

Peptide Neuroregulators: The Opioid System as a Model

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Aaron Lerner's work provides a stunning set of examples of substances that help to transmit information in the brain and body. His characterization of alpha-MSH and melatonin and his sparking of interest in the further discovery of previously unknown substances have been of inestimable value for the field of neurobiology. Efforts such as those that Lerner undertook so successfully in the field of investigative dermatology now constitute a major research thrust in the field of behavioral neurochemistry and are directly related to advances in psychiatry and neurology.

This review considers aspects of research on the neuropeptides, with particular attention to the endogenous opioid (morphine-like) peptides that are active on neural tissue. Neuropeptide research can be categorized broadly as efforts to discover and characterize new families and classes of active agents, investigations of their genetic and molecular processing, and studies of their relationships to behavior in animals and human beings. This review selectively considers some key research questions and strategies that arise from such research.

INTRODUCTION

Neuroregulators are substances that help to control chemical transmission between and among nerve cells. They include both neurotransmitters, which are released from one nerve cell to act on an adjacent one, and neuromodulators, which affect transmission in a variety of other ways. Many classes of compounds fall into these categories, including not only such classical agents as acetylcholine and the biogenic amines but also the neuropeptides. Lerner's discovery of alpha-MSH [1] and melatonin [2], members of quite different chemical families, were two important steps in the characterization of previously unrecognized neuroregulators. Both are fascinating substances that have proven to be of great interest to those dedicated to understanding how the brain works, and yet they are only two of well over one hundred neuroregulators that researchers have already identified [3].

For the past decade, neuropeptides have been especially crucial to the rapid advances that have been occurring in basic and clinical neuroscience [4-6]. From studies of neuropeptides have come new concepts and approaches for studying both animal and human behavior and mental and neurological disorders [7]. For this review, we have used the opioid peptides to illustrate some of the issues involved in the study of the neuropeptides. We will consider current concepts of neuroregulation, methods that have been used to find previously unrecognized neuropeptides, interrelationships among the opioid neuropeptide systems, and clinical aspects of those systems.

THE CONCEPT OF NEUROREGULATION

Now a cornerstone of neurobiology, the hypothesis that nerve cells communicate via chemicals was first proposed only at the turn of this century [8]. Most of the first tests of this hypothesis were done on peripheral nerve systems that use acetylcholine as the neurotransmitter, and models of chemical neurotransmission are still strongly influenced by the results of work on such peripheral systems. Neurotransmitters may be defined as those compounds that relay an electrically promulgated signal from one nerve cell to another that is closely adjacent to it via a structure—the synapse—designed for that purpose; they are made and released presynaptically and act through specific post-synaptic receptors to exert their effects. Typically, neurotransmitters are viewed as rapidly turning nerve cells on and off. Gamma-aminobutyric acid (GABA) and acetylcholine are prime examples of such substances.

More recent studies of neuroregulation, especially that mediated by neuropeptides and other neuroactive substances in the brain, have added greatly to the number of known ways in which substances can affect neuronal intercommunication. Discovery of such additional mechanisms has demanded the introduction of the term neuromodulation to round out the description of neuroregulatory processes [9–14]. Although remarkably little still is known about their specific functions, neuromodulators may act on many cells or may fine-tune neurotransmitter action. They must act to permit specific alteration of nerve cell activity, rather than through a general increase or decrease of neuronal metabolism. Neuromodulators can probably be subdivided into two broad classes: synaptic neuromodulators, which act in synapses by altering the dynamics of a neurotransmitter, and hormonal neuromodulators, which can act at a distance, and in several sites, possibly through specific receptors.

In recent years a further elaboration of the concepts of neuromodulation has arisen from recognition that multiple substances may be co-localized from a nerve cell and act by various mechanisms on adjacent or regional nerve cells [15,16]. Many questions remain unanswered about how these substances interact [17–19]. For example, it now is clear that a single peptide precursor can contain a number of neuroactive substances and that that precursor may be processed differentially in various areas of the brain [20–24]. The resulting possibilities for complex interactions among neuroregulators within cells and brain regions are astonishing and still incompletely understood.

