A Molecular and Cellular Theory of Depression

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Recent studies have begun to characterize the actions of stress and antidepressant treatments beyond the neurotransmitter and receptor level. This work has demonstrated that long-term antidepressant treatments result in the sustained activation of the cyclic adenosine 3′, 5′-monophosphate system in specific brain regions, including the increased function and expression of the transcription factor cyclic adenosine monophosphate response element-binding protein. The activated cyclic adenosine 3′, 5′-monophosphate system leads to the regulation of specific target genes, including the increased expression of brain-derived neurotrophic factor in certain populations of neurons in the hippocampus and cerebral cortex. The importance of these changes is highlighted by the discovery that stress can decrease the expression of brain-derived neurotrophic factor and lead to atrophy of these same populations of stress-vulnerable hippocampal neurons. The possibility that the decreased size and impaired function of these neurons may be involved in depression is supported by recent clinical imaging studies, which demonstrate a decreased volume of certain brain structures. These findings constitute the framework for an updated molecular and cellular hypothesis of depression, which posits that stress-induced vulnerability and the therapeutic action of antidepressant treatments occur via intracellular mechanisms that decrease or increase, respectively, neurotrophic factors necessary for the survival and function of particular neurons. This hypothesis also explains how stress and other types of neuronal insult can lead to depression in vulnerable individuals and it outlines novel targets for the rational design of fundamentally new therapeutic agents.

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Depression is thought to be a heterogenous illness that can result from the dysfunction of several neurotransmitter or metabolic systems. Basic and clinical studies have provided evidence that the serotonin and norepinephrine (NE) neurotransmitter systems are involved in the treatment of depression. These studies have led to a series of hypotheses concerning the mechanism of the action of antidepressant treatments, as well as the pathophysiology of depression, that have focused on alterations in levels of these monoamines or their receptors. Although these models have guided research efforts in the field for 3 decades, they have not generated a compelling model of antidepressant action or the pathophysiology of depression. For example, studies to date have failed to identify a common action of antidepressant treatments at the level of monoamines or their receptors. This is not surprising given that different types of antidepressant treatments exert widely dif-

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ferent effects on the serotonin and NE systems. It is also possible that there is more than a single mechanism by which antidepressant treatments exert their therapeutic actions.

An updated hypothesis that builds on previous work suggests that the long-term, therapeutic action of antidepressant treatments is mediated by postreceptor intracellular targets. Advances in molecular and cellular biology have elucidated the intracellular machinery through which monoamines ultimately act to control the functioning of neurons. These advances have made it possible to begin the process of delineating the specific signal transduction pathways that mediate the short-term effects of different types of antidepressant treatments and that underlie the adaptations in neuronal function responsible for the long-term effects of these treatments.

We present a brief review of the basic and clinical work about the role of serotonin and NE systems in the treatment of depression. We also discuss, in greater detail, recent advances that demonstrate a role for intracellular signal transduction pathways and the regulation of specific target genes in antidepressant treatments. Specifically, we describe recent findings that demonstrate that the transcription factor cyclic adenosine 3′, 5′-monophosphate (cAMP) response element-binding protein (CREB) is one intracellular target of long-term antidepressant treatment and that brain-derived neurotrophic factor (BDNF) is one target gene of CREB. These studies support an emerging hypothesis of the mechanisms by which stress and other environmental insults may damage specific populations of neurons and, thereby, contribute to the pathophysiology of depression in vulnerable individuals. This provides a framework for future studies needed to test this potentially unifying hypothesis for antidepressant action and the pathophysiology of at least certain forms of depression.

MONOAMINES AND DEPRESSION

Early studies indicated that agents that deplete monoamines, such as reserpine, could lead to depression in a small percentage of individuals. This led to the theory that reduced availability of monoamine neurotransmitters, particularly NE and serotonin, could play a role in the formation of depression.[1-3] This hypothesis was supported by the discovery that prototypical antidepressant treatments, the tricyclics and monoamine oxidase inhibitors, cause a short-term increase in synaptic levels of monoamines. However, despite extensive research, it remains unclear whether long-term antidepressant treatments result in the increased or decreased function of the many monoamine pathways located throughout the brain.

The role of monoamines in depression has been further examined with the use of serotonin and NE depletion paradigms in normal and drug-remitted individuals with depression.[4-6] The results of these studies demonstrate that although the depletion of serotonin or NE does not lead to depressive symptoms in normal individuals, patients who experienced remission on either serotonin or NE selective reuptake inhibitors are vulnerable to relapse on depletion of the corresponding monoamine. This indicates that serotonin and NE are somehow involved in the maintenance of an antidepressant response, but cannot alone explain either the mechanism of action of antidepressant treatments or the pathophysiology of depression. This conclusion is also supported by the time required for the therapeutic action of antidepressant treatments (several weeks), even though levels of monoamines are increased rapidly by these treatments. Together, the results suggest that additional factors contribute to antidepressant responses and the onset of depression.

