Learning and Behavior (II): The Hypothalamic Peptides

Evidence accumulating for the last few years indicates that a number of peptide hormones directly influence animal—including human—behavior in addition to producing their better known endocrine effects. Some of these agents are synthesized or stored in the pituitary gland (*Science*, 24 October); others, the topic of this article, are secreted by a portion of the brain called the hypothalamus. They are among the ten or more hypothalamic factors or hormones that regulate secretion of hormones by the pituitary gland. There are indications that certain of these agents may be clinically useful for treatment of such conditions as Parkinson's disease and depression.

The behavioral work has been done with the hypothalamic control factors of known structure. These are all peptides, consisting of 3 to 14 amino acids, and have been synthesized. Thus, the investigators can work with substances of known chemical composition.

Most of the hypothalamic agents stimulate the release of pituitary hormones, but there are exceptions. One of these is the factor that inhibits the release of melanocyte-stimulating hormone (MSH); this factor is abbreviated MIF. In 1967, Abba J. Kastin of Tulane Medical School and the Veterans Administration Hospital in New Orleans suggested that MIF might be of value in treating Parkinson's disease. He based his suggestion on observations in his laboratory and others that MSH aggravates the disease in some patients and that certain drugs that may produce symptoms like those of Parkinson's disease also stimulate release of MSH. Consequently, Kastin hypothesized that MIF might improve the condition of patients with Parkinsonism by inhibiting MSH release. Experiments now indicate that MIF does help these patients, although possibly by an effect on brain that is independent of MSH.

Nicholas Plotnikoff of Abbott Laboratories and Kastin acquired further evidence for a potential role of MIF in therapy for Parkinsonism when they determined the activity of the tripeptide called MIF-1 in a number of tests that are used to screen for compounds with the capacity to alleviate the characteristic symptoms of the disease. These symptoms are tremors, rigidity of the body, and abnormal lack of movement. The investigators found that MIF-I potentiates to a high degree the behavioral effects of dihydroxyphenylalanine (dopa) in mice. This means that the tripeptide further increases the increased irritability, reactivity, jumping, squeaking, and fighting caused by administration of dopa. (Dopa, the drug now most often used for therapy of Parkinsonism, is the precursor of the neurotransmitter dopamine. The absence of dopamine, brought about by degeneration of certain neurons, in the caudate nucleus of the brain is thought to produce the symptoms of the disease.) The pituitary peptide also alleviates the tremors induced in mice by the drug oxotremorine. In contrast to what Kastin originally hypothesized, however, he showed that the effects of MIF-I were independent of its action on MSH release. They occurred even in mice without pituitary glands.

In a preliminary clinical trial, André Barbeau of the Clinical Research Institute of Montreal and Kastin found that a single injection of 30 milligrams of MIF-I did improve the condition of patients suffering from Parkinson's disease by relieving their tremors and rigidity. When they gave the peptide to patients already taking dopa, it did not further potentiate the effects of dopa but it did appear to reduce the abnormal involuntary movements that are a side effect of dopa treatment. Attempts by other investigators to confirm these results met with mixed results; some confirmed them but others did not.

Recent Studies on MIF-I and Parkinsonism

These problems may have been due to differences in dosage and the route of administration of the peptide. In a recent study, Barbeau increased the dose of MIF-I to 200 milligrams given in a single intravenous injection. He found that the peptide significantly alleviated all the symptoms of Parkinson's disease in patients not receiving dopa. In patients who were taking dopa, MIF-I potentiated its beneficial effects but not its side effects. Barbeau, who is one of the pioneers of dopa therapy, said that the improvement was greater than any he had ever seen with dopa alone. In fact, five of the six individuals tested attained normal scores on tests of motor activity. Barbeau says that this never occurs with just dopa. There were no significant side effects associated with the MIF-I, but further studies of the long-term toxicity of this agent are required.

A drug that can be given by mouth would be much more convenient than one that must be injected. In a double-blind study (neither the investigators nor patients knew who were getting the MIF-I and who the control material) lasting 4 months, Barbeau and Kastin found that MIF-I given orally did slightly improve the condition of the patients but only during part of the study; moreover, the improvement was not statistically significant. Barbeau thinks that the material is less active when given orally because it is not absorbed well or because it is broken down quickly in the blood. He is now looking for analogs of MIF-I that are active when given orally.