THE SEARCH FOR PREVIOUSLY UNRECOGNIZED NEUROPEPTIDES

Until only a few years ago, the neuroactive substances widely acknowledged to be neuroregulators in the brain consisted of acetylcholine and the biogenic amines. As we noted earlier, many additional classes of substances now appear to be neuroregulators, and the neuropeptides, in particular, have added to the number and complexity of these identified systems. Various investigators have identified several dozen neuroactive peptides over the past few decades, and additional dozens almost certainly still await discovery.

The vigorous search for such substances is vital for continued progress in behavioral neurochemistry. Until the active substances are known, it is likely to be impossible to relate neuroregulators to the range of behaviors, both normal and disturbed, with which neuroscientists must be concerned. Often, key neuroregulator systems have crucial homeostatic relationships with other systems, and optimal studies of their physiology and pharmacology require a detailed knowledge of such relationships. The

development of this area is hampered not only by the complexity of the natural systems but also by the nomenclature and the lack of a "table of elements" with which to predict relationships. As new substances are found, they generate a whole series of investigations to determine their localization and functional relationships with other neuroregulators, and that information in turn helps to define key features of the entire system.

Neuroscientists have utilized six basic strategies to find endogenous neuropeptides. They have searched for the substance responsible for a specific biological activity or used a screening approach to identify a compound with a particular pharmacological activity. More recently, some have used antibodies to recognize substances that are structural analogs of another compound of interest and chemical assays with which to recognize an important characteristic of peptides. Still others are using molecular biological approaches, including the identification of precursor molecules, to detect previously unknown neuroactive substances and likely cleavage products. Each of these methods is providing valuable knowledge about important substances.

Lerner's own work beautifully illustrates the power of searching for an endogenous substance with a highly specific biological action. His interest in compounds that could stimulate melanocytes led to the discovery of alpha-MSH [1]. The finding contributed immediately to an understanding of skin physiology and later yielded insights into brain function. Initially thought to be associated only with the pituitary, alpha-MSH has been found more recently in other tissues, including the brain. Structural variants of MSH are known to exist, and such variants may alter its activity in different tissues or animal species. For example, in the rat, an acetylated form of MSH is present in the intermediate lobe of the pituitary but not in the brain [25]. Lerner's work also led to the discovery of the important nonpeptide neuroregulator melatonin, which has some biological actions that oppose those of alpha-MSH [2,26] and is important in biological rhythms and some forms of depression. Investigators continue to demonstrate the power of using specific biological activities to isolate neuropeptides, as recently demonstrated in the identification of corticotropin releasing factor [27].

Less specific biological activities also can be pivotal in attempts to isolate neuroactive peptides. For instance, a number of quite different neuroregulators may have readily identifiable effects on a particular test system of broad general interest [28]. Thus, searches continue for substances that change blood pressure or alter smooth muscle contractility.

The increasing facility with which researchers can identify specific receptors involved in neural activity makes feasible a useful variant of this approach. Using a receptor that typically is characterized by its interactions with one or more pharmacological agents, they search for endogenous ligands that act as specific agonists or antagonists to that receptor activity [29].

Another effective approach uses immunological similarity among related substances. For example, an antibody raised against a key portion of a neuropeptide may have substantial cross-reactivity with similar peptides. This approach has been used successfully to find new opioid-like peptides, using an antibody that recognizes the "universal" portion of known opioid peptides. Once detected by these methods, newly discovered peptides are characterized by other methods [30-33].

An especially powerful technique for identifying new neuropeptides employs chemical assays for specific derivatives. This approach has been pioneered in relation to the search for endogenous amidated peptides—many of which have considerable

biological activity. The development of a universal means of detecting and isolating such peptides was the key step in identifying a series of new substances that appear to have great importance [34]. This method does not permit researchers to predict *a priori* what physiological or pharmacological activity a peptide will have, so some neuroscientists have questioned whether the search for such peptides is justifiable. We reject that view. Each substance identified to date [35] has proven to be of great interest, and the technique is likely to remain invaluable for uncovering the existence of a number of peptides of biological interest that might otherwise have remained unknown for many years.

