Neurobiology: Researchers High on Endogenous Opiates

The hottest growth industry in the neurosciences is the study of a group of substances called endorphins—for endogenous morphinelike substances. These endorphins occur naturally in the brain or the pituitary gland, which is closely associated with brain, and mimic the effects of opiates such as heroin and morphine. Neuroscientists think that the substances may be a new class of neurotransmitters (chemicals that transmit impulses between neurons) or at least substances that modify neuronal activity. In either case the endorphins could have profound effects on behavior. And learning how they function should contribute significantly to understanding how the brain works, especially in regard to the mechanisms of pain perception. Suggestions that certain endorphins may be involved in producing the aberrant behavior called schizophrenia have in no way diminished the attractiveness of the research.

The discovery of the endorphins grew out of the identification in brain and other tissues of opiate receptors, that is, cell components with which opiates must combine in order to produce their characteristic biological effects of analgesia and euphoria (*Science*, 29 August 1975, p. 708). Since opiates do not occur naturally in animals, neurobiologists hypothesized that, if the receptor had a physiological role, there should be an endogenous material—a neurotransmitter or neuromodulator—that binds to it.

Investigators may have succeeded beyond their wildest dreams in their attempts to find the material. At present, they have detected at least seven such substances. These include a family of five structurally related peptides that are present in the brain, the pituitary, or both; a pituitary peptide of unknown structure that is apparently unrelated to the others; and a low-molecular-weight peptide in blood. All of these mimic at least some of the actions of opiates in the commonly used assays, but there are differences of opinion about which are the most important physiologically.

The enkephalins, which are small peptides, were the first endorphins to be characterized. John Hughes and Hans Kosterlitz of the University of Aberdeen, Scotland, isolated the material from pig brains and determined that it contained two pentapeptides with similar amino acid sequences. The sequences are H-Tyr-Gly-Gly-Phe-Met-OH (methionine-enkephalin) and H-Tyr-Gly-Gly-Phe-Leu-OH (leucine-enkephalin). Shortly afterward, Solomon Snyder and Rabi Simantov, at Johns Hopkins University Medical School, determined the structures of enkephalins isolated from beef brains and found the same sequences.

The small size of the enkephalins meant that they could be easily synthesized. Neurobiologists found that fact gratifying for two reasons. There would be plenty of material available for study. And it might be possible to produce an analgesic drug that is as effective as the opiates but lacks their addictive potential. The latter goal has been one of the major stimuli for the investigations, but recent results indicate that it is not going to be easy to achieve because the enkephalins appear to be addictive.

The Enkephalins and β-Lipotropin

The second striking aspect of the structure of Met-enkephalin is that the same sequence of five amino acids appears in a polypeptide called β-lipotropin. Choh Hao Li of the University of California at San Francisco isolated this material from pituitary glands and determined the sequence of its amino acids in 1964. Since then, it has more or less been a hormone in search of a function. The sequence of amino acid residues 41 to 58 in β-lipotropin is identical to that of β-melanocyte–stimulating hormone (β-MSH), and there were suggestions that the larger polypeptide is the precursor of this hormone. The function of β-MSH in mammals is still uncertain, although there is evidence that it, too, may act on the brain to affect learning or behavior (*Science*, 24 October 1975, p. 367). In addition, β-lipotropin promotes the breakdown of fats, but many other hormones do this; the biological significance of the polypeptide remained unclear.

The recognition by Hughes and Kosterlitz that β-lipotropin contains the sequence of Met-enkephalin touched off a series of investigations to determine whether the polypeptide has opiate activity. Brian Cox and Avram Goldstein of Stanford University Medical School and the Addiction Research Foundation in Palo Alto, and Li found that the whole molecule was virtually devoid of activity in two assays but that a fragment encompassing amino acids 61 to 91 [β-lipotropin (61–91)] is active. The sequence 61 to 65 is that of Met-enkephalin.

Meanwhile, Roger Guillemin and his colleagues at the Salk Institute observed that extracts of material comprising both pituitary and hypothalamic tissue (the hypothalamus is the part of the brain to which the pituitary is attached) contain a number of peptides with opiate activity. They identified the peptides as β-lipotropin (61–76) or α-endorphin, β-lipotropin (61–91) or β-endorphin, and β-lipotropin (61–77) or γ-endorphin.