MONOAMINE RECEPTORS AND DEPRESSION

A consequence of antidepressant treatment and elevated levels of serotonin or NE is the activation of monoamine receptors. One possibility is that persistent activation of these receptors would lead to adaptations in the receptors that would then contribute to the delayed therapeutic action of antidepressant treatments.[7-9] Indeed, early studies demonstrated that long-term antidepressant treatments down-regulate the density of receptor sites for serotonin and NE. The best-characterized example is that the long-term, but not short-term, administration of many types of antidepressant treatments decreases levels of β-adrenergic receptor (βAR) ligand–binding sites in limbic brain regions, such as the cerebral cortex and the hippocampus.[10,11] The ability of βARs to stimulate the formation of cAMP is similarly decreased in these regions by long-term antidepressant treatments. Serotonin$_2$-receptor–binding sites were also found to be down-regulated by many antidepressant treatments.[12] These and other receptor studies led to various receptor sensitivity hypotheses—for example, that the action of antidepressant treatments is dependent on the down-regulation of βAR or serotonin$_2$ receptors and that enhanced sensitivity of these receptors may lead to depression.[7,8]

However, there are many problems with these hypotheses. First, not all antidepressant treatments effectively regulate the levels of βAR or serotonin$_2$-receptor sites.[9] This could mean that the action of different antidepressant treatments is mediated by different receptors or that other sites are more relevant. Second, the time course for down-regulation of βAR and serotonin$_2$ receptor–binding sites is more rapid than the therapeutic onset of these treatments.[9,13,14] Third, levels of serotonin$_2$ receptors are increased, not decreased, by long-term electroshock seizure treatment, one of the most effective therapies for depression.[9,14] Fourth, the reduction of βAR function by the administration of selective βAR receptor antagonists is not an effective treatment for depression and actually produces depression in some individuals.[15,16] In fact, the activation or facilitation of βAR function by the administration of thyroid hormone or a specific receptor agonist can have antidepressant efficacy in some patients.[17]

A serotonin$_{1A}$ receptor sensitivity hypothesis has also been put forth by Blier and de Montigny.[18] This hy-

pothesis states that long-term antidepressant treatments increase the function of postsynaptic serotonin$_{1A}$ receptors in the hippocampus. Depending on the type of antidepressant treatment, they propose that this could occur by either the increased sensitivity of postsynaptic serotonin$_{1A}$ receptors or the desensitization of serotonin autoreceptors. One problem with this hypothesis is that direct-acting serotonin$_{1A}$ receptor agonists are not clearly effective antidepressant treatments, although the serotonin$_{1A}$ agonists tested (buspirone hydrochloride and gepirone) are thought to be partial agonists at postsynaptic serotonin$_{1A}$ receptors and may not adequately test the hypothesis. Another possibility is that increased serotonin$_{1A}$ neurotransmission is necessary, but insufficient, for antidepressant efficacy and that the activation of additional factors is required.

Several other monoamine receptor subtypes are reported to be regulated by antidepressant treatments.[8,9] However, as with the βAR, serotonin$_2$, and serotonin$_{1A}$ receptors, regulation of these other monoamine receptors alone cannot account for the mechanism of action of antidepressant treatments. Rather, alterations in the levels of these receptors or in their functional sensitivity probably represent adaptations to increased levels of monoamines. In fact, it can be argued that the observed receptor down-regulations are indicative of continued receptor activation secondary to continued elevations in monoamine levels after long-term antidepressant treatments. Indeed, levels of these receptors are decreased, and not completely eliminated, by long-term antidepressant treatments, raising the possibility that there could be sufficient levels of receptor remaining to respond to the elevated levels of serotonin and NE (**Figure 1**). If this is the case, the functional output of the monoamine receptors would be increased, not decreased, by long-term treatments, a possibility that was entertained in an early review.[19] This would suggest that long-term antidepressant treatments result in the sustained activation of the intracellular signal transduction cascades that mediate the actions of monoamine receptors. Such intracellular factors represent potential common targets for many different types of antidepressant treatments because they could be regulated by the activation of either the serotonin or NE receptor systems. This could include the activation of serotonin and NE receptor subtypes that are not themselves regulated by antidepressant treatments. It will be important in future studies to determine which serotonin and NE receptor subtypes mediate the relevant therapeutic actions of antidepressant treatments, although identification of the critical intracellular targets will likely facilitate this process.

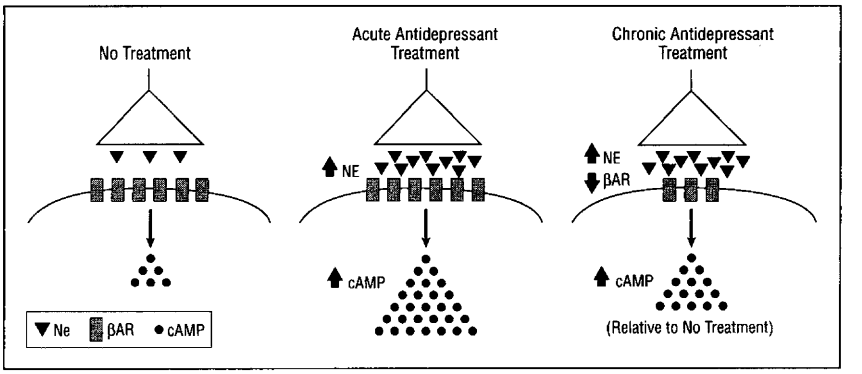

Figure 1. *A model demonstrating antidepressant regulation of the β-adrenergic receptor (βAR)–coupled cyclic adenosine 3', 5'-monophosphate (cAMP) system. When there is no treatment, basal levels of norepinephrine (NE) stimulate the βAR–coupled cAMP system. Short-term administration of certain types of antidepressants elevates levels of NE, increasing levels of βAR–stimulated cAMP formation. Long-term administration of many types of antidepressants causes down-regulation of the number of βAR-binding sites available to stimulate cAMP production. This in turn leads to a decrease in the maximal level of βAR–stimulated cAMP formation. However, with synaptic levels of NE still elevated as a result of continued antidepressant treatment, the level of cAMP formed remains elevated relative to the no-treatment condition. This possibility requires further testing to determine if levels of βAR–stimulated cAMP formation are indeed elevated. However, this model provides a mechanism to explain how long-term antidepressant treatments activate the cAMP pathway even though levels of βAR are decreased.*

