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Behavioral Neurochemistry: Neuroregulators and Behavioral States

Jack D. Barchas, Huda Akil, Glen R. Elliott
R. Bruce Holman, Stanley J. Watson

Summary. There is compelling evidence that behavioral events alter neurochemical function and that altered neurochemical function can change behavior. Such processes have been related both to neurotransmitters and to neuromodulators, together termed neuroregulators. Available research tools and theoretical constructs have begun to permit studies of certain types of behavior, primarily those related to emotional states and drives. This work is changing long-held concepts about severe mental disorders and the treatment of them.

Preparing a general review of behavioral neurochemistry is a striking experience. Only two decades ago, no such discipline existed; and yet today it addresses topics that range from biochemical aspects of behavioral mechanisms to severe neurochemical abnormalities that have behavioral sequellae, including psychiatric disorders and mental retardation. The field is concerned with relationships of behavior to levels of neuronal organization ranging from nerve networks to cytoplasmic and nuclear events; it makes use of many disciplines, including biochemistry, analytical chemistry, neuropharmacology, neurophysiology, histology, neuroanatomy, physiological psychology, and psychiatry. Basic investigation has enhanced our understanding of biochemical aspects of brain function, provided new insights into certain basic behavioral processes, produced testable hypotheses about the causes of several severe mental disorders, and yielded effective pharmacological treatments for these illnesses.

The field of behavioral neurochemistry deals with a wide range of behaviors and related neuronal processes. For purposes of this article, we have chosen to focus our discussion on the relation of neuroregulators in mammalian systems to emotional states and drives. Our purpose is to demonstrate the multiple ways in which information regarding neuroregulators has developed and has affected the general concepts and approaches in behavioral neurochemistry. We give examples of some problems, substances, and hypotheses which have received particular attention. We consider clinical problems with which neuroregulators have been linked. Some of the recent work dealing with opiate-like substances in the brain, which may function as neuroregulators, is considered as a case that demonstrates the rapid advances within the general field. Finally, we will touch upon some general considerations related to health maintenance problems.

Neuroregulators: Neurotransmitters and Neuromodulators

An underlying assumption in behavioral neurochemistry is that certain substances, neuroregulators, play a key role in communication among nerve cells. These compounds may be subdivided into those which convey information between adjacent nerve cells (neurotransmitters) and those which amplify or dampen neuronal activity (neuromodulators). Table 1 presents a partial list of some of the compounds which are known or hypothesized to be present in brain and may function as neuroregulators (*1*). The idea of chemicals being involved in neuronal communication is usually credited to T. R. Elliott, a Cambridge graduate student who, in 1904, suggested that stimulation of peripheral autonomic nerves might release small amounts of a chemical substance to produce effects on the target organ (*2*). However, Loewi's (*3*) 1921 demonstration of the release of *vagusstoff* following vagal stimulation provided the first compelling evidence for chemical neurotransmission. That same year Cannon and Uridil (*4*) described the properties of "sympathin," a substance released from the liver on stimulation of sympathetic nerves. These compounds subsequently were identified as acetylcholine and norepinephrine, respectively, the first two neurotransmitters to receive extensive investigation.

Early suggestions that chemical neurotransmission might occur in the central nervous system (CNS) generally were

Dr. Barchas is Nancy Friend Pritzker Professor in the Department of Psychiatry and Behavioral Sciences at Stanford University School of Medicine, Stanford, California 94305, and is director of the Nancy Pritzker Laboratory of Behavioral Neurochemistry in which Dr. Akil, Dr. Elliott, Dr. Holman, and Dr. Watson are senior staff members.

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discounted on the grounds that only electrical processes would permit the necessarily rapid transfer of signals (*5*). However, two developments in the early 1950's made it clear that chemical, rather than electrical, processes must be involved (*6*). Results from microelectrode recordings of single cells (*7*) and microiontophoretic applications of substances onto neurons (*8*) were irreconcilable with constructs of purely electrical junctions. Also, after von Euler's (*9*) discovery of norepinephrine, Vogt (*10*) demonstrated that norepinephrine and epinephrine in the CNS were related to brain function and could be changed by drugs and physiological states. From this early work, our information about CNS neurochemistry has expanded rapidly over the past 20 years. Still, most of our concepts about neurotransmitters rely heavily on information obtained from peripheral nervous systems, which have the advantage of relative accessibility and homogeneity. The criteria by which a compound is classified as a neurotransmitter are presented in Table 2 (*11*). In the CNS, the chemical and anatomical mechanisms of formation, release, reuptake, enzymatic destruction, and effects on the pre- and postsynaptic receptors in the brain have been demonstrated most clearly for the catecholamine systems (*1*, *12*). However, even for the catecholamines, we have yet to demonstrate that all criteria for a neurotransmitter are satisfied.

Figure 1 is an idealized representation of the current model for a dopaminergic synapse. A brief discussion of this model will help to define current concepts of a chemical neurotransmitter and to summarize our present state of knowledge. Dopamine is synthesized in two enzymatic steps from the amino acid tyrosine. The first enzyme, tyrosine hydroxylase, present only in catecholaminergic neurons, is the rate-limiting step; the second, dopa decarboxylase, is ubiquitous and catalyzes the decarboxylation of several important amino acids. We have indicated that this process occurs in a synthesis pool; however, the actual physical environment in which the reactions occur is unclear. Presynaptic structures called vesicles or granules are specialized for the uptake and storage of dopamine and are thought to be involved in its release into the synaptic cleft. Through mechanisms which still are understood imprecisely, depolarization of the presynaptic membrane by an action potential causes the release of dopamine into the synaptic cleft, where it diffuses across to the postsynaptic receptor. Transmitters appear to interact with receptors in a "lock-and-key" arrangement to either facilitate or inhibit the action of the second neuron. Dopamine is thought to induce a change in receptor conformation which produces changes in membrane permeability to ions and initiates a complex chain of intracellular reactions, probably mediated by the production of cyclic adenosine monophosphate (AMP) (*13*). Although dopamine binding sites have been demonstrated in the CNS, their specific relation to postsynaptic receptors remains to be elucidated. The signal is terminated by the destruction or removal of the neurotransmitter. For dopamine and several other neurotransmitters, there is a specific reuptake mechanism which actively transfers the neurotransmitter from the synaptic cleft back into the presynaptic cell. The transmitter can either be degraded enzymatically or stored in vesicles for reuse.

