off, although some evidence shows that the peptide is not taken up again by the neurons that release it. There has to be a way to rapidly break down a neurotransmitter or to remove it from the junction between the cell releasing it and the target neuron. Otherwise, the target cell would continue to be stimulated even after incoming impulses had stopped. Nor have investigators clearly identified any substance P

A Potpourri of Brain Peptides

Neurobiologists looking into the brain opened a box that released not all the evils of the world but a seemingly never-ending stream of peptides. Many of them are also found in other regions of the body, where they have well-defined roles as hormones or other regulators of physiological processes. A sampling of some of the peptides turning up in the brain includes the following.

• Angiotensin, a powerful elevator of blood pressure, is found in the bloodstream. It is produced by the action of renin, an enzyme made in the kidney. All the components of the renin-angiotensin system have now been found in the brain, and there are indications that they are part of the brain's mechanism for regulating blood pressure.

• Cholecystokinin is a peptide hormone produced by the small intestine in response to the movement of food from the stomach into the intestine. It causes the contraction of the gallbladder, thus releasing bile into the small intestine, where the enzymes and other components of bile aid digestion. What cholescystokinin is doing in the brain is unclear, but there are hints that it may help to regulate feeding.

• Hormones such as adrenocorticotropin and melanocyte stimulating hormone, which are produced by the pituitary gland, and vasopressin, which is produced by the hypothalamus but transported to and released by the pituitary, are present in several regions of the brain. A body of evidence suggests that these hormones facilitate some facets of memory and learning.

• Endorphins and enkephalins, the brain's own opiates, are also found in such peripheral areas of the body as the myenteric plexus of the gut. The endogenous opiates, possibly acting as neurotransmitters in the brain and spinal cord, suppress pain perception under some conditions and may also have other effects on behavior. Their role in the periphery is unknown.

• Neurotensin is a peptide consisting of 13 amino acid residues that has several actions in the periphery. For example, it helps to regulate blood glucose concentrations by its effects on a number of hormones, including insulin and glucagon. A variety of evidence suggests that the central nervous system contains pain-suppressing pathways that do not depend on the endorphins. Neurotensin may mediate some of this endorphin-independent analgesia.

• Releasing factors, which are produced by the hypothalamus and then travel to the pituitary where they evoke the release of the appropriate hormones, are being found in additional brain regions. Among those that have been found are luteinizing hormone releasing hormone (LHRH) and thyroid hormone releasing factor (TRF). Both TRF and LHRH have behavioral effects when they are administered to experimental animals; LHRH, for example, enhances mating behavior.

• Vasoactive intestinal peptide (VIP) is also present in both the gut and the brain. Its peripheral effects include lowering blood pressure by causing vasodilation, suppressing secretion of stomach acid, and stimulating secretion in the small intestine and colon. VIP is a neurotransmitter candidate that may play a role in effecting arousal. It also stimulates the release of a number of pituitary hormones, including growth hormone and prolactin, and may thus help to regulate the endocrine system.

Neurobiologists are just beginning to understand what these and the other peptides are doing in the brain. The agents need not—and probably will not—all turn out to be neurotransmitters, however. There are a number of ways in which they might influence nerve cell activity besides carrying nerve impulses between specific neurons—an activity that Jeffery Barker of the National Institute of Neurological and Communicative Disorders and Stroke has likened to a telephone conversation between two people. Both neurotransmission and the telephone conversation are private and require hard-wiring; in the case of neurotransmission, the communicating neurons must actually be in contact through a junction called the synapse.

But a peptide might be a neuromodulator or neurohormone rather than a transmitter. By Barker's analogy, a neuromodulator, which alters the response of a nerve cell to a neurotransmitter, would be akin to the control for adjusting the volume of the signal picked up by the telephone receiver. Finally, Barker compares neurohormonal communication, which does not require direct contact between the nerve cell releasing the agent and the target cells, to a radio broadcast, where there is public transmission of a signal that can be picked up by any properly equipped receiver within range.

In addition, a peptide or other agent could affect neuronal activity by increasing or decreasing the synthesis, release, or breakdown of neurotransmitters, -modulators, or -hormones.

Finally, there is no reason to think that neurobiologists have identified all the peptides working in the brain or all the ways they might work there. At least a few scientists and engineers understand how a computer works. It will be some time before anyone can say the same for the brain.

—J.L.M.

receptors. Such receptors are needed on the target nerves for a neurotransmitter to exert its effects.

Several researchers point out that substance P research is greatly hampered by the lack of an inhibitor that specifically blocks the peptide's effects. Says Iversen, "Until you have a blocker, you will have trouble finding out what it does." An inhibitor that blocked an animal's response to pain, for example, would support the theory that substance P transmits pain signals.