INTRACELLULAR MESSENGERS, NEUROTROPHIC FACTORS, AND DEPRESSION

Advances in molecular and cellular biology have paved the way for studies to determine how antidepressant treatments ultimately regulate the function of target neurons in the brain. There are many comprehensive reviews on intracellular signal transduction pathways,[20-25] and this information will not be discussed in detail herein except as it pertains to antidepressant treatments. In general, these pathways can be divided into 2 broad categories. The first category includes those pathways that are controlled by receptor-coupled second messengers (eg, cAMP, inositol triphosphate, Ca^{2+}, and nitric oxide) and are usually regulated by classic neurotransmitters, such as the monoamines, amino acids, and neuropeptides (**Figure 2**). The second category includes those pathways that are controlled by receptors that contain, or interact with, protein tyrosine kinases and are usually regulated by neurotrophic factors and cytokines; activation of these receptors leads to the regulation of other intracellular cascades, such as the mitogen-activated protein kinase pathway (Figure 2). As the protein kinases and phosphatases, phosphoproteins, transcription factors, and target genes that make up these pathways are increasingly identified and characterized, it is becoming possible to examine how adaptations of these complex pathways are involved in the long-term actions of antidepressant treatments and other psychotropic drugs.

The ability to study these adaptations is a critical advance because these pathways control all aspects of neuronal function and ultimately underlie the ability of the brain to adapt and respond to pharmacological and environmental inputs. The functional consequences of such molecular adaptations can be determined at the cellular level by analysis of the electrophysiological and morpho-

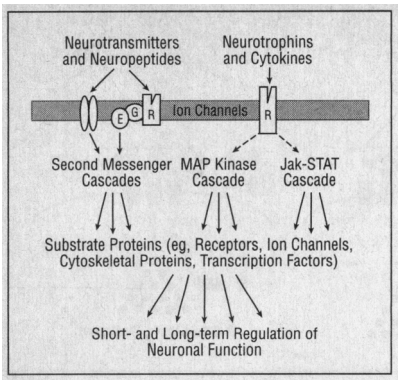

Figure 2. *A general model of receptor-coupled intracellular signal transduction pathways. Intracellular pathways can be subdivided into 2 general categories. Intracellular pathways in the first general category are the second messenger-regulated signaling cascades. These pathways are activated by neurotransmitter or neuropeptide receptors that couple to effector (E) proteins via G proteins. Examples of second messenger cascades include cyclic adenosine monophosphate, cyclic guanosine monophosphate, Ca^{2+}, inositol triphosphate, and nitric oxide. Neurotransmitter regulation of ion channels can also result in regulation of second messenger-dependent pathways. These second messengers lead to the regulation of second messenger-dependent protein serine–threonine kinases. Intracellular pathways in the second general category are the tyrosine kinase–mediated cascades. These pathways are regulated by neurotrophins and cytokines via the activation of receptors (R) that have intrinsic tyrosine kinase activity on the intracellular aspect of the receptors (eg, trkB) or interact with cytoplasmic tyrosine kinases. Activation of these kinases leads to association with other proteins that subsequently activate the mitogen-activated protein (MAP) kinase and Jak-STAT cascades. Regulation of these and the second messenger-dependent protein kinase cascades results in the short- and long-term regulation of neuronal function via the phosphorylation of specific substrate proteins.*