The concept of neuromodulation is new and, as yet, incompletely developed. It arises from the discovery of a number of compounds which are important in general communication between nerve cells but which act not in a transsynaptic fashion but in a hormonal-like manner (*14*). Thus, in contrast to a neurotransmitter, a neuromodulator is not responsible for direct transfer of a nerve signal from the pre- to the postsynaptic element; instead, it alters neuronal activity in other ways. There are at least two potential types of neuromodulation. In the first, substances could be released from neurons, glia, or true secretory cells to amplify or dampen, that is, set the "tone" of local synaptic activity by altering the effectiveness of a neurotransmitter. Unlike neurotransmitters, a neuromodulator need not have specific receptors; instead, it might affect neurotransmitter synthesis, release, receptor interactions, reuptake, or metabolism. The adrenal glucocorticoids, neuromodulators of peripheral origin, are illustrative of one such effect; these steroids influence the steady-state levels of tyrosine hydroxylase in the brain, which, in turn, affects the activity of catecholaminergic neurons (*15*). In contrast to such indirect effects through actions on a neurotransmitter, a neuromodulator could be released either within the brain or from other parts of the body to act directly on a large number of neurons at some distance from the release site. Potentially, such effects could be quite long-lasting, helping to influence either baseline activity or response to other neuronal input. Table 2 presents a preliminary set of criteria for neuromodulators.

Table 1. Possible CNS neuroregulators.

Dopamine
Norepinephrine
Epinephrine
Tyramine
Octopamine
Phenylethylamine
Phenylethanolamine
Dimethoxyphenylethylamine (DMPEA)
Tetrahydroisoquinolines
Serotonin (5-hydroxytryptamine)
Melatonin
Tryptamine
Dimethyltryptamine (DMT)
5-Methoxytryptamine
5-Methoxydimethyltryptamine
5-Hydroxydimethyltryptamine (bufotenin)
Tryptolines
Acetylcholine
Histamine
γ-Aminobutyric acid (GABA)
γ-Hydroxybutyrate (GHB)
Glycine
Taurine
Purine
Aspartate
Glutamate
Prostaglandins
Corticosteroids
Estrogens
Testosterone
Thyroid hormone
Enkephalins
β-Endorphin
Substance P
Somatostatin
Angiotensin
Luteinizing hormone releasing hormone (LHRH)
Vasoactive intestinal polypeptide (VIP)
Adrenocorticotropic hormone (ACTH)
Thyroid releasing hormone (TRH)
Sleep factor delta

A seemingly diverse group of substances, neuroregulators have in common a role in communication processes among neurons. In that respect, they differ from substances such as glucose and oxygen, which are involved primarily in the metabolic maintenance of the cell. They also differ from second messengers, such as cyclic AMP and cyclic guanosine monophosphate (GMP), which help to translate neurotransmitter or neuromodulator signals into metabolic events (*13*). It only recently has become clear that many neuroregulators in the brain do not satisfy the criteria for neurotransmitters, and we are just beginning to explore the potential importance of neuromodulators. Thus, we still do not know whether it is possible for a single cell to secrete both neurotransmitters and neuromodulators or more than one substance in each category (*16*). In addition, we need to determine whether a given substance may have more than one role in the brain. Fortunately, even as we explore these difficult but important is-

sues, we have available several powerful techniques that already permit us to study the interrelationships of several neuroregulators with behavior and with psychiatric disorders.

Approaches to the Study of Neuroregulators

Only a few decades ago most studies of neuroregulators relied on bioassays. Although some of these assays rival the specificity and sensitivity of the best modern techniques, they can be cumbersome and unpredictable; they also require a specific biological activity for each substance. Introduction of spectrophotometric and spectrophotofluorometric assays (*17*) permitted rapid, reproducible measurement of a variety of important substances. However, special separation procedures often are needed to eliminate interference among substances with similar spectral properties, and sensitivity is not always adequate. Gas chromatographic assays generally are sensitive, but they can lack specificity, particularly with complex biological samples (*18*). Added to existing techniques, three relatively new approaches have made it possible to devise sensitive and specific assays for virtually any desired substance. Radioenzymatic assays utilize a series of enzymatic reactions to add a specific radioactive label to the substance of interest; quantitation depends upon comparison of the radioactivity of the sample with that of known standards (*19*). Radioimmunological assays require the development of specific antibodies to the substance of interest; various techniques then can be used to quantitate the amount of antigen-antibody complex formed in the sample (*20*). Finally, mass spectrometry and fragmentography depend on recognition of characteristic molecular fragments of the substance of interest; particularly when combined with gas liquid chromatography or high pressure liquid chromatography, this technique can permit identification and analysis of a large number of substances at low concentrations (*21*).

Improved neuroanatomical procedures have also aided efforts to uncover the physiological functions and interactions of major neuroregulatory systems. Initial histochemical studies were restricted to substances for which unique spectral patterns could be induced (Fig. 2a) (*22, 23*). The resulting anatomical maps for dopamine, norepinephrine, and serotonin (Fig. 3) provided valuable new insights into their potential functions and interactions. More recently, introduction of immunological methods has permitted visualization of many new substances and important enzymes (Fig. 2b) (*24*). Another powerful anatomical technique has entailed the use of microelectrodes and micropipets to record the activity of single cells or of cell groups and to observe their response to the application of neuroregulators or pharmacological agents (*7, 8, 25*). Used by skilled investigators, such techniques have permitted the dissection of the component parts of complex interactive systems. For example, our current understanding of the effect of LSD (lysergic acid diethylamide) and other indole hallucinogens on serotonergic systems rests largely on differentiation between the presynaptic and postsynaptic effects of these drugs (*26*).