One important note of caution must be sounded about studies involving MIF-I. A number of investigators question whether this tripeptide (prolyl-leucylglycinamide) is the naturally occurring inhibitor of MSH release. Roderich Walter, now at the University of Chicago, and S. Taleisnik and Maria Celis of the Instituto de Investigacion Medica in Cordoba, Argentina, have evidence that it is. They showed that in vitro preparations of the median eminence (part of the hypothalamus) contain an enzyme that hydrolyzes peptides and catalyzes the formation of an MIF. The peptide hormone oxytocin and a number of other peptides resembling oxytocin in structure can serve as substrates for the enzyme. All the structures have a common amino acid sequence—the prolyl-leucylglycinamide sequence; peptides lacking it do not produce the inhibitor of MSH release when incubated with the enzyme.

Kastin and his colleagues have identified this tripeptide in hypothalamic tissues. But several investigators have been unable to detect an inhibitory action of MIF-I on MSH release in their assays. Despite these doubts, MIF-I does appear to have behavioral effects and a potential therapeutic value in the treatment of Parkinsonism.

The mechanism by which MIF-I produces its effects is as yet unclear. At least some of its actions are compatible with MIF-I having an effect on the kind of neural receptors that respond to the neurotransmitter dopamine. These include the potentiation of the behavioral effects of dopa, a precursor of dopamine, and also, according to Barbeau, of those of apomorphine, a drug that acts by specifically combining with dopamine receptors. Barbeau does not think that MIF-I itself acts as a neurotransmitter but rather that it affects the membrane bearing the receptors in an as yet unknown way. Investigators are now trying to determine whether MIF-I alters the concentrations of neurotransmitters in the brain. There is preliminary evidence that it increases concentrations of dopamine and norepinephrine (another neurotransmitter structurally related to dopamine) in some parts of the brain. More work is needed to confirm this; other investigators have found no such effects.

Thyrotropin releasing hormone (TRH) is another hypothalamic peptide that is attracting attention because of its behavioral effects. This tripeptide (pyroglutamyl-histidylprolinamide) stimulates the release from the pituitary gland of thyrotropin, a

hormone that in turn stimulates the release of thyroxine from the thyroid gland. TRH is active in the dopa potentiation test in mice, according to Plotnikoff and Arthur Prange of the University of North Carolina in Chapel Hill, although it is not as active as MIF-I; it does not reverse the effects of oxotremorine, however. The effects of TRH occur in hypophysectomized and thyroidectomized mice and are thus independent of these endocrine glands.

Although Prange says that it is difficult to demonstrate behavioral effects of TRH alone, its capacity to counteract the effects of a number of drugs that depress the central nervous system constitutes further evidence that the tripeptide does influence brain activities. Prange and George Breese, also at Chapel Hill, showed that TRH antagonizes the sedative action of a number of barbiturates and of alcohol. The investigators found that animals given both the peptide and one of the depressant drugs have shorter sleeping times than those given depressants alone. Certain derivatives of TRH have effects similar to those of the parent molecule but none of the three amino acids that compose the tripeptide do. Thus the behavioral activities of TRH appear to depend on a specific structure.

The North Carolina investigators have suggested that TRH may be clinically useful in treating a number of disorders. One possibility is that the peptide may be an antidote for barbiturate overdoses because of its capacity to counteract the sedative effects of the drugs. Another potential use of TRH is as an adjunct to phenobarbital therapy for epilepsy. The sedation induced by phenobarbital is a drawback to this therapy. TRH would not be of much value in this application if it also antagonized the anticonvulsant activity of the drug; however, Prange finds that it actually potentiates the anticonvulsant properties of phenobarbital in mice.

The dopa potentiation test is relevant for depression as well as for Parkinson's disease. Prange, in collaboration with Morris Lipton of the University of North Carolina and Ian Wilson of the North Carolina Mental Health Department in Raleigh, have examined the effects of TRH in humans. They find that patients suffering from depression experienced prompt but brief relief of their symptoms after a single injection of the peptide. In normal women it produced feelings of relaxation, mild euphoria, and a sense of increased energy. The investigators hypothesize that TRH produces these effects directly and not by causing stimulation of the thyroid gland because there was no correlation between the euphoria and increased concentrations of thyrotropin or thyroid hormone in the subjects.