Molecular neurobiology has led to new methods of detecting possible new neuroregulators by "reading" the gene sequences for neuroactive substances [36–38]. Often, active substances, including much of their structure, have been predicted even before the native peptides were isolated [39]. Related to these techniques have been predictions of the structure of neuroactive peptides based on the structure of isolated proteins or larger peptides. The methods of gene cloning enable researchers to discover related neuropeptides much more rapidly once one member of a family has been isolated. Yet, even with these powerful techniques, peptide sequencing must be used to determine the actual structure of the peptide because of possible post-translational modifications of peptides. Functional groups may be modified in key ways to produce marked differences in biological activity [40–45].

THE OPIOID NEUROPEPTIDES AS A MODEL

The discovery of the opioid neuroregulators highlights many aspects of the search for endogenous neuropeptides [46–49]. Three gene families (Table 1) are known to yield opioid peptides in brain, an organ which contains over a dozen bioactive opioid peptides [50]. Opioid neuropeptides also are present in many other tissues, including the pituitary and adrenal [51]. Discovery of these fascinating substances has entailed the use of several of the methods described above. For instance, discovery of enkephalins was a result of the successful use of a pharmacological assay [46], discovery of beta-endorphin entailed prediction from the structure of a larger precursor [52], and metorphamide was predicted largely from data about gene and protein structure [53].

Interestingly, the various opioid neuropeptides have quite distinct neuronal effects, with many acting on receptors that appear to be specific for them. Even among a group of closely related neuropeptides, neuroregulatory effects may differ markedly. The dynorphin family of neuropeptides illustrates this point nicely: one form acts much more rapidly and in different ways than another [54–56]. As noted earlier, specific brain regions process the opioid peptide precursors in characteristic ways that may differ markedly from other brain regions; as a result, even for a single gene family, the ratio of active substances may vary greatly from region to region [21,57]. Learning how neuropeptides are processed and what controls those mechanisms, especially when neuroregulators are localized in the same neuron [58–64], are important directions for future research. Elucidating the regulation of these steps will depend in part on successful cloning of the genes for the enzymes involved in the processing.

Molecular biology may also provide essential tools for gaining a clearer understanding of the role of receptors in mediating neuroregulators such as the neuropeptides [54,65–68]. Studies of receptor development and analysis of receptor localization with *in situ* techniques should become possible [69]. These and other methods such as

TABLE 1
Endogenous Opioid Peptides

From Proenkephalin	From Prodynorphin	From Proopiomelanocortin
Met-enkephalin (5)	Leu-enkephalin (5)	α -Endorphin (16)
Leu-enkephalin (5)	Dynorphin A (1-8) (8)	γ -Endorphin (17)
Met-enkephalin-Arg-Phe (7)	β -Neoendorphin (9)	β -Endorphin (1-26) (26)
Met-enkephalin-Arg-Gly-Leu (8)	α -Neoendorphin (10)	β -Endorphin (1-27) (27)
Metorphamide (8)	Dynorphin B (13)	β -Endorphin (1-31) (31)
Bam 12 (12)	Dynorphin A (17)	
Bam 18 (18)	Dynorphin B 29 (29)	
Bam 22 (22)	Dynorphin 32 (32)	
Peptide E (25)		
Amidorphin (26)		
Peptide F (34)		

Note: The number of amino acids in each peptide is indicated in parentheses.

receptor labeling can enable researchers to obtain meaningful answers to a number of fascinating questions about receptors for particular neuroregulators. How many receptors are there? Is receptor function a result solely of protein sequence and structure, or can local environmental effects alter activity? In what ways are the receptors modified physiologically? Under what conditions does the number of receptor molecules change, and how important are such changes? What regulatory mechanisms control the formation and inactivation of receptors?