Findings from the general field of biochemistry also have impact on the way in which problems in neurochemistry are conceptualized. For example, although the formation of catecholamines involves only a few enzymes, its regulation is immensely complicated, with some steps affected by feedback inhibition and others influenced by other endogenous inhibitors or activators. The rate-limiting first step, involving tyrosine hydroxylase (Fig. 1), can respond both to stress and to neuronal activation (*27*). With chronic stress there is, over a period of hours, an increase in the number of enzyme molecules, thereby enchancing the ability of catecholamines to be formed. However, with short-term activation, there can be a severalfold increase in activity of the enzyme, with no concomitant change in the number of enzyme molecules. The purification of the enzyme, which has proved quite difficult, led to findings which suggest that one mechanism of activation of the brain enzyme might involve its phosphorylation (*28*). The delineation of these complicated mechanisms of activation and deactivation now may prove very important in attempts to understand neurochemical correlates of behavior. For example, we have known for many years that neuroregulators respond differentially to stress (*29*). Individual differences in stress response may be associated with differences in degree of activation and deactivation of a rate-limiting step. In addition, genetic controls over this process may exist. If so, differences in activation could prove important. Also, some severe mental disorders have important genetic factors and may have stress-related components.

Table 2. Criteria for establishing the identity of a neuroregulator in the central nervous system.

Neurotransmitter
The substance must be present in presynaptic elements of neuronal tissue, possibly in an uneven distribution throughout the brain
Precursors and synthetic enzymes must be present in the neuron, usually in close proximity to the site of presumed action
Stimulation of afferents should cause release of the substance in physiologically significant amounts
Direct application of the substance to the synapse should produce responses which are identical to those of stimulating afferents
There should be specific receptors present which interact with the substance; these should be in close proximity to presynaptic structures
Interaction of the substance with its receptor should induce changes in postsynaptic membrane permeability leading to excitatory or inhibitory postsynaptic potentials
Specific inactivating mechanisms should exist which stop interactions of the substance with its receptor in a physiologically reasonable time frame
Interventions at postsynaptic sites or through inactivating mechanisms. The effects of stimulation of afferents or of direct application of the substance should be equally responsive
Neuromodulator
The substance is not acting as a neurotransmitter, in that it does not act transsynaptically
The substance must be present in physiological fluids and have access to the site of potential modulation in physiologically significant concentrations
Alterations in endogenous concentrations of the substance should affect neuronal activity consistently and predictably
Direct application of the substance should mimic the effect of increasing its endogenous concentrations
The substance should have one or more specific sites of action through which it can alter neuronal activity
Inactivating mechanisms should exist which account for the time course of effects of endogenously or exogenously induced changes in concentrations of the substance
Interventions which alter the effects on neuronal activity of increasing endogenous concentrations of the substance should act identically when concentrations are increased by exogenous administration

Neuroregulators and Behavior

There have been few attempts to study biochemical changes associated with behavioral states (*30*). Even for simple studies, investigators must attempt to select accurate, sensitive, and appropriate

neurochemical measures and devise a system in which those measures can be obtained reliably in association with a specific behavior. Choice of a specific behavior requires application of criteria of reliability and validity in the context of the species-specific repertoire of the animal. Behavioral states and processes need as careful delineation and articulation as do neurochemical events (*31*). The examples selected and described below exclude most of the behavioral world, but we believe that they illustrate principles by which behavior and neurochemistry can be studied.

In many studies, brain tissue is obtained at various times relative to a chosen event; it is assumed that neurochemical differences among groups reflect a relation between the neuroregulator and the behavior or behavioral state under investigation. For example, investigations of neuroregulator utilization and metabolism associated with aggressive behavior in animals suggest changes in utilization rate and metabolic pathways through time, specific to the brain region studied. These changes are dependent on the psychological and social situation under which the animal is tested. If paired rats are given aversive stimuli (a paradigm which results in fighting behavior), the neurochemical changes are quite different from those occurring if matched animals are given the same amount of aversive stimuli in isolation (*32*). There are also profound neurochemical differences from the effects of escapable and inescapable aversive stimuli (*33*).

In addition to immediate changes in the formation, concentration, and pathway of metabolism of the neuroregulators, long-term changes in biochemical mechanisms can result from behavioral states. Certain stresses, such as exposure to aversive stimuli, seem to alter the reuptake mechanisms for norepinephrine, a finding which suggests that there are changes in the way in which a neuroregulator functions at synapse, with potential alterations in its effects on receptors (*34*). Thus, a behaviorally induced change in reuptake mechanisms could lead to subsequent long-term behavioral changes due to receptor alterations no longer responsive to immediate behavioral states. Whether such mechanisms are relevant to the processes of depression, a disorder most effectively treated by drugs which alter reuptake, remains to be determined.

Studies requiring the death of the animal prevent examination of a single animal throughout the time-course of a behavior. Recently developed techniques permit in vivo studies of behaving animals; methods for measuring relatively small amounts of neuroregulators have been combined with sampling procedures in which indwelling cannulae are used for perfusion either of the ventricular space or of specific brain sites (*35*). Site specificity and potential damage to tissue continue to be troublesome issues raised about these techniques. Still, they already have been invaluable in documenting release of neurotransmitters and in demonstrating the role of specific neurotransmitters in self-stimulation behavior (*36*) and in temperature regulation (*37*). Potentially, they also could be extremely useful in furthering studies of numerous other behaviors, such as sleep, revealing changes in release patterns of major neuroregulators through time in various sites of the brain (*38*).