Prange thinks that the experiments with TRH and depression may be more important as clues to the etiology and diagnosis of this condition than as guides to a new therapy for it. He points out that a number of investigators have found that TRH stimulates thyrotropin release less effectively in depressed patients than in normal subjects. This could be explained if the patients had overactive thyroid glands. By a feedback mechanism, high levels of thyroid hormones in these patients would depress thyrotropin secretion by the pituitary even in the presence of TRH. But Prange says that, by all criteria, the depressed patients had normal thyroid activity. Consequently, the investigators think that the abnormal pituitary response of depressed patients may indicate abnormal hypothalamic activity.

Somatostatin and Depression

The activity of yet another hypothalamic peptide—somatotropin release inhibiting factor (SRIF or somatostatin)—may also be involved in the explanation of the lowered response of the pituitary to TRH in depressed persons. Somatostatin is known to inhibit the release of both growth hormone (somatotropin) and thyrotropin by the pituitary gland. Some investigators have evidence that growth hormone release is impaired in depressed patients. Moreover, Prange, Plotnikoff, and Breese have found that somatostatin slightly, but significantly, prolongs barbiturate-induced sedation in the mouse. Thus the effects of somatostatin appear to oppose those of TRH and an excess activity of the former peptide may be involved in the etiology of depression.

In one study, Kastin and his colleagues also found that TRH alleviated the symptoms of depression, but they could not confirm this result in later experiments. Kastin points out that all clinical studies of drugs that affect the central nervous system are complicated by large placebo effects. This means that the control alone produces a large improvement—sometimes of more than 40 percent—in the patients receiving it. In order for results with the test drug to be significant, it must produce even greater improvements in the patients' condition.

Sexual activity is another form of behavior that appears to be directly influenced by a hypothalamic hormone, luteinizing hormone releasing factor (LHRH). The control of sexual cycles in the female is very complex and involves the interaction of several hormones or factors, including hypothalamic factors and pituitary and ovarian hormones. In animals other than humans, sexual receptivity in the female and mating occur only around the time of ovulation. Release of LHRH by the hypothalamus not only triggers the events leading to ovulation but, according to Robert Moss and S. M. McCann of Southwestern Medical School and to Donald Pfaff of Rockefeller University, the peptide also plays a direct role in producing sexual receptivity in the female.

Moss and McCann showed that the peptide would induce mating behavior in ovariectomized rats provided that the animals were first primed by injection of a small quantity of estrogen. Estrogen and progesterone together, or large amounts of estrogen alone, will induce mating behavior in ovariectomized rats. In their experiment with LHRH, however, Moss and McCann used a priming dose of estrogen that by itself was too low to produce the behavior. Pfaff obtained similar results and, in addition, showed that the behavioral change did not depend on the presence of the pituitary gland because it occurred in hypophysectomized rats. Moss and McCann also found that the peptide evokes mating behavior in castrated male rats. This, too, requires a priming dose of testosterone (the male sex hormone) that by itself does not produce the behavior.

The investigators conclude that LHRH functions not only to trigger the discharge of luteinizing hormone that evokes ovulation but also acts in conjunction with sex hormones to bring about mating behavior. The result would be a reinforcement of the synchronization between mating and ovulation. Moss points out that the brain centers regulating mating behavior are located near those producing LHRH; in fact, they overlap. The proposed role of LHRH is consistent with this anatomy.

The mechanism by which LHRH affects brain activity is unknown. Moss says that it may act as a neurotransmitter; however, there is a latent period of about 2 hours between LHRH injection and the development of the behavior, which lasts for about 6 hours. He thinks that an effect on protein synthesis would be a more likely explanation of these findings.

More work needs to be done to determine how the hypothalamic peptides produce their behavioral effects and to locate the target neurons. Investigators especially want to know whether the agents function as true neurotransmitters or whether they modulate nerve functions in some other way. Iontophoretic experiments may help solve these problems. Iontophoresis involves the use of tiny, multibarreled pipettes that can both inject a test substance into a single neuron and measure whatever electrical changes may occur. Such experiments are currently under way in several laboratories. When the structures of more hypothalamic factors become known and materials become available in sufficient quantities for use in experiments, it will be interesting to see whether they, too, will have behavioral effects.—JEAN L. MARX