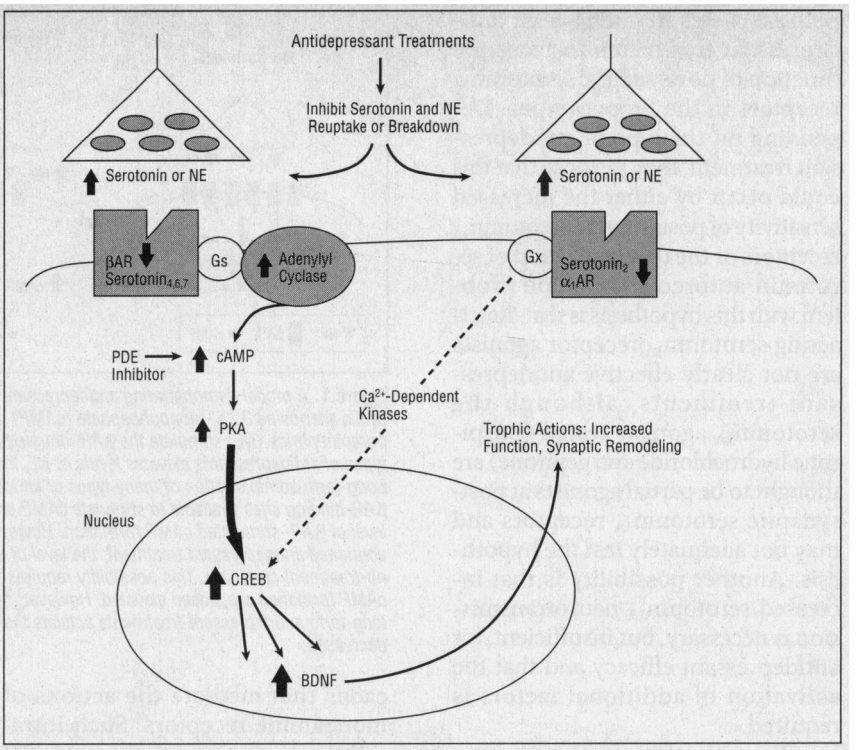

Figure 3. *A model for the molecular mechanism of action of long-term antidepressant treatments. Antidepressants cause a short-term increase in levels of serotonin and noreprinephrine (NE) by inhibiting the reuptake or breakdown of these monoamines. Long-term antidepressant administration decreases the function and expression of certain serotonin and NE receptors (eg, β-adrenergic receptor [βAR] and serotonin$_2$), but the cyclic adenosine 3', 5'-monophosphate (cAMP) signal transduction pathway is increased by long-term antidepressant treatments, including increased levels of adenylyl cyclase and cAMP-dependent protein kinase (PKA), as well as the translocation of PKA to the cell nucleus. Moreover, recent studies demonstrate that expression and function of the transcription factor cAMP response element–binding protein (CREB) is increased by different types of antidepressant treatments, suggesting that CREB is a common postreceptor target for antidepressants. Cyclic adenosine monophosphate response element–binding protein could be regulated by monoamine receptors that couple to the cAMP-PKA cascade (serotonin$_{4,6,7}$ and βAR) or via receptors that lead to the activation of Ca^{2+}-dependent kinases (eg, serotonin$_2$ and α_1-adrenergic receptor [α_1AR]). Increased activity of the cAMP signal transduction cascade indicates that the functional output of serotonin and NE are up-regulated at least in some brain regions, even though levels of certain serotonin and NE receptors are partially down-regulated. This conclusion is further supported by the finding that expression of brain-derived neurotrophic factor (BDNF) and trkB, 2 potential targets of CREB, is also increased by long-term antidepressant treatment. Up-regulation of BDNF and trkB could influence the function of hippocampal neurons or the neurons innervating this brain region, such as serotonin and NE neurons. This could include increased neuronal survival, function, and remodeling of synaptic or cellular architecture.*

logical properties of neurons. Examples of these types of adaptations are the enhanced or diminished synaptic efficacy that is observed in cellular models of learning and memory.[26-29] Morphological changes include atrophy or the sprouting of neurons in response to damaging or growth-promoting stimuli.[30-32]

These types of responses can be viewed as prototypical ways in which the brain adapts to repeated perturbations. Our hypothesis is that similar types of molecular and cellular adaptations occur in response to antidepressant treatments and are involved in the pathophysiology of depression. These types of adaptations are complicated and, in many cases, are difficult to identify, but recognition of their potential importance has stimulated studies that have investigated the role of intracellular pathways and their cellular adaptations in antidepressant action and in depression. Characterization of such adaptations is at a relatively early stage, but has already led to notable conceptual advances in the field.

ANTIDEPRESSANT TREATMENTS ACTIVATE THE cAMP SIGNAL TRANSDUCTION PATHWAY

There are many potential intracellular pathways that could mediate the action of antidepressant treatments. One such pathway that is regulated by the serotonin and NE systems is the cAMP signal transduction cascade (**Figure 3**). Receptor activation leads to the formation of cAMP by coupling to adenylyl cyclase via the stimulatory G protein subtype, Gs. Certain forms of adenylyl cyclase can be activated independent of Gs in response to elevated cellular Ca^{2+} levels.[24] The effects of cAMP are then mediated by the activation of cAMP-dependent protein kinase, which, in turn, leads to the regulation of cellular function by the phosphorylation of specific proteins. One of these is the transcription factor CREB, which mediates many of the actions of the cAMP system on gene expression.[33,34] In addition to its regula-