Another way of examining behavioral neurochemical interactions is through

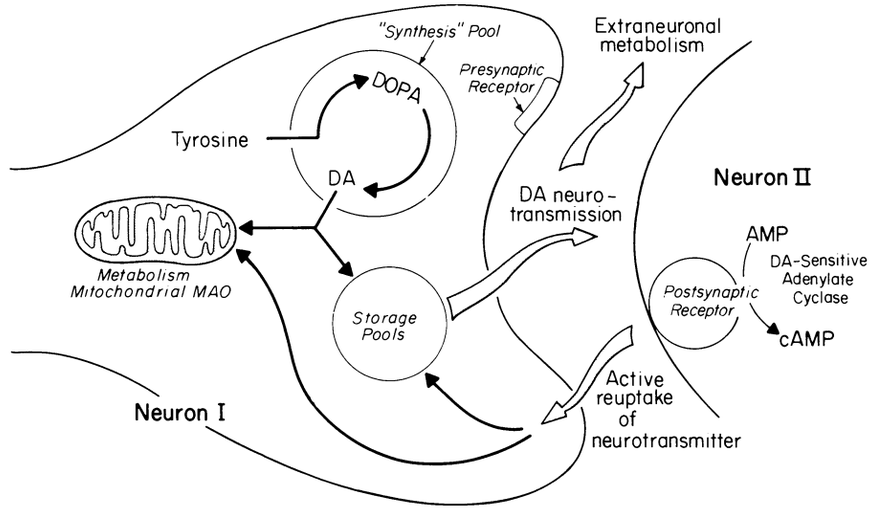

Fig. 1. Idealized model of a dopaminergic synapse. Dopamine (*DA*) is synthesized in two steps from the amino acid tyrosine and then stored in synaptic vesicles. An action potential depolarizes the presynaptic membrane, resulting in the release of dopamine into the synaptic cleft, which it diffuses across to interact with specific postsynaptic receptors. Interaction of dopamine with its receptor initiates a series of complex events, including changes in membrane permeability to ions and formation of cyclic AMP, which, in turn, activates other intracellular reactions. There also is some evidence for presynaptic receptors which may help to regulate dopamine synthesis as a function of synaptic activity. The signal is terminated as dopamine is removed from the synaptic cleft, primarily by an active mechanism which takes dopamine back up into the presynaptic terminal, where it is metabolized by monoamine oxidase (*MAO*) or restored for future use.

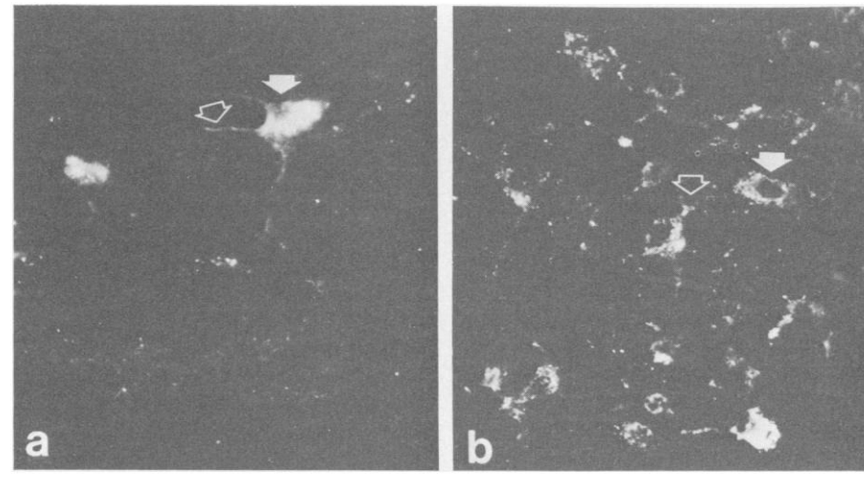

Fig. 2. Histo- and immunofluorescent visualization of catecholaminergic and β-endorphin cells in rat brain. (a) Catecholamine-containing neuron from the subcoeruleus region of medulla pons (×450). The solid arrow indicates the nucleus within a continuously fluorescent cytoplasm, leading into three processes (open arrow). The brain from an untreated rat was prepared by glyoxylic acid condensation (*81*). (b) β-Endorphin-immunoreactive cells in the periarcuate region of the hypothalamus. The solid arrow indicates an unstained nucleus; the open arrow indicates branched processes of the cell body. The rat was first treated with 0.05 mg of colchicine intraventricularly 48 hours before preparations for immunofluorescence were made (*82*).

Table 3. Criteria for linking one or more neuroregulators to a specific psychiatric disorder.

Each neuroregulator must be an endogenously formed substance for which receptors exist in nerve cells or which alters neuronal function
A characteristic pattern of neuroregulator activity should occur in relation to the psychiatric disorder
Alteration of the activity of neuronal systems involving one or more neuroregulators or alteration of the balance between them should affect the psychiatric disorder
Appropriate manipulation of the neuroregulatory system or systems should induce the psychiatric disorder
Unless the physiological process is irreversible, appropriate restoration of neuroregulator activity or balance should ameliorate the psychiatric disorder

genetic analysis. For example, selective breeding of inbred mouse strains can either increase or decrease the tendency toward aggression in some behavioral tests. Using such inbred strains, investigators can begin to differentiate biochemical characteristics which are truly related to the behavior being studied from traits which are associated with it only casually. Genetic factors influence some aspects of neuroregulator function. For example, it has been shown that steady-state levels of the catecholamine synthesizing enzymes in mouse adrenal glands and brain are under genetic control (*39*). Genetic factors also might affect many other critical neuroregulatory processes such as release, metabolism, reuptake, and receptor interactions (*40*). Combined studies of genetic control of behavior and neuroregulator function are particularly promising. Studies of mice suggest that high levels of certain forms of aggression are inherited along with high levels of adrenal catecholamine synthesizing enzymes as well as with high concentrations of brain cyclic AMP (*41*). Studies demonstrating the limits within which behavior may function in relation to differing biochemical and genetic structures are rare but are a critically important area for future research.