Much of the current effort is aimed at determining the physiological role of these assorted peptides. One school of thought holds that the enkephalins are physiologically significant, possibly as neurotransmitters, and that the larger peptides may serve as precursors, at least of Met-enkephalin. (There are now no candidates for a precursor for Leu-enkephalin.) Many physiologically active small peptides are produced by the cleavage of larger ones, and there are plenty of enzymes in the brain and elsewhere that split peptides.

The other school of thought holds that the larger endorphins, principally β-endorphin, are physiologically active and that the enkephalins are merely degradation products of the former. The β-lipotropin molecule could be the original precursor of any of these smaller peptides. In that event, the mystery of its physiological function would be solved. The Salk investigators showed that when β-lipotropin is incubated with extracts of rat brain, peptide fragments with opiate activity are generated. Although the structure of the fragments has not yet been determined, this finding supports the hypothesis that β-lipotropin is a precursor of endorphins.

One of the problems with the idea that the enkephalins play an important role in the brain is their relative lack of effect in producing analgesia when injected into the brains of rats or other experimental animals. Several investigators have found that very large doses of the enkephalins are required to produce transient analgesia that lasts less than 5 minutes. In the same assays, much smaller doses of morphine produce more distinct effects that last up to 4 hours. In vitro assays, however, indicate that the enkephalins have more profound opiate effects, and they do bind to receptors about one-half as avidly as morphine. In all pharmacological assays, the effects of the enkephalins are blocked by naloxone or other specific antagonists of opiate action. These agents are known to bind

to the receptors and thus prevent binding of opiates but the antagonists have none of the characteristic behavioral and biological effects of opiates. Consequently, blockage of the effects of a drug by naloxone is one of the criteria for establishing that binding to opiate receptors is necessary for the drug's action.

Researchers who favor the hypothesis that the enkephalins are physiologically important think that the apparent lack of analgesic activity does not necessarily disprove the hypothesis. Evidence indicates that enzymes in brain break down the pentapeptides very rapidly; this could account for the low activity. Some investigators have suggested that the enkephalins may be true neurotransmitters. Such breakdown is compatible with this putative role; a mechanism is required for rapid removal of neurotransmitters from synapses (the region of contact between two neurons through which neurotransmitters carry nerve impulses) to prevent the continuous firing, or inhibition of firing, of the target neurons.

The evidence that opiate receptors are actually receptors for a neurotransmitter, whether this proves to be an enkephalin, one of the larger endorphins, or some as yet unidentified substance, includes demonstrations by a number of investigators that the receptors are not randomly distributed in brain. Rather they are concentrated in discrete regions, some of which are associated with pain perception or the generation of moods. For example, Snyder, with Candace Pert, who is now at the National Institute of Mental Health (NIMH), and Michael Kuhar, also at Johns Hopkins, used a radioautographic technique to show this nonrandom type of distribution. Snyder and Pert also found that the receptors are located in the subcellular fraction of brain cells that contains the synaptic membranes. The synapse is the logical site for receptors for a neurotransmitter.

Moreover, the Johns Hopkins group found that the distribution of the enkephalins parallels that of the receptors. Goldstein points out that the distribution of the enkephalins might merely reflect their capacity to bind to opiate receptors. Recently, however, Tomas Hökfelt of the Karolinska Institute in Stockholm used antibodies to the enkephalins to show that they are located in nerve terminals in the same regions of the brain where the receptors are found. Since neurotransmitters are stored in the terminals until they are released when the nerve fires, this would support the hypothesis that the enkephalins are neurotransmitters.

One reason that other investigators, including Goldstein and Guillemin, think that the larger endorphins, β-endorphin in particular, and not the enkephalins are the physiologically significant agents is that β-endorphin is much more potent than the pentapeptides in producing analgesia when injected directly into the brain. This is probably due to a less rapid breakdown of β-endorphin in vivo. In addition, some, but not all, researchers have found that β-endorphin has an even higher affinity for opiate receptors than does morphine or Met-enkephalin.

The brain does appear to contain large peptides with opiate activity in addition to the enkephalins. In fact, Goldstein says that when care is taken to minimize protein degradation during preparation and analysis, approximately two-thirds of the opiate activity of both the brain and the pituitary is associated with polypeptides. Goldstein and his colleagues have not yet completely characterized these larger materials, however.

The α- and γ-endorphins are generally much less active than β-endorphin in both in vitro and in vivo assays. A number of investigators have suggested that β-endorphin is the active agent and that the smaller peptides, including the enkephalins, are breakdown products, possibly formed during isolation.