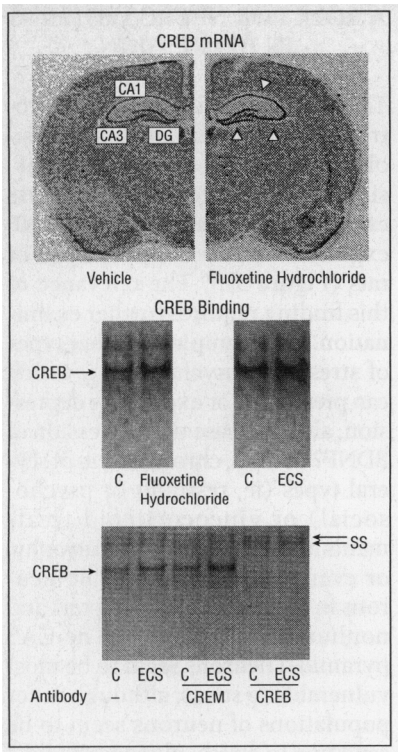

Figure 4. *Long-term antidepressant treatments increase levels of cyclic adenosine 3′, 5′-monophosphate (cAMP) response element–binding protein (CREB) messenger RNA (mRNA) and binding in the hippocampus. The finding that long-term antidepressant treatments increase the expression and function of CREB in the hippocampus is illustrated. The influence of long-term (21 days) fluoxetine hydrochloride administration on levels of CREB messenger RNA was determined by in situ hybridization analysis. Levels of CREB messenger RNA were increased in the major subfields of the hippocampus, including CA3 and CA1 pyramidal and dentate gyrus granule cell layers. Levels of CREB binding were also increased by long-term fluoxetine treatment, as well as electroshock seizures (ECS) (10 days). For this assay, homogenates of the hippocampus are incubated with a radiolabeled, synthetic fragment of DNA that contains a consensus cAMP response element. Binding of CREB shifts the migration of the radiolabeled DNA through the gel. The identity of CREB is confirmed in several ways. First, the CREB binding is reduced by competition with unlabeled DNA containing the cAMP response element, but not with DNA containing a mutated cAMP response element sequence (not shown). Second, supershift (SS) studies (bottom) demonstrate that a specific antibody to CREB, but not CREM (cAMP response element modulatory protein), alters the cAMP response element–binding band. For ss analysis, the homogenate is preincubated with antibody before the radiolabeled DNA is added. If the specific antibody binds to the protein-DNA complex, it either disrupts the complex or supershifts the migration of the complex through the gel.*[44]

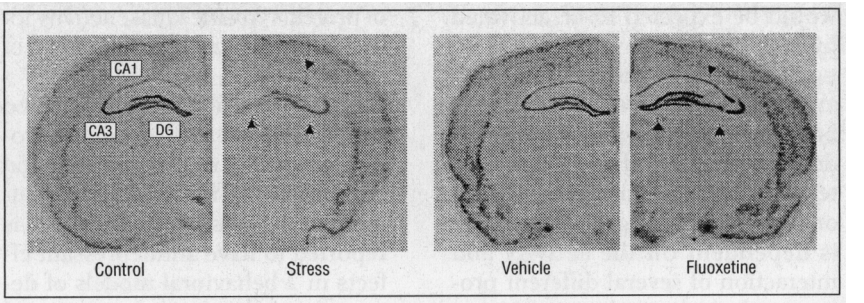

Figure 5. *Stress decreases and antidepressant treatments increase the expression of brain-derived neurotrophic factor in the hippocampus. The influence of acute restraint stress (90 minutes) or long-term fluoxetine hydrochloride administration (21 days) on levels of brain-derived neurotrophic factor messenger RNA were determined by in situ hybridization analysis. The CA3 and CA1 pyramidal and dentate gyrus granule cell layers of the hippocampus are indicated. Pretreatment with fluoxetine or other antidepressants also blocks the down-regulation of brain-derived neurotrophic factor in response to stress.*[52]

tion by phosphorylation, recent studies have demonstrated that expression of the total amount of CREB is another mechanism by which the function of CREB can be regulated.[35-37] The cAMP system is a potential common target for serotonin and NE because there are receptor subtypes for both monoamines that stimulate the formation of this second messenger (Figure 3). In addition to the βAR, there are 3 serotonin receptor subtypes that stimulate the cAMP system (serotonin$_{4,6,7}$), and one of these (serotonin$_7$) is reported to be regulated by long-term antidepressant treatments.[38] Moreover, CREB may represent a common intracellular target for antidepressant treatments because other serotonin or NE receptors (eg, serotonin$_{2A,C}$ and α_1-adrenergic receptors) could also activate CREB via the stimulation of Ca^{2+}-dependent protein kinases (**Figure 4**).[33,34]

Several studies have demonstrated that the postreceptor components of the cAMP system are regulated by long-term antidepressant treatments (Figure 3). Levels of guanine nucleotide–stimulated adenylyl cyclase and cAMP–dependent protein kinase enzyme activity are reported to be increased by long-term antidepressant treatments.[39-43] In addition, we have found that the levels of CREB messenger RNA and protein in the hippocampus are increased by long-term antidepressant treatments, including serotonin and NE selective reuptake inhibitors (Figure 4 and **Figure 5**).[44] The time course for the induction of CREB is consistent with that for the therapeutic actions of antidepressant treatments (ie, 10-21 days of treatment). To further test the hypothesis that CREB is involved in antidepressant actions, it will be important to determine whether the time course for the reversal of these effects is consistent with the offset of therapeutic actions. Increased expression of CREB could be mediated by the up-regulation of cAMP function discussed previously because CREB expression can be induced in cultured cells by the activation of the cAMP cascade.[35] These findings contrast to those of a recent study of CREB function in PC12 cells, an adrenal chromafin cell line.[45] However, in the latter study, the short-term incubation of PC12 cells with antidepressant treatments inhibited depolarization-induced activation of CREB, an effect owing to the blockade of Ca^{2+} channels. To our knowledge, such inhibition of CREB has not been demonstrated in vivo.

Increased function and expression of CREB provides direct support for the proposal that the cAMP signal transduction system is increased in the hippocampus by long-term antidepressant treatments. Induction of the intracellular components of the cAMP cascade may also explain the requirement for long-term antidepressant treatments, even though levels of cAMP may be elevated by short-term antidepressant treatments (Figure 1 and Figure 3). Our results also indicate the types of specific gene elements likely to be regulated by long-term antidepressant treatments; that is, those with functional cAMP response elements. However, not all genes with a cAMP response element

would be expected to be activated by antidepressant treatments. First, up-regulation of the cAMP system and CREB is not observed in all brain regions (there is evidence for down-regulation of the cAMP system in the locus coeruleus[46]). Second, regulation of gene expression is dependent on the activity and interaction of several different promoter elements and transcription factors. Thus, activation of the cAMP system and CREB may lead to the regulation of a specific set of genes in the hippocampus that are involved in the action of antidepressant treatments. One goal of current studies is to identify these genes and to determine their relevance to the clinical responses to antidepressant treatments. Recent studies have identified BDNF and its receptor, trkB, as 2 potential target genes of interest.