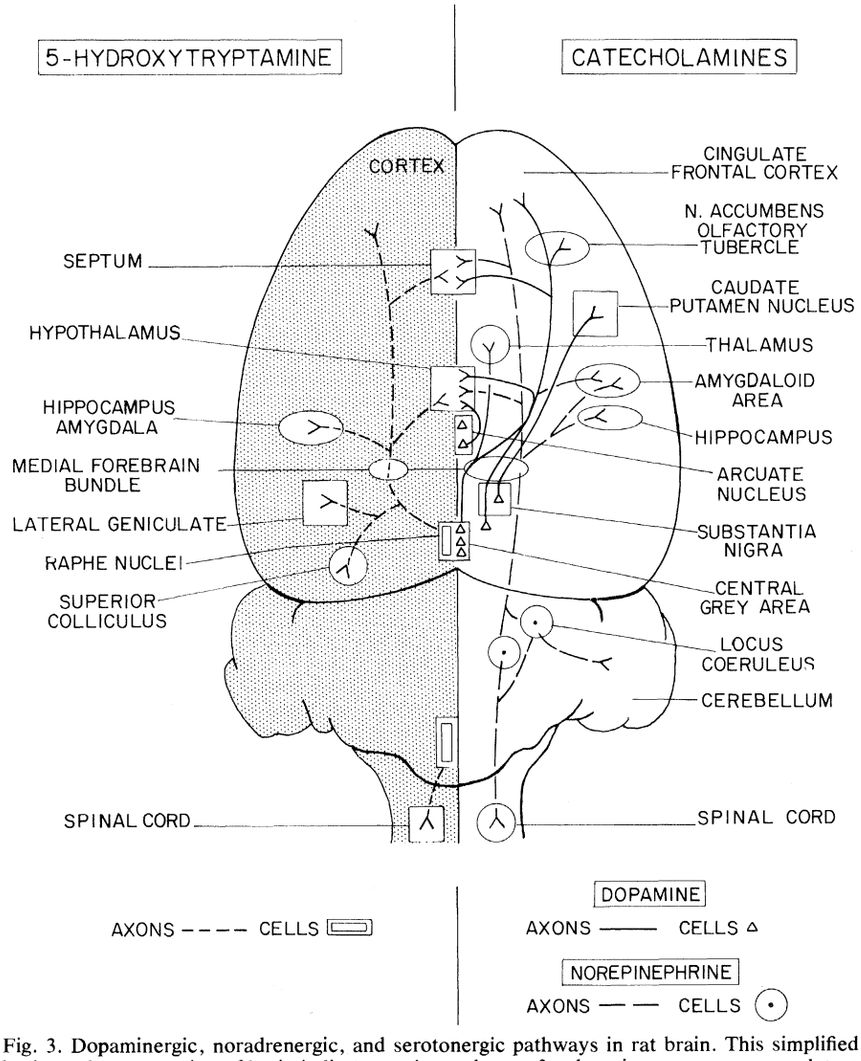

Fig. 3. Dopaminergic, noradrenergic, and serotonergic pathways in rat brain. This simplified horizontal cross section of brain indicates major pathways for three important neuroregulators (*83*).

As the mutual interactions of neurochemistry and behavior become clearer, the field probably will need to develop new concepts to recognize, characterize, and analyze these interrelations. Formerly, we have focused mainly upon the effects of neurochemistry on behavior, tending to discount the importance of reciprocal influences. If a resting organism engages in a behavior as a result either of external stimuli or from its inner experiences, then a sequence of biochemical changes occurs. In turn, those biochemical changes alter the relative probabilities of specific future behaviors—which then will alter the biochemistry yet again. While one tends to imagine these processes as fixed steps, in each case they are in a state of constant flux. Simple causal notions are inadequate to disentangle the two processes, constraining us to view only limited portions of what may or may not be a continuum.

Neuroregulators and Mental Illness

The diversity of neurochemical regulatory mechanisms offers multiple sites for alteration; the resulting changes in behavior may sometimes contribute to an "abnormality" designated as a psychiatric disability. These processes of regulatory mechanisms could be highly individual, such that the neurochemical balance necessary for "normal" behavior may vary in different people. In terms of psychiatric disorders, the reciprocal interaction of behavioral and biochemical events over time is critical.

The dynamic relationships between neuroregulators and psychiatric disorders do not preclude, then, recognition of the important role of psychological factors. There may well be a spectrum of interactive psychological and biochemical processes, even within illnesses labeled similarly according to symptomatology. Thus, there may be some forms of schizophrenia or depression which involve disorders in both psychological and biochemical processes, while others may reflect problems primarily in one or the other. Although we believe that genetic factors do influence some important behavioral processes, the biochemical mechanisms involved probably display considerable plasticity. It may well be that effects of early experience on subsequent behavior—an important idea in behavioral sciences—might be reflect-

ed in biochemical changes that alter communication between neuronal units later in life. In this view, within limits set by genetic factors, experiences at critical stages may influence the setting of "regulostats," biochemical mechanisms which may establish the normal range within which biochemical events occur.

In susceptible individuals certain psychological states may lead to changes in neuroregulatory activity. That alteration then may produce changes leading to what we perceive as disordered function, perhaps by the "locking in" of a biochemical process. Such a situation may pertain only to a portion of those individuals with severe psychiatric disorders. This view may be relevant to considering some schizophrenic patients, who are particularly vulnerable to life stress, including the stress of social interaction (*42*) and some depressed patients, who often experience their disorder in reaction to life events involving social loss. Such an approach to the disorders has implications not only for development of pharmacological treatment but also for development of improved environmental treatments involving psychological and social factors.

Some criteria by which a neuroregulator may be related to severe mental illness are listed in Table 3; these are derived from Reis' criteria for associating a neurotransmitter with a specific behavior (*43*). There are neuroregulator hypotheses for several psychiatric disorders, and a large body of data links changes in neuroregulator activity to such states. In this article we focus on depression and schizophrenia, although hypotheses have been offered for such disorders as anxiety (*44*) and alcoholism (*45*) as well.

Among the most active current biochemical hypotheses involving depression is that of a relative deficiency of one or more neuroregulators in neuronal systems that utilize the particular substances (*46*). The hypotheses are usually stated in terms of norepinephrine or serotonin but have also been proposed with reference to other substances. While the specific evidence is still circumstantial, and indeed often indirect, the hypotheses have been particularly stimulating to current research efforts. Further, they have led to the development of improved modes of therapy, as described by Berger (*47*). It is important to investigate not only the concentrations of selected neuroregulators in such situations but also the other factors involved in neuroregulator function including release, reuptake, and receptor processes, as well as steps following receptor activation.

Perhaps schizophrenia, one of the most baffling of human states and one which forces us to consider basic qualities of human mental processes, has had the most intensive recent consideration at a biochemical level. The two major biochemical theories of schizophrenia are the dopamine hypothesis and the endogenous psychotogen hypothesis.

The dopamine hypothesis, currently the most widely accepted biochemical hypothesis of schizophrenia, postulates that the symptoms of the disorder result from a relative excess of dopaminergic transmission in critical cell groups within the brain. Its support rests largely on the fact that all known antipsychotic agents block dopaminergic neurotransmission; in addition, amphetamine, which can cause a psychotic-like paranoid state even in nonschizophrenics, appears to enhance dopaminergic activity (*48*). The psychomimetic actions of amphetamine, which are thought to occur in part through dopaminergic systems, have been used to develop an animal model of paranoia within a social setting (*49*). To date, efforts to demonstrate an actual increase in dopamine concentrations, turnover, or synthetic enzymes in schizophrenics have been unsuccessful, or the results have been inconsistent (*50*). However, for both schizophrenia and depression, many regulatory steps, such as release, reuptake, receptors, and metabolic enzymes remain to be studied fully. Suggestions of possible differences in the total number of dopamine receptors or in other postsynaptic mechanisms constitute an area of particularly intense research (*51*).