Despite the absence of an ironclad case for substance P as a pain transmitter, most investigators are optimistic that the final proof will be forthcoming. Meanwhile, research on the peptide is shedding some light on how the endorphins suppress pain perception.

Jessell and Iversen have shown that a synthetic analog of the endorphin called Met-enkephalin blocks the release of substance P in isolated preparations of the trigeminal nucleus, a region of the brainstem that contains the terminals of pain fibers and is especially rich in substance P neurons. The effect of the synthetic enkephalin was blocked by naloxone, which is thought to be a specific inhibitor of opiate action. Leeman, with Ann Mudge and Gerald Fischbach, also of Harvard Medical School, found a similar inhibition of substance P release in sensory neurons grown in tissue culture.

The investigators suggest that enkephalin suppresses pain perception by blocking substance P release from the pain-transmitting neurons. As Leeman puts it, "It makes physiological sense for the enkephalins to work this way."

If the endorphins do block substance P release in living animals, the analgesic peptides ought to be present in the same regions of the spinal cord as the neurons containing the pain transmitter. In addition, the substance P neurons should have receptors for the opiates. There is evidence for both of these circumstances. Receptors for the endogenous opiates have been identified, and their distribution in the spinal cord closely parallels that of the substance P neurons. So does that of the enkephalins.

The substance P neurons may contain binding sites for the enkephalins. When the sensory neurons into the spinal cord are cut, the neurons containing substance P degenerate; at the same time the enkephalin receptors also vanish.

Jessell and Iversen think the relationship between enkephalins and substance P may be the physiological basis of the "pain gate" postulated some years ago by Ronald Melzack of McGill University in Montreal and Patrick Wall of University College, London. To explain how it happens that people occasionally do not feel pain even from serious injuries, Melzack and Wall proposed that there is a "gate" in the spinal cord that lets pain impulses through to the brain. In certain circumstances the gate may swing shut to block pain transmission. Jessell and Iversen are proposing that prevention of substance P release by enkephalin may be what closes the gate.

Other investigators have also suggested that the endorphins block pain transmission in the spinal cord. They have found that stimulation of certain nerves that come down the spinal cord from the brain produces analgesia. Since the analgesia is prevented by naloxone, they hypothesize that it is mediated by endogenous opiates.

These descending neurons are known to use the neurotransmitter serotonin. Recently, Hökfelt found substance P in some spinal cord neurons that also contain serotonin. If these are the same cells that suppress pain signals by triggering enkephalin release, then the role of substance P may be more complicated than that of a simple pain signal transmitter.

Investigators still have a lot to learn about the activity of these nerve cells, however. Substance P appears to excite neurons, whereas serotonin usually inhibits them. No one knows whether the two agents are released at the same time and whether they act on the same target cells. But one thing is clear—the existence of two neurotransmitters in the same nerve cell is a phenomenon being observed with increasing regularity these days. The possibility that one neuron might send out two (or more, for that matter) kinds of chemical signals is not going to make life any easier for neurobiologists.

In addition to being present in the dorsal horn of the spinal cord, substance P is concentrated in several areas of the brain—as many as 25 or 30, according to Hökfelt. One brain region with particularly high concentrations is the substantia nigra, which contains nerve tracts that help to control movement.

Huntington's chorea is a degenerative brain disease characterized by uncontrollable movements and progressive mental deterioration. Loss of neurons containing the neurotransmitter γ-aminobutyric acid (GABA) has been observed in patients with Huntington's chorea, but the deficiency does not appear to be the sole cause of their neurological symptoms because treatment with agents that increase GABA concentrations has no effect on the patients.

Loss of substance P-containing neurons of the substantia nigra may also contribute to the abnormalities of Huntington's chorea, according to Ichiro Kanazawa of Tsukuba University in Japan, who found a decrease in the neurons in brains of patients who had died of the disease. Since the condition has so far proved intractable to treatment, any clues to its cause will be given close attention.

Schizophrenia is another often intractable disorder. Thus, suggestions that substance P may play some role in the genesis of schizophrenia are attracting interest. The evidence for the link depends partly on observations from the laboratory of Jacques Glowinski at the College de France in Paris that suggest that substance P stimulates release of the neurotransmitter dopamine. Overproduction of dopamine has been implicated as a cause of schizophrenia.

Finally, Hökfelt has identified substance P and some of the other suspected peptide neurotransmitters in nerves of the autonomic nervous system. The only proved neurotransmitters for this system are acetylcholine and norepinephrine, whose roles have been well established for many years—so well established, in fact, that no one really expected any additional neurotransmitters to turn up. Even in the autonomic nervous system substance P appears to transmit sensory information from the periphery to the gut.

All in all, research on substance P and the many other peptides found in nerve cells is enlarging our understanding of some of the functions of the nervous system.—JEAN L. MARX

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