ANTIDEPRESSANT TREATMENTS REGULATE NEUROTROPHINS

Brain-derived neurotrophic factor is a member of the nerve growth factor family, which also includes the prototype nerve growth factor as well as neurotrophin-3 and neurotrophin-4. These growth factors are involved in the differentiation and growth of many types of neurons in the developing brain as well as the maintenance and survival of neurons in the mature brain.[30-32,47] For example, the survival and growth of serotonin neurons in the adult brain is increased by BDNF and, to a lesser degree, by neurotrophin-3, but not by the nerve growth factor.[48] Also, neurotrophins can rapidly influence the function of neurons, as demonstrated by the finding that short-term exposure of hippocampal slices to BDNF increases the strength of certain synaptic connections.[28,29] These types of findings, along with an increasing appreciation of the neurotrophic actions of the monoamines,[49,50] highlight the large overlap between the roles played by neurotrophins and monoamines in the regulation of brain function. The actions of neurotrophins are mediated by binding to specific receptors, referred to as trkB receptors, which lead to the activation of protein tyrosine kinase activity located on the intracellular domain of the receptors.

Recent studies have provided support for the notion that neurotrophins and neuronal plasticity and survival may be involved in the treatment of depression. First, BDNF is reported to have antidepressant effects in 2 behavioral models of depression, the forced swim and learned helplessness paradigms.[51] Second, long-term, but not short-term, antidepressant administration, including serotonin and NE selective reuptake inhibitors, a monoamine oxidase inhibitor, an atypical antidepressant, as well as electroshock seizures, increases the expression of BDNF and trkB in the hippocampus (Figure 3 and Figure 5).[44,52] Third, BDNF is reported to enhance the growth of serotonin and NE neurons, as well as to protect these neurons from neurotoxic damage.[48,53] While these findings support a role for BDNF in the actions of antidepressant treatments, studies of additional antidepressant and nonantidepressant agents are required to further test this hypothesis. In addition, the role of other neurotrophic factors (eg, nerve growth factor, neurotrophin-3, and neurotrophin-4) should be examined.

The possibility that increased expression of BDNF results from the activation of CREB is supported by several lines of evidence. First, the time course for the up-regulation of CREB is similar to that of BDNF. Second, the regulation of CREB and BDNF is observed in the same populations of neurons in the hippocampus (Figure 4 and Figure 5). Third, studies in primary neuronal cultures have demonstrated that the stimulation of cAMP- or Ca^{2+}-activated protein kinases increases the expression of BDNF.[54,55] Finally, the reduction of CREB levels in the hippocampus by the administration of antisense oligonucleotides to CREB messenger RNA decreases basal levels of BDNF and blocks the induction of BDNF by antidepressant treatments.[49] Additional in vitro and in vivo studies are needed to demonstrate a direct link between CREB and the expression of BDNF.

A ROLE FOR NEUROTROPHINS IN DEPRESSION

There is also evidence of a neurotrophic element in the pathophysiology of certain forms of depression. First, immobilization stress causes a dramatic reduction in BDNF expression in the hippocampus of rats (Figure 5).[56] The relevance of this finding requires further examination. For example, do other types of stress (eg, psychosocial), which can precipitate or exacerbate depression, also decrease the expression of BDNF? Second, chronic stress of several types (ie, restraint or psychosocial) or glucocorticoid treatments are reported to cause atrophy, or even death, of vulnerable neurons in the hippocampus in rats and nonhuman primates.[57-63] The CA3 pyramidal neurons seem to be most vulnerable to stress, although other populations of neurons seem to be sensitive to stress, glucocorticoids, cytokines, and other types of neuronal insult, such as hypoxia-ischemia or hypoglycemia.[64] Third, the ability of the hippocampus to inhibit the hypothalamic–pituitary–adrenal axis is reduced in certain patients with depression, consistent with a deficit of hippocampal function in these individuals.[65] Fourth, there is a small decrease in hippocampal volume as determined by magnetic resonance imaging in patients with depression or posttraumatic stress disorder.[66-68] In addition, a recent review of the literature provides evidence to suggest that there may be a decrease in the size of certain brain structures in depression.[69] Down-regulation of BDNF may contribute to the atrophy of neurons in the hippocampus in response to stress. However, not all episodes of depression are associated with stress, and the model presented may be limited to certain subtypes of depression. Postmortem studies to demonstrate atrophy or neuronal death directly in the hippocampus of patients with depression are required to further test this hypothesis.

These studies form the basis of a molecular and cellular model of depression and antidepressant action (**Figure 6**). Depression, particularly stress-associated cases, may re-

sult from the atrophy or death of vulnerable pyramidal neurons in the CA3 region of the hippocampus. This could be a consequence, at least in part, of the decreased levels of BDNF available to these neurons. Elevated levels of glucocorticoids are also known to play a notable role in stress-induced damage of CA3 neurons.[57-63] Antidepressant treatments could reverse this atrophy by increasing BDNF expression and function. In fact, we have found that long-term antidepressant pretreatment blocks the down-regulation of BDNF in response to stress.[52] Normalization of glucocorticoid levels by antidepressant treatments in some individuals could be an additional mechanism for the prevention of further neuronal damage.[70] Further studies are required to determine whether antidepressant treatments have direct neuroprotective effects or neurotrophic actions on hippocampal neurons as suggested by early studies.[71,72] Recent studies are encouraging. We have found that long-term electroshock treatment causes sprouting of granule neurons in the hippocampus.[73] Unlike the sprouting observed in response to kindling or excitotoxin treatment, there was no obvious cell damage after long-term electroshock treatment. In addition, electroshock-induced sprouting was markedly attenuated in mutant knockout mice that express reduced levels of BDNF. These results indicate that electroshock induces sprouting of certain hippocampal neurons and that the response is mediated by the increased expression of BDNF in these neurons. The observation that antidepressant treatments can lead to the reinstatement of hippocampal control of the hypothalamic–pituitary–adrenal axis in some patients further supports the possibility that the function of these neurons, indeed, can be improved.[65]