Hypotheses about endogenously formed psychosis-producing neuroregulators (psychotogens) derive from the existence of potent hallucinogens. Several of the hallucinogens are derivatives, often methylated, of known neuroregulators such as serotonin (Fig. 4). Therefore, investigators are trying to determine whether schizophrenics might be characterized by methylated psychotogens (*52*). Some methylated hallucinogens have been detected in low concentrations in body fluids of schizophrenics, but with no greater frequency than that seen in controls and at concentrations that are far lower than those associated with effects when the compounds are administered pharmacologically, although they may be acting at very specific sites. Investigators have demonstrated several enzymes that appear to be capable of forming such compounds in brain or other tissues, again both in schizophrenics and in controls (*52*). It is not clear why these enzymes and substances are present or what their significance is. It may be that methylated derivatives are present normally, having properties that are relevant to the psychotic process only under certain conditions. Or it may be that, under such conditions, compounds

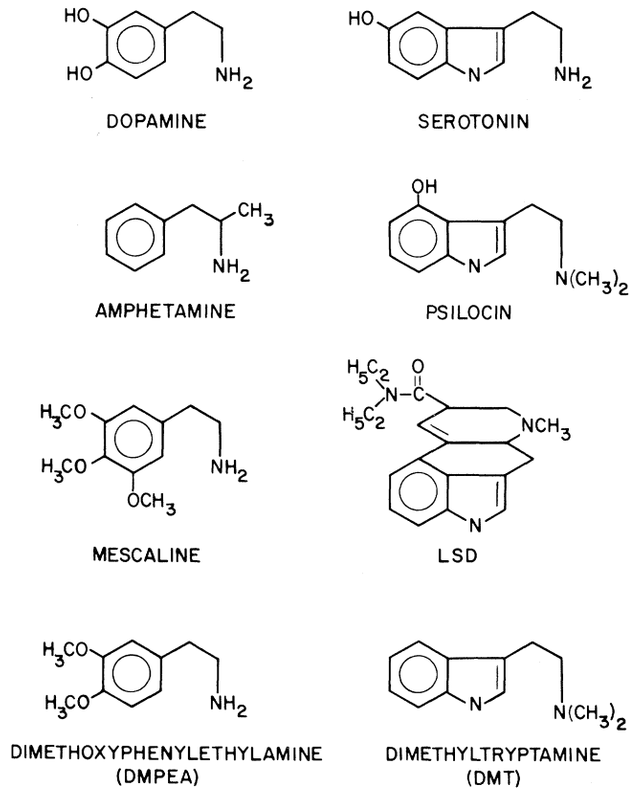

Fig. 4. Important substances related to the neuroregulators dopamine and serotonin. Many potent hallucinogens essentially are methylated analogs of known neuroregulators. This observation has led to one major biochemical hypothesis about schizophrenia.

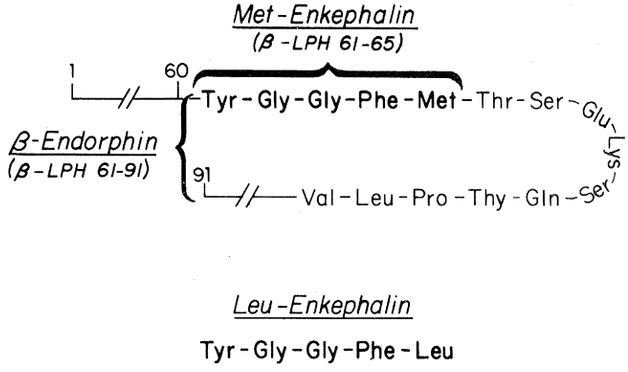

Fig. 5. Endogenous opioids and their structural relationships to β-lipotropin. β-Lipotropin (β-LPH 1 to 91) is a pituitary hormone whose physiological functions remain to be established. Its carboxyl terminus (β-LPH 61 to 91) is an endogenous opioid called β-endorphin. The first five residues of β-endorphin (β-LPH 61 to 65) form another opioid peptide, methionine-enkephalin. A second pentapeptide with opiate-like properties, leucine-enkephalin, differs from the first only by substituting the leucine residue for the methionine residue.

are formed which normally are not present and which alter normal neuronal communication. By enhancing or by inhibiting processes involving neuroregulators usually present, these compounds could function as neuromodulators or even as "false" neuroregulators, replacing some other neuroregulatory agent. It is important to recognize that a chemical substance need not be "abnormal" to be involved in a disorder such as schizophrenia. A compound normally present could be utilized differently by a person in a psychotic episode, at least during the episode. Such indeed would currently be the hypothesis for dopamine and might also be valid for those related to endogenous substances.

Some important brain function may be thought of as resulting from a relative balance of activity between two or more neuroregulators, rather than from the activity of a single system. A commonly used example of this concept is Parkinson's disease, a movement disorder that is thought to reflect a relative preponderance of cholinergic over dopaminergic activity; thus, symptoms improve when cholinergic activity is decreased and when dopaminergic activity is increased. Several current hypotheses of severe psychiatric disorders also invoke imbalances between important neuroregulators.

For schizophrenia, investigators have suggested that symptoms might result from imbalances between dopamine and any of several other neurotransmitters, including acetylcholine, serotonin, and γ-aminobutyric acid (GABA) (*53*). A balance hypothesis involving dopamine and serotonin (or a methylated derivative of serotonin) has the effect of combining the two major hypotheses of schizophrenia into one. Each of the balance hypotheses about schizophrenia posits that, while absolute activity of the dopamine system is either unchanged or increased, its activity relative to some other system is relatively increased, so that antipsychotic drugs, which inhibit dopaminergic transmission, act to restore the proper balance. Balance hypotheses seem to offer more flexibility than do "single" neurotransmitter hypotheses, and they potentially allow for more ingenuity in the treatment situation. They permit the possibility that there may be a change in whatever system is primarily altered and also that possible treatment could be directed at a system with which it is in balance. None of the hypotheses can yet account for the range of behaviors seen in a disorder such as depression or schizophrenia.