The opioid neuropeptides have been linked to a range of physiological processes and behaviors [70-77]. A brief list includes such diverse behaviors as eating, pain perception, stress-induced analgesia, learned helplessness, social behaviors, and some forms of learning; however, efforts to study their roles in such processes pose serious methodological difficulties [78]. The problems are illustrated in the next section, which outlines some of the difficulties of linking the opioid neuropeptides to mental disorders.

Successful behavioral investigations of the neuropeptides and other newly discovered neuroregulators must be based on a firm understanding of genetic and biochemical regulatory mechanisms involved [79]. The complex interactions among the opioid neuropeptides underscore the strong need to elucidate carefully the patterns of involvement among the components of the opioid systems and with other neuroregulator systems. Molecular neurobiology will markedly enhance the range of studies that can be conducted, once it becomes feasible to investigate the kinetics of the expression of the various steps involved in the formation and degradation of key elements of the system, including the neuroregulator, its receptors, and essential synthetic and degradative enzymes. Study of these complex interactions and of how behavior changes them may be especially important for the future development of behavioral neurochemistry.

ISSUES AND APPROACHES INVOLVED IN RELATING ENDOGENOUS OPIOIDS TO MENTAL ILLNESS

The opioid neuropeptides provide an excellent example of the opportunities and pitfalls of trying to link a neuroregulator to some form of mental illness [80]. Criteria for linking a neuroregulator to a mental disorder include the following: (1) the

neuroregulator must be formed endogenously and interact with receptor mechanisms or other aspects of neuronal communication; (2) a characteristic pattern of neuroregulator activity or action should exist in relation to the mental disorder; (3) alteration of the neuroregulator activity or of a balance between the activities of related neuroregulator systems should alter the mental disorder; (4) appropriate manipulation of the neuroregulator system, or those to which it is linked, should induce the disorder; and (5) appropriate restoration of neuroregulator activity or balance should ameliorate the mental disorder, unless the physiological process is irreversible. To date, no neuroregulator system has been shown to meet even most of these criteria for a specific mental disorder, but such concepts provide a framework for hypothesis-oriented research [81,82].

A possible link between endogenous opioid neuropeptides and mental disorders is appealing for several reasons. First, opiate drugs can have potent psychoactive effects, including mood alteration and even hallucinations, and they sometimes have been used in clinical treatment of major mental disorders [83]. Second, the presence of endogenous opioids in brain regions associated with mood and behavior suggests hypotheses regarding the possible involvement of endogenous opioids in mental disorders, especially since they appear to be co-localized with other neuroregulator systems also thought to be involved.

Hypotheses connecting neuroregulators with mental and addictive disorders typically posit that the latter result from a change in the former. Possible trouble sites include neuroregulatory synthesis, storage, release, and inactivation, as well as receptor mechanisms. For instance, a relative excess in the activity of an opioid neuropeptide system, or a change in the balance between it and some related system, could be offered as the etiological agent for some major mental disorder such as schizophrenia, depression, or infantile autism. Tests of such hypotheses for the opioid neuropeptides have employed two broad strategies [84]. One involves correlations between the presence or absence of a given mental illness and concentrations of an endogenous opioid neuropeptide of interest in samples of tissue, blood, hemodialysate, or cerebrospinal fluid. Another entails the administration of an opioid agonist or antagonist to patients with such major mental disorders as depression or schizophrenia, to assess the clinical effects of altering the neuropeptide system. Results from each of these strategies highlight both why they are so attractive to investigators and some of the serious pitfalls that they must try to avoid.

Any study of human psychopathology demands careful attention to the clinical status of the patients who volunteer for the research [85]. That seemingly straightforward statement has been a major source of error for a great many neurobiological studies of severe mental illness. The resources of a specialized mental health clinical research center typically are essential, for they enable investigators to recruit patient volunteers to a safe environment where they can discontinue medications if necessary and where standardized, reliable methods are available for diagnosing the disorders and identifying and rating the severity of relevant symptoms. Only a handful of such centers exist in this country, and the lack of qualified facilities and investigators has been one of the problems with which the field has dealt.