Synthetic Analogs of Met-enkephalin

On the other hand, Pert says that when synthetic pentapeptide analogs of Met-enkephalin that resist degradation are injected into rat brain, they cause long-lasting analgesia almost as effectively as morphine. She did receptor-binding assays for a series of synthetic pentapeptides prepared by Jaw-Kang Chang and Bosco Fong of the Beckman Instrument Company in Palo Alto, California. A peptide in which the D-isomer of alanine was substituted for the first glycine of Met-enkephalin bound to the receptors almost as tightly as the parent compound. And Agu Pert, also of NIMH, found that low concentrations of the analog produced analgesia that lasted 3 to 4 hours—the same as morphine-induced analgesia—although the effects of the alkaloid were somewhat more intense than those of the peptide. The Perts think that these results suggest that the analgesic effects of the enkephalins, although slight, are real and not artifacts.

The investigators hypothesize that the introduction of D-alanine into the molecule makes it resistant to breakdown by enzymes. The D-isomers of amino acids are rarely found in nature, and the enzymes that split peptides are specific for the L-isomers.

One of the many questions regarding the endorphins that remains to be answered is what is the source of the materials in the brain. If β-lipotropin, synthesized by the pituitary, is the precursor of the materials, a mechanism for moving either the precursor or the endorphins themselves from the blood into the brain would be required. According to Horace Loh and Eddie Wei, of the University of California at San Francisco, and Li, β-endorphin is at least 20 times more potent than morphine in producing analgesia when injected into the brain, and, in addition, is still three times more potent when injected intravenously.

This latter finding is somewhat surprising since peptides of the size of β-endorphin do not normally pass from blood into the brain. Floyd Bloom of the Salk Institute, who is collaborating with Guillemin in assessing the behavioral properties of the endorphins, says that they did not observe any analgesic effects of the peptide after intravenous injection. This point is an important one and more work will be required to resolve the discrepancy.

Many investigators think that the precursor for the brain endorphins may be synthesized in the brain. Goldstein and his colleagues have shown that the concentration of endorphins in rat brains does not decline after removal of the animals' pituitary glands. If the pituitary materials do not enter the brain, they would exert their effects only on those peripheral tissues that are innervated by nerves with opiate receptors. There are several precedents for hormones and other agents that act both peripherally and in the central nervous system.

A major reason for the interest in the endorphins has been the possibility that they, or their analogs, might turn out to be nonaddictive pain-killers that could replace opiates in medical practice. So far, the results have not been encouraging in this regard. Addiction is characterized by tolerance and dependence. Tolerance means that the person who takes the drug requires progressively more of it to achieve the same effect, whether this be degree of pain-killing or euphoria. Dependence means that the person who stops taking the drug will suffer symptoms that are the reverse of the effects evoked by the agent. Several investigators now have evidence that the enkephalins and β-endorphin produce both tolerance and dependence in experimental animals. When the antagonist naloxone is given to the animals to block the effects of the endorphins, the withdrawal symptoms they experience are quite similar to those of morphine or heroin withdrawal. Agu Pert has obtained similar results with the D-alanine analog of Met-enkephalin. He also says that it is with-

out effect when given intravenously because not enough of it gets into the brain. Nevertheless, it may be possible to chemically tinker with the molecule to produce a pain-killing analog that does enter the brain from the bloodstream and is not addictive.

Understanding the physiological mechanism underlying tolerance and dependence is another goal of investigators. Recently, Snyder and Simantov showed that the enkephalin content of rat brains increased when the animals were implanted with a pellet that slowly released morphine; the increase was 75 percent over control values by 5 days after the implantation. The concentrations returned to normal within 1 hour after administration of naloxone. The animals' withdrawal symptoms also subsided by 60 minutes after naloxone injection.

In accordance with a previous suggestion by Kosterlitz and Hughes, the investigators hypothesize that the exogenous opiate morphine inhibits, by a feedback mechanism, the firing of neurons that would normally release enkephalins as neurotransmitters. If the peptides are not released they should accumulate in the nerve terminals and their concentrations should increase. This abnormal state, in which there is only exogenous opiate present in the synapses to react with the target neurons, would correspond to tolerance, according to Snyder and Simantov. On abrupt cessation of opiate administration or when naloxone is injected into the animals, the receptors would be temporarily deprived of both enkephalin and morphine. Withdrawal symptoms would result until the enkephalin neurons began firing and releasing the peptides at a normal rate.

The validity of this hypothesis obviously depends on the validity of the assumption that the enkephalins actually are neurotransmitters. In support of the hypothesis, the investigators point out that when the neurons that release the known neurotransmitters acetylcholine and dopamine stop firing, the concentrations of the agents do increase.