Contrary to popular opinion, there actually can be high reliability among psychiatrists asked to judge the impairment and qualities of a patient's illness. At present, investigations tend to characterize populations; however, the final label of identification may differ both because of different taxonomies and because many different illnesses may present the same clinical pattern. Therefore, neurochemical isolation of subsets may be especially vital for the study of psychiatric disorders. Somewhat like those who studied pneumonia a century ago, we may find that there are multiple causal processes, as well as factors involved in labeling the symptomatology. Ultimately, there may be ways to separate those causes and factors, thus facilitating treatment.

Endogenous Opioids as an Example of Neuroregulator Systems

A new class of compounds that has created a great deal of research activity in the neurosciences are the so-called "endogenous opioids," naturally occurring substances in the brain and other mammalian tissues whose actions resemble opiate alkaloids, such as morphine and heroin. Since work on these substances is illustrative of the many perspectives from which neuroregulators can be studied, we use this burgeoning field as an example of the promising developments that can arise in behavioral neurochemistry.

In 1973, several research groups independently demonstrated the presence of opioid receptors in the mammalian CNS which were not responsive to any known neurotransmitters (*54*). During this same period, investigators at the University of California at Los Angeles demonstrated that electrical stimulation of the brain could relieve pain in animals (*55*). Pharmacological studies revealed marked similarities between this stimulation-produced analgesia and the effects of morphine administration, including the ability of naloxone, an opiate antagonist, to block both effects (*56*). Together, these results prompted speculation about an endogenous ligand.

The search for an endogenous opioid proved to be far more rewarding than might have been anticipated. In 1974, Hughes, using a bioassay for opiates, reported some evidence for the presence of an opiate-like substance in brain extracts (*57*); by 1975, he and his co-workers had isolated and identified the active principle of that extract as two pentapeptides, a finding later confirmed by Snyder and co-workers (*58*). Since those substances were derived from the head and differed only by a terminal amino acid, they were called methionine-enkephalin (Met-enkephalin) and leucine-enkephalin (Leu-enkephalin) (Fig. 5).

In their original paper on Met- and Leu-enkephalin, Hughes and associates noted that the peptide sequence for the former corresponded to positions 61 to 65 of a pituitary hormone, β-lipotropin (β-LPH). Teschemacher *et al.* (*59*) reported that the pituitary contained an endogenous opioid with a molecular weight greater than that of the enkephalins. When Li and co-workers isolated and sequenced such a substance from camel pituitary, they found that it corresponded to positions 61 to 91 (the C fragment) of β-LPH (*60*). The opiate properties of C fragment were simultaneously described by Bradbury *et al.* (*61*) (Fig. 5). Guillemin *et al.* (*62*) isolated yet another endogenous opioid, and it proved to be identical with fragment 61 to 76 of β-LPH. Following the suggestion of Eric Simon, scientists have adopted the convention of referring to the entire family of endogenous opioids as endorphins. In addition to the enkephalins, this includes α-endorphin (β-LPH 61 to 76) and β-endorphin (β-LPH 61 to 91). Most recently, Mains *et al.* (*63*) have demon-

strated that a single molecule with a molecular weight of about 31,000 is a common precursor for both β-LPH and adrenocorticotropic hormone (ACTH), suggesting possible links between the endorphins and other important hormonal systems. Fervent efforts to isolate and characterize additional endorphins continue to represent one important focus of research in this area (*64*).

To date, all of the endorphins resemble each other and opiates in their ability to produce analgesia and specific motor effects and to induce tolerance and dependence (*65*); also, most are blocked by opiate antagonists such as naloxone. However, they do have different potencies; and, at least in pharmacological doses, enkephalins are degraded rapidly, while endorphins with higher molecular weights produce effects that are longer lasting. Structural and pharmacological similarities of the opioid peptides pose important questions about the relations among them. For example, is β-endorphin a neuroregulator for which enkephalin is a metabolite; or is it primarily a precursor for the neuroregulator enkephalin? Alternatively, are the two independent, with β-endorphin acting as a pituitary hormone, while enkephalins serve as neurotransmitters in the brain? If so, is β-LPH a precursor for either, both, or neither?

Over the past 2 years, efforts to obtain answers to these questions have begun to bear fruit. We and others (*66, 67*) have shown that pituitary and brain endorphins are synthesized independently. Enkephalin predominates in brain, while β-endorphin is present in highest concentrations in pituitary; but the latter also is present in brain (Fig. 2b) and probably is more than merely a precursor for Met-enkephalin. So far, two independent endorphin pathways have been delineated—a β-endorphin system with a single cell group in the hypothalamus and long axons innervating midbrain and limbic structures and an enkephalin system with multiple cell groups throughout the spinal cord and brainstem and relatively short axons (*68, 69*) (Fig. 6). Immunocytochemical mapping of Leu- and Met-enkephalins carried out independently by several groups (*70*) suggests that the two pentapeptides have virtually identical distributions and may, in fact, be in the same neurons. Immunocytochemical studies of brain indicate that neurons containing β-endorphin also contain β-LPH and ACTH, suggesting that the 31,000-molecular-weight precursor may be present both in brain and in pituitary (*66, 69, 71*).

The physiological role of the two identified opioid systems remains to be elucidated. The enkephalins are found in high concentrations in brain areas involved in pain transmission, respiration, motor activity, endocrine control, and mood; their role in all these behaviors is being actively explored. The suggestion of interconnections between the endorphins and ACTH has become even more intriguing as a result of behavioral studies showing that stress increases the concentrations of endorphin in blood and brain, with parallel changes in pain threshold (*72*). Histochemical localization also suggests that the opioid substances may have potentially important relations to noradrenergic and dopaminergic systems (*70*). If the endorphins are part of the basic systems which modulate responses to pain and stress, they well may play critical roles in behavioral and emotional responses to the environment. More information is needed about other possible endorphins and about their synthetic and degradative pathways. Tools are essential for studying their turnover. Although we do have an extensive array of drugs with which to manipulate the "opiate receptor," evidence is emerging that this too may prove to be a complex problem in which multiple receptor populations will have to be carefully identified and characterized (*73*).