Many of the most prevalent mental disorders are probably syndromes that may be the end result of a number of dissimilar processes. Only some forms may be predominantly biological, with others having major psychological or social contributing factors, and even within the mainly biological subtypes, only a fraction may result

from disturbance of a single neuroregulatory system. Unfortunately, researchers often must treat these heterogeneous subtypes as a unitary entity because no valid and reliable methods yet exist for identifying the subgroups. In the following descriptions about opioid neuropeptides and schizophrenia and depression, no distinction is made about subtypes for either of these disorders; yet, if only a small portion of the total population has a disorder in the specific neuropeptide of interest, the abnormality in that subgroup may be hidden in normal results for the vast majority of subjects.

Solutions to the conceptual problem of differentiating subtypes have not come easily. Efforts to distinguish them clinically have not been completely satisfactory, although they have been helpful at times. Similarly, biological tests or measures using urine, blood, skin, and cerebrospinal fluid have not yet yielded the valid and reliable markers needed—and psychiatric researchers naturally cannot obtain tissue samples of brain in living patients. It seems likely that some of the newer methodologies for *in vivo* studies of brain function and structure, including positron emission tomograph (PET), computer-analyzed EEG, magnetic resonance spectroscopy and imaging, and X-ray computed axial tomography, will provide new dimensions to clinical syndromes that may be useful for subtyping.

The Measurement of Opioid Peptides in Tissue, Blood, and CSF

Most studies of changes in the concentrations of opioid-like substances in patients with mental disorders have used cerebrospinal fluid or plasma [86–90]. For example, investigators have evaluated profiles of endorphin-like immunoreactivity in schizophrenic patients, with contradictory results [86,91–100]. Several studies that used cerebrospinal fluid reported interesting changes in the concentrations of certain peaks and fractions; yet, agreement among the studies is relatively low with respect both to the concentrations and to the characteristics of the opioid-like substances found.

Such contradictory results emphasize the need for more basic research to identify endogenous opioid peptides in the cerebrospinal fluid, plasma, and tissue. Many researchers have used radio receptor assays that have relatively poor specificity, so that apparent differences between patients and controls may arise from factors completely unrelated to the neuropeptide of interest. Radioimmunoassays that currently are available for measuring opioids in a tissue, cerebrospinal fluid, and plasma also pose problems. Often, radioimmunoassays are performed on crude or semicrude extracts, with little effort to characterize the substance for which measurement is being sought. When our group [101] attempted to measure opioid peptides in cerebrospinal fluid, we detected measurable displacement in our radioimmunoassays but analysis of that immunoreactivity showed that it did not behave identically with a standard curve for the opioid peptides under study.

Our finding raises questions about what investigators are measuring in cerebrospinal fluid. Salt, lipid, or other compounds may interfere with the antibody and antigen complex, a similar peptide with a different affinity for the antibody may interfere, or a biologically inactive metabolite of the peptide under study may compete for the antibody. Most publications that purport to measure neuropeptides in cerebrospinal fluid using radioimmunoassays have not considered these problems. Until the validity of an assay method for a specific peptide is confirmed, its use to study the role of that peptide in a mental disorder is premature and likely to yield unreliable results.

For opioid neuropeptides, as with other neuropeptides, researchers who are interested in clinical applications will need to ensure that the peptides under study have

been isolated from the fluid under study and characterized definitively and that parameters of valid assay are established before the studies begin. It is unlikely that measurements of the opioid neuropeptides as a class will be useful or feasible; rather, studies are needed that quantitate specific substances or groups of substances from the three known gene families. Measures of the substances as a group could provide a general assessment of whether opioid peptides are "increased" or "decreased," but a negative finding might mask an important change in the ratio of two or more key substances that did not result in an overall change in total concentration.