One challenge in depression research is the difficulty of identifying specific anatomical substrates involved in the disorder. The hippocampus is involved in the control of emotion, learning and memory, and the regulation of the hypothalamic–pituitary–adrenal axis, as well as other vegetative processes. Changes in the structure or function of hippocampal neurons could be involved in the affective, neuroendocrine, cognitive, and vegetative abnormalities observed in depression. However, these abnormalities are also likely to be influenced by several other limbic brain structures and it is possible that stress alters the structure and function of neurons in these brain regions as well.

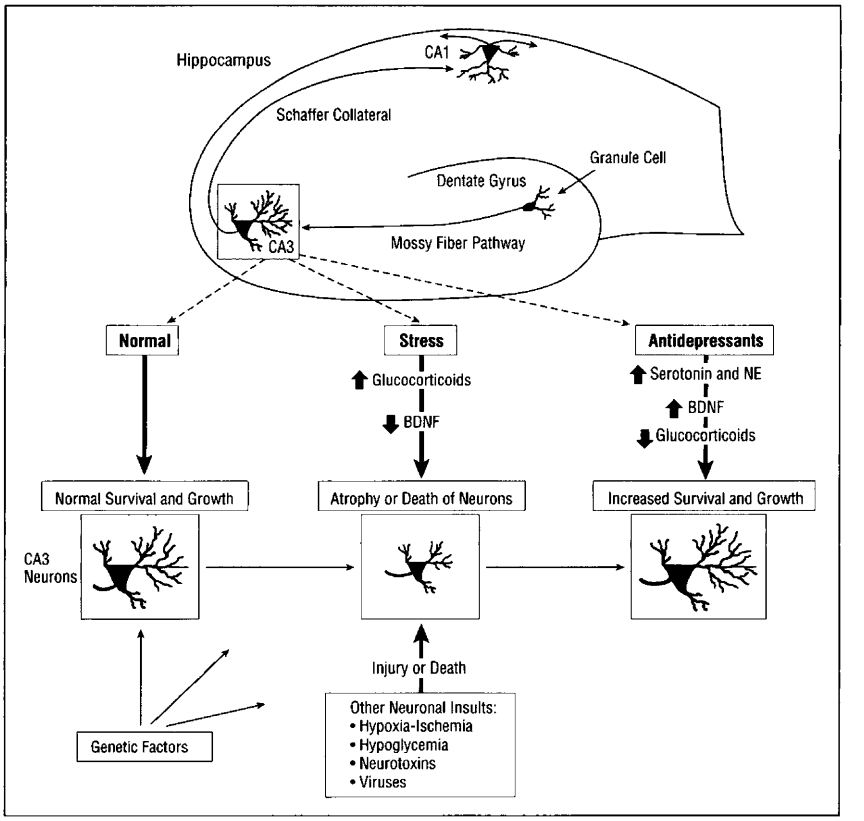

Figure 6. *A molecular and cellular model for the action of antidepressant treatments and the pathophysiology of stress-related disorders. This model of the hippocampus shows the major cell types in the hippocampus and how stress and antidepressant treatments may influence CA3 pyramidal cells. The 3 major subfields of the hippocampus (CA3 and CA1 pyramidal cells and dentate gyrus granule cells) are connected by the mossy fiber and Schaffer collateral pathways. Recent studies demonstrate that chronic stress decreases the expression of brain-derived neurotrophic factor (BDNF) in the hippocampus. This could contribute to the atrophy or death of neurons in the CA3 pyramidal cell layer of the hippocampus. Long-term elevation of glucocorticoid levels is also known to decrease the survival of these neurons. Other types of neuronal insult such as hypoxia-ischemia, hypoglycemia, neurotoxins, and viral infections could also cause atrophy or damage of neurons and could, thereby, make an individual vulnerable to subsequent insults. These types of interactions could underlie the observations of decreased function and volume of hippocampus in patients with affective disorders and could explain the selective vulnerability of certain individuals to become depressed. Long-term antidepressant treatments increase the expression of BDNF, as well as trkB, and prevent the down-regulation of BDNF elicited by stress. This could increase the growth or survival of neurons, or help repair or protect neurons from further damage. Increased expression of BDNF and trkB seems to be mediated by the sustained elevation of the serotonin and norepinephrine (NE) systems and the cyclic adenosine monophosphate cascade. Normalization of glucocorticoid levels by long-term antidepressant treatments could also contribute to the recovery of CA3 neurons.*

A HYPOTHESIS FOR GENETIC AND ENVIRONMENTAL VULNERABILITIES

It is widely believed that depression results from a combination of genetic and environmental factors. Yet, virtually nothing is known about the specific genes that may predispose certain individuals to depression and render others relatively resistant. Studies of the cAMP pathway, CREB, and BDNF, discussed previously, demonstrate the enormous number of genes that could be involved. Indeed, these studies highlight the naivete of candidate gene strategies to identify depression vulnerability genes, which remain focused on monoamine metabolic enzymes, receptors, and reuptake proteins. These studies indicate the need for more open-ended investigations of the genetic factors involved in depression.