There has been an early and continued interest in elucidating specific functions of the endogenous opioids in human behavioral states. To date, studies have been suggestive but inconclusive. We have found that human subjects receiving electrical stimulation in medial thalamus for relief of chronic pain exhibit a rise of enkephalin-like activity in their cerebrospinal fluid (*74*). Others have reported that endorphin concentrations are altered in some psychiatric disorders

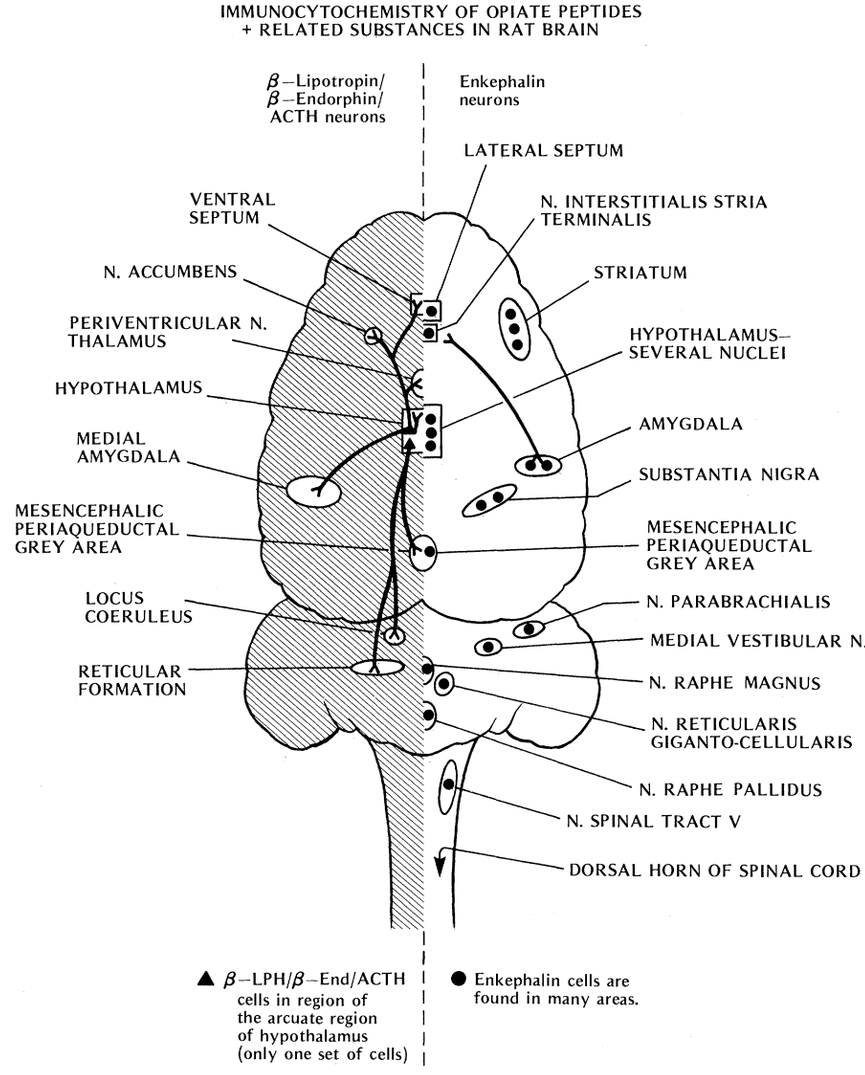

Fig. 6. Endogenous opioid localization in rat brain. This simplified horizontal cross section of brain shows pathways of cells which are immunoreactive to β-LPH, β-endorphin, and ACTH. The right side indicates some locations of cells reacting to antibodies against enkephalin.

(*75*). Also, there have been reports that naloxone decreases symptoms in some manics and in some schizophrenics (*76*), although others found no such effects (*77*). One study of the effects of administering β-endorphin suggests that it might have therapeutic value for selected patients (*78*); carefully designed, thoroughly controlled studies of this possibility are needed. Finally, one group has suggested that a previously unknown peptide (leucine-endorphin) might be implicated in the reported ability of chronic kidney dialysis to relieve schizophrenic symptoms (*79*). Much work remains to be done but it is possible that once artifacts are separated from facts, the basis for an endogenous opioid hypothesis of some forms of psychosis will emerge.

Clearly, the endogenous opioids have powerful effects, are involved in behavior, and interact with other neuronal systems. They appear to offer a perfect example of the need for increasingly broad concepts of neuroregulators. While enkephalins potentially appear to conform to definitions of a neurotransmitter, β-endorphin may more appropriately be thought of as a neuromodulator. The enkephalins seem to be ideally situated to influence basic processes such as pain transmission, respiration, motor integration, endocrine responses, and limbic functions involved in the elaboration of emotions; β-endorphin, residing in a single system with widespread influences, seems more designed to affect various structures by interacting with other local neuroregulators. Thus, not only has the discovery of these substances opened a new and exciting chapter in the study of pain, drug addiction, and psychiatric disorders but it also has forced us to reassess our conception of basic brain functions.

General Considerations

Even at this early stage, behavioral neurochemistry has manifested its potential importance to the health sciences in the form of improved treatments for those suffering from some forms of schizophrenia, mania, and depression. Unfortunately, some have assumed that this important progress is sufficient. A distressing percentage of people suffering from these and other severe psychiatric disorders either receive no benefit from existing drugs or are unable to take them because of disturbing or dangerous side effects. That should not overshadow the immense good that has accrued to millions of individuals. Greater understanding of the processes involved in these and other illnesses should permit both more accurate diagnosis and better treatment.

Although it is our main thesis that behavioral neurochemistry strives to extend basic human knowledge and thus to improve the human condition, that effort will also have profound effects on health care. Government studies suggest that the current combined economic costs of just two of the major disorders with which behavioral neurochemistry is concerned, depression and schizophrenia, are about $50 billion per year, while costs of drug abuse and alcoholism add another $50 billion [see (*80*)]. In contrast, federal support for all research in alcoholism, drug abuse, and mental health (of which behavioral neurochemical studies and related disciplines were only a small portion) amounted to $160 million in 1977 (or 0.16 percent of the economic costs of the disorders) (*80*). This comes to 75 cents per year per citizen. Expressed as research dollars per number of people affected, the amount that our nation spends is between $5 and $10 for each individual immediately afflicted with one of these severe health problems and less than $5 for those with alcoholism; for heart disease and cancer the amount spent on each is more than ten times greater. As was true in the prevention of polio, the cost of long-term care is immense compared to the cost of research. By improving diagnosis and providing more effective means of treatment for severe mental disorders, there are immense benefits not only to the public purse but also for the well being of people.

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