The endorphins may do more than modulate responses to pain. Bloom and Guillemin, with David Segal of the University of California at San Diego, have found that α-, β-, and γ-endorphins produce behavioral effects in addition to analgesia when they are injected into rat brains. In particular, β-endorphin induces a state that resembles catatonic schizophrenia. The animals experience extreme muscular rigidity, they cannot right themselves, and they stop moving spontaneously. While in this state the animals stop blinking and lose their normal lid and corneal reflexes. Their rectal temperatures drop by more than 2°C. Naloxone completely reverses these effects within a few seconds.

Several investigators have pointed out that high doses of morphine cause a similar state in rats but Bloom says there are significant differences between rats given β-endorphin and those given comparable or larger doses of morphine. The morphine-treated animals are not rigid, they retain spontaneous movements, and their temperatures drop less. The behavioral effects of the α- and γ-endorphins are less dramatic than those of β-endorphin. Bloom and Guillemin suggest that the endorphins are involved in maintaining normal behavior and that derangements in their activities could lead to the symptoms of mental illness.

Endorphins and Schizophrenia

The Swedish investigators, Lars Terenius, A. Wahlstrom, and L. M. Gunne of Uppsala University are beginning experiments to determine whether there are alterations in the endorphin content of spinal fluid in patients with schizophrenia. Their early results indicate that it may be elevated in individuals with this mental disorder.

Wahlstrom and Gunne have preliminary evidence that naloxone may improve the condition of schizophrenic patients by decreasing their hallucinations and improving the clarity of their thought processes. However, at this time they have studied only a very small number of patients and the study was not controlled. Thus, their results require further substantiation. Several investigators, including those at the Salk Institute, are now planning controlled double-blind trials of naloxone therapy for schizophrenia and other psychotic states.

Although most of the attention has thus far focused on peptides that are structurally related to the enkephalins, these are not the only endogenous materials that have opiate activity. The Perts and John Tallman of NIMH have identified such a substance in both human and rat blood. The material is apparently a peptide with a molecular weight of about 600 but it differs chemically and biologically from the enkephalins. They have named it anodynin (from anodyne, a drug that calms and allays pain). Anodynin is degraded by brain much more slowly than the enkephalins, and it produces analgesia lasting for at least 1 hour—compared to a few minutes for enkephalin-induced analgesia—when small quantities are injected into rat brains. Since naloxone blocks the analgesic effects of the material, it apparently interacts with opiate receptors to produce its effects.

Because removal of the pituitary glands of rats results in almost complete disappearance of anodynin from blood, the Perts conclude that either the material is stored in and released from this gland or the pituitary produces some substance required for the maintenance of anodynin.

The function of anodynin is as yet unknown, but the investigators hypothesize that it may somehow be involved in the activity of opiate receptors in nerves of peripheral tissues. The NIMH investigators are now studying the effects of stress, pain, sleep, and other physiological states on the concentration of anodynin in blood.

According to Goldstein, the pituitary and the brain produce other polypeptides with opiate activity in addition to β-endorphin. One of these materials is more potent than β-endorphin in certain assays, it has more basic amino acid residues than β-endorphin, and its activity is destroyed by trypsin, an enzyme that splits proteins only at certain sites, whereas that of β-endorphin is not. However, the activity of both types of endorphins is destroyed by cyanogen bromide, a reagent that chemically alters methionine residues. This indicates that both materials require a critical methionine residue to produce their effects. β-Endorphin is known to contain the sequence of Met-enkephalin, which is required for its activity, and the same may be true for the trypsin-sensitive material.

Another polypeptide with opiate activity detected by the Goldstein group is not affected by cyanogen bromide. The researchers speculate that this one may contain Leu-enkephalin but this speculation will not be confirmed until its amino acid sequence is known. The amino acid sequences of these materials are now being determined in Goldstein's laboratory; these data should clarify the relationships, if any, between these materials and the other endogenous opiates, including anodynin.

Much additional work will be required to sort out the roles of all these endorphins. Many questions remain: Which endorphins are physiologically active and by what mechanisms do they produce their behavioral effects? What are the relationships between the endorphins? Where are they synthesized and by what enzymes? Are there specific enzymes for converting β-lipotropin, for example, to β-endorphin or the enkephalins? More and more investigators are beginning to do research on the endorphins. If progress is as rapid as it has been in the year since the enkephalins were discovered, the answers should be forthcoming.—JEAN L. MARX