Despite their brief history, opioid neuropeptides already are associated with some controversies in psychiatric research. For example, one finding stemmed from an uncontrolled trial that suggested that hemodialysis might decrease symptoms in patients with schizophrenia [102]. An analysis of the changes in the dialysates over time led to a report that a leucine form of beta-endorphin decreased as the patients improved, leading promptly to the hypothesis that *leu*-endorphin was causing symptoms of schizophrenia [103]. The issues were several: (1) does hemodialysis improve schizophrenia; (2) does dialysis result in removal of *leu*-endorphin; and (3) is the latter compound involved in the symptoms of schizophrenia? The answer to each of the questions to date has been a clear-cut "no." Other researchers were unable to confirm the effectiveness of dialysis in schizophrenia [104,105]. Furthermore, no investigator has been able to confirm the finding of a leucine form of beta-endorphin in any species; indeed, it is not found in any of the three gene families of opioid neuropeptides that have been isolated and characterized [106]. Also, a double-blind study showed that the total concentration of beta-endorphin-like substances is not elevated in plasma of patients with schizophrenias and that the concentrations of such substances are not consistent with the levels of the endorphin-like material reported earlier in the dialysates [107]. Although clinically relevant changes in the pattern of opioid neuropeptide concentrations in the plasma of schizophrenic patients may ultimately be identified, existing results strongly suggest that the original report was not valid.

Studies of opioid peptides in plasma and tissue present some of the same problems as those done with cerebrospinal fluid. Plasma, in particular, has provided relatively nonspecific measurements. Investigators typically describe their results in terms of "opioid-like" activity, which may be inadequate. The wide range of values reported by different investigators and the apparent nonspecificity of the assays underscore these difficulties. Even so, such studies eventually may be of interest, for there is reason to believe that plasma contains a number of endogenous opioid substances. The pituitary and adrenal glands may both release opioid peptides in times of stress, and a thorough understanding of what substances are there, what they are doing, and how their release patterns change in relation to stress therefore may be of considerable importance.

Studies of neuropeptide concentrations and distributions in human autopsy material may be particularly valuable, especially in these nascent phases of research (see [7] for a series of papers measuring various peptides in which autopsy material was utilized from patients with neurological conditions). With such samples, it is possible not only to investigate patterns of neuropeptide distribution but also to characterize their receptor distribution and activity. Such studies may require material obtained quite soon after death, and further work is needed to define what happens to these substances at the time of and immediately after death [108]. Particularly promising would be the use of the tools of molecular neurobiology to investigate the altered patterns of

neuropeptide systems. Evidence increasingly suggests that such studies can be conducted even in autopsy material.

Administration of an Opioid Agonist—Studies with Beta-Endorphin

Studies of beta-endorphin probably have attracted the most attention of any of the clinical trials of opioid agonists. The first reports [109,110] suggested that a single intravenous injection of beta-endorphin could produce dramatic, lasting symptom relief in patients with depression and schizophrenia, but these were not performed with adequate controls. Subsequent, more carefully controlled studies have not supported the initial findings. For example, in a double-blind study in which patients were administered 20 mg of beta-endorphin intravenously, no change was observed in schizophrenic symptoms either by the research staff or by the patients [111,112]. The researchers were able to show that this dosage of beta-endorphin did have central effects, however, as evidenced by a marked increase in prolactin secretion [111] and by an increase in alpha rhythm on EEG [113]; both of these central effects also occur with morphine administration. The physiological effects of beta-endorphin, which lasted about 30 minutes, might have reflected either peripheral changes that were then transmitted to the brain or direct central actions of the drug on receptors at locations in the brain where the blood-brain barrier is open to macromolecules. Other double-blind studies of schizophrenic subjects confirm that behavior is not improved after a single dose of beta-endorphin [114–116]. Research on a non-opioid endorphin *des*-tyrosine-gamma-endorphin has also been disappointing, despite early enthusiasm [117–123].