However, it could be immediately informative to examine how

various types of environmental factors may predispose certain individuals to mood disorders based on our hypothesis. One possibility is that many individuals who become depressed may have had a prior exposure to stress that causes a small amount of neuronal damage, but not enough to precipitate a behavioral change. If additional damage occurs, either as a result of normal aging or further stressful stimuli, these effects may then be manifested in the symptoms of a mood disorder. These types of events could explain the decreased volume of specific brain structures in depression. This model could also explain how other types of neuronal insult predispose an individual to mood disorders. Insults such as hypoxia-ischemia, hypoglycemia, neurotoxins, or viral infections could cause direct neuronal damage or render neurons more vulnerable to psychosocial stress (Figure 6). Such changes could lead to depression at later times or, if severe enough, could immediately precipitate a depressive episode. For example, a high incidence of depression is well documented in patients who have had a stroke.[74-76] The possible involvement of myriad environmental factors implicates many additional sets of genes that could conceivably alter an individual's inherent responses to stress and establish a diathesis for depression. The possible role of a subtle neurodegenerative contribution to the pathophysiology of depression should be directly examined by analysis of postmortem tissue, as discussed previously.

RATIONAL DESIGN OF NOVEL THERAPEUTIC AGENTS

New information indicating a role for the cAMP pathway and CREB in the actions of antidepressant treatments suggests novel approaches to develop faster-acting and more effective agents. The development and testing of such agents will also help to further test our hypothesis. First, receptor agonists could be targeted at the specific serotonin or NE receptors that stimulate cAMP- or Ca^{2+}-activated protein kinases and mediate the antidepressant induction of BDNF. These receptor sites must first be identified, although the βAR and the serotonin$_{4,6,7}$ receptor sites are likely candidates. Second, identification of other neurotransmitter receptors that stimulate cAMP- or Ca^{2+}-activated protein kinases in the hippocampus could be additional targets. Third, agents that directly stimulate cAMP- or Ca^{2+}-activated kinases, or that directly activate CREB, could be developed. Fourth, the inhibition of cAMP breakdown would enhance the function of CREB and increase BDNF expression. Fifth, agents that activate the BDNF-trkB signaling pathway may be of some use. The first 2 possibilities have the potential for regulating the function of CREB and the expression of BDNF specifically in the hippocampus, depending on the distribution of the receptors targeted, but may not be much more effective than available treatments. Conversely, the last 3 possibilities may be less specific because they would be expected to influence many brain regions as well as peripheral tissues that express these intracellular targets, but may be more effective. There has been legitimate concern regarding the specificity and safety of such agents. However, it is impossible to predict the effects of such agents until prototypical compounds are available for evaluation. Moreover, some agents, such as lithium and nonsteroidal anti-inflammatory agents (which affect arachidonic acid metabolism), are enormously effective for their respective indications despite their generalized actions.

The possibility that the inhibition of cAMP breakdown increases BDNF expression has been examined. Long-term administration of inhibitors of phosphodiesterases, the enzymes that metabolize cAMP, increases the expression of CREB and BDNF in the hippocampus of rats.[44] Moreover, coadministration of a phosphodiesterase inhibitor with a tricyclic antidepressant results in a more rapid induction of CREB and BDNF.[44] These findings provide additional support for the hypothesis that the cAMP system regulates the expression of BDNF and suggest that the inhibition of cAMP metabolism may provide a mechanism for a more rapid treatment of depression. The notion that the inhibition of cAMP metabolism may have antidepressant effects was raised several years ago[77] and was supported by preliminary clinical studies with rolipram, a potent phosphodiesterase inhibitor.[78] The lack of further progress on the therapeutic potential of phosphodiesterase inhibitors raises questions about the efficacy of these agents in the treatment of depression. However, given the recent observations, it may be time to reevaluate the therapeutic potential of phosphodiesterase inhibitors. One potential application that has not been investigated is the use of phosphodiesterase inhibitors as augmenting agents with other antidepressant treatments that block the reuptake or metabolism of monoamines. This approach could allow the cAMP system to be augmented in specific brain regions that contain the appropriate serotonin and NE receptors.

COMMENT

Although the regulation of CREB and BDNF may be important in the actions of antidepressant treatments, it would be preliminary and naive to suggest that these are the sole targets of these treatments. There are many genes that are known to be regulated by the cAMP system and certain antidepressant treatments.[70,79-86] In addition, the cAMP cascade is just one of many intracellular pathways that could be regulated by serotonin and NE and could be important to the action of antidepressant treatments. One example is the regulation of the phosphatidylinositol system and protein kinase C by lithium.[87-91]

The findings discussed herein indicate that antidepressant action is not mediated by the simple up- or down-regulation of monoamines and their receptors. Elucidation of the role of the cAMP system and BDNF in the response to antidepressant treatments provides fundamentally new leads in understanding antidepressant action and the pathophysiology of depression. Although the studies discussed provide a framework for a unifying hypothesis, it requires further testing of its several components. However, the model

incorporates the important role of the monoamines in these phenomena and begins to elaborate the many molecular and cellular mechanisms influenced by the monoamines that are more proximal to the complex adaptations in brain function that underlie depression and its treatment.

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