Opioid agonists may be more useful for treating depression than schizophrenia [124–133]. The administration of opioid agonists appears to cause some depressed patients to switch into mania, and exogenous opioids are reported to affect mood in ways that are consistent with such findings. Again, results of the studies are somewhat contradictory, and the numbers of subjects have been quite limited in each study, so that varying findings may reflect, in part, the characteristics of the patients or of their subtype. Nevertheless, on the basis of the evidence currently at hand, one might hypothesize that a relative deficiency of beta-endorphin or a change in the ratios of endogenous opioid peptides could play an etiological role in some forms of depression.

Although the reports to date on beta-endorphin have been largely negative, the basic strategy of using agonists appears to have merit for studying connections between opioid neuropeptides and specific forms of mental illness. A number of investigators are trying to develop various peptide and nonpeptide analogs that can be studied directly in analogous research designs [124–126].

One of the problems with intravenous injections of beta-endorphin and other neuropeptides is their relative impermeability to the blood-brain barrier, so that little of the administered drug gets into the brain. One way of avoiding these difficulties would be to administer beta-endorphin or other endogenous opioid peptides directly into the cerebrospinal fluid. A precedent for such studies is the evidence that intrathecal injections of beta-endorphin or morphine by lumbar puncture produce a profound analgesia [134–139]. Some of these studies were performed on patients who had required substantial doses of morphine by more traditional routes for even partial pain relief. Others used intrathecal beta-endorphin to produce analgesia in obstetrical patients. Taken together, the results suggest that the effects of beta-endorphin when administered in this way may be long-lasting. The reports indicate that patients have

appeared normal behaviorally, although a few have felt drowsy; however, little specific attention has been given to the psychological responses of these patients. The evidence is at least suggestive that higher doses may induce a manic psychosis.

In general, available studies seem to support the value of a new trial of beta-endorphin in depression, using intrathecal administration of low doses, e.g., 1 mg, of the drug. Other endogenous opioid neuropeptides or their derivatives could also be studied, in efforts to uncover possible relationships between specific opioid systems and forms of depression. Individuals who are resistant to other forms of treatment might be appropriate candidates for a single dosage in a double-blind, cross-over design that could thereby permit evaluation of the results with fewer problems of artifact.

The studies with beta-endorphin highlight many problems associated with investigating effects of a particular neuroregulator in relation to mental illness. The necessity of double-blind studies, including the use of placebo trials, is clear, as is the need to establish that the agent is having a definable central effect. In the early studies, beta-endorphin cost an estimated \$10,000 per injection. With the starkly limited budgets for mental health research, key questions simply could not be addressed. For example, to what extent do differences in drug dosage and route alter the outcome of various treatments? Would results differ if the peptide or an analog could be administered on a more prolonged basis? Perhaps the development of less expensive but more effective derivatives would make it possible to address such questions more directly.

The impact on public expectations of some of the early studies of endorphin and mental disorders also raises broader questions about the communication of medical information at its most preliminary stages. Research efforts performed may deal with patients who sometimes are untreatable with current methods. The families of persons with chronic schizophrenia are particularly susceptible to new "cures," even if the evidence is slim or nonexistent. The use of dialysis in schizophrenia illustrates the potential impact of this situation concerning the necessity for care in obtaining data and in presenting it.

Studies Utilizing Opioid Antagonists—The Use of Naloxone

The strategy of using opioid antagonists in studies of mental disorders is both interesting and potentially powerful. Naloxone and naltrexone are the only two antagonists to receive widespread use in studies to date. Of these, naloxone, which has a more complete opiate antagonist effect, has been tested more extensively, despite its short duration of action and the need to administer it intravenously.

Limited trials of naloxone in depression and mania have been inconclusive, and the data generally indicate no change. However, a series of reports suggest that naloxone can induce quite specific changes in patients with schizophrenia. This topic now has an extensive though as yet unresolved history [140–155]. The first report of an attempt to reverse schizophrenic symptoms with naloxone came from the Swedish group who had earlier reported that certain endorphin-like fractions were elevated in cerebrospinal fluid. In a single-blind study, low doses of naloxone reduced auditory hallucinations in some patients. Several groups tried to replicate the result with mixed success at that dose. One interesting study found an altered pain threshold in some schizophrenics, which opioid antagonists appeared to alter [156].

The Stanford study of naloxone and schizophrenia provided the first extensive, double-blind study of the effects of a relatively high dose of naloxone on hallucinations

[157,158]. The study, which still is ongoing, has involved screening of 1,800 individuals to identify 18 subjects who met the Research Diagnostic Criteria for schizophrenia, exhibited chronic and nearly constant hallucinations, and were able and willing to participate in the study. Patients with chronic hallucinations were used because of naloxone's presumed short period of action. The study has utilized a double-blind cross-over design in which patients received either saline or istered 10 mg of naloxone and then were followed for two days after the infusion.

In the Stanford study, naloxone reduced or eliminated hallucinations in half of the patients for about two hours after administration. Typical was the response of a patient who commented, "The hallucinations are like a little radio attached to my ear that is always on, only now I cannot hear the radio." The result was a surprise. Many of the earlier studies had not examined time points beyond the traditionally short period of action normally associated with naloxone in opiate overdose situations. Since the initial report from Stanford, other groups have confirmed the positive findings using longer time periods and higher doses. The most extensive replication has been a multicenter study conducted by the World Health Organization (WHO), which reported a significant reduction of schizophrenic hallucinations [159].

Taken as a whole, the body of literature about reduction of hallucinations in individuals with schizophrenia is relatively consistent, at least for those studies that utilized a double-blind paradigm with a relatively high dose of naloxone. The positive studies report improvement in auditory hallucinations, psychotic symptoms, unusual thought content, or tension. Nevertheless, these signs and symptoms are not normally thought of as involving the same underlying factors. It is also interesting that the effects occur at a time period beyond that normally associated with the effects of naloxone and at quite high doses, raising the question of whether the effect is indeed due to a blockade of the opiate system or to a secondary effect on that system of an effect on some other system. It is clear that further studies of opiate antagonists in schizophrenia are merited. Ultimately such attempts must be based on the use of antagonists that act on specific receptors which have been described for the endogenous opioid systems.

CONCLUSIONS

A decade after the discovery of the opioid-peptide neuroregulators, few would contest their importance for understanding brain function and behavior. A number of areas appear ripe for further study, including resolution of issues regarding the regulation and processing of neuropeptides in brain regions and delineation of the controls of those mechanisms. Also important is elucidation of the genetic limits on such regulation and the ways in which developmental, behavioral, and environmental factors can influence processing. Although a variety of opioid neuropeptides have been found, others await discovery. Some of these newly discovered neuroregulators will fit within the three genes already known to provide progenitors of the opioid peptides, but others may offer clues to other origins. The multiplicity of the opioid neuropeptides and the diversity of their receptors may enable researchers to study the ways in which related systems function and interact. The complexity of the systems highlights the importance of anatomical and neurophysiological studies of them individually and together, as well as in combination with the various other neuroregulators with which they are co-released.

The opioid neuropeptides are already known to be important in a variety of

behaviors. Nevertheless, underlying mechanisms, including the relationships to other systems, remain to be established. In the future, researchers must study how behavior alters neurochemical processes and in turn is altered by them. Consideration of these reciprocal relationships, which may involve changes at any stage in neuroregulator mechanisms from formation to release and receptor interactions, will be critical to the continued advances of behavioral neurochemistry as a discipline. In the long term, identification of such interactions between behavior and neurochemistry will also prove critical to understanding severe mental disorders. Causal processes between endogenous opioid peptides and forms of depression, schizophrenia, and opiate addiction have already been posited.

The ability to study relationships between peptides and other neuroregulators and behavioral states, including disorders of behavior, rests heavily on the development of basic information about the characterization and regulation of neuropeptide systems. In that context, discovery of previously unrecognized neuroactive agents is a key step, as is basic research on such substances once they have been identified.

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