Neural Plasticity to Stress and Antidepressant Treatment

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Adaptations at the cellular and molecular levels in response to stress and antidepressant treatment could represent a form of neural plasticity that contributes to the pathophysiology and treatment of depression. At the cellular level, atrophy and death of stress-vulnerable neurons in the hippocampus, as well as decreased neurogenesis of hippocampal neurons, has been reported in preclinical studies. Clinical studies also provide evidence for atrophy and cell death in the hippocampus, as well as the prefrontal cortex. It is possible that antidepressant treatment could oppose these adverse cellular effects, which may be regarded as a loss of neural plasticity, by blocking or reversing the atrophy of hippocampal neurons and by increasing cell survival and function. The molecular mechanisms underlying these effects are discussed, including the role of the cAMP signal transduction cascade and neurotrophic factors. Biol Psychiatry 1999;46: 1181–1191 © *1999 Society of Biological Psychiatry*

Key Words: Norepinephrine, serotonin, neurotrophic factors, neurogenesis, cyclic AMP, phosphodiesterase, MAP kinase

Introduction

An evolving hypothesis of the pathophysiology and treatment of depression involves adaptation or plasticity of neural systems. Depression could result from an inability to make the appropriate adaptive responses to stress or other aversive stimuli. This could be attributed to dysfunction of the normal mechanisms underlying neural plasticity. Antidepressant medications may act by correcting this dysfunction or by themselves directly inducing the appropriate adaptive responses. A role for plasticity in the actions of antidepressant treatment has been recognized for quite some time. This is based on the observations that the therapeutic action of antidepressants requires long-term administration even though these treatments block the reuptake or metabolism of norepinephrine (NE) and serotonin (5-HT) much more rapidly. This suggests that adaptations or plasticity to the acute actions of antidepressants are required.

A role for neural plasticity also indicates that the underlying mechanisms for the therapeutic action of antidepressants, as well as the etiology of depression, are much more complex than simply changing synaptic levels of monoamines. Recent studies have begun to characterize adaptations of neuronal morphology and survival at the cellular level, and the intracellular signal transduction cascades at the molecular level, that underlie the response to antidepressant treatments. Our hypothesis proposes that these adaptations oppose the actions of stress and environmental factors, as well as genetic factors, that lead to depression. In this review, we will describe some of actions of stress and antidepressant treatments on hippocampal neurons and the intracellular mechanisms that are thought to underlie these effects.

Evidence for Neuronal Atrophy and Loss of Plasticity in Response to Stress

Stress is known to influence a wide range of neuronal systems that in the acute phase result in beneficial endocrine and behavioral responses; however, repeated or severe stress or increased vulnerability due to genetic factors can lead to adverse effects on neuronal function. The hippocampus is one brain structure that has been extensively studied with regard to the actions of stress, depression, and antidepressant actions. Dysfunction of the hippocampus could result in some of the vegetative and endocrine abnormalities, as well as cognitive and memory deficits, observed in depressed patients. Hippocampal neurons are reported to be damaged by exposure to stress or activation of the hypothalamic-pituitary-adrenal (HPA) axis and elevation of glucocorticoids.

Stress Results in Atrophy and Death of CA3 Pyramidal Neurons in the Hippocampus

CA3 pyramidal neurons in the hippocampus have been demonstrated to be extremely vulnerable to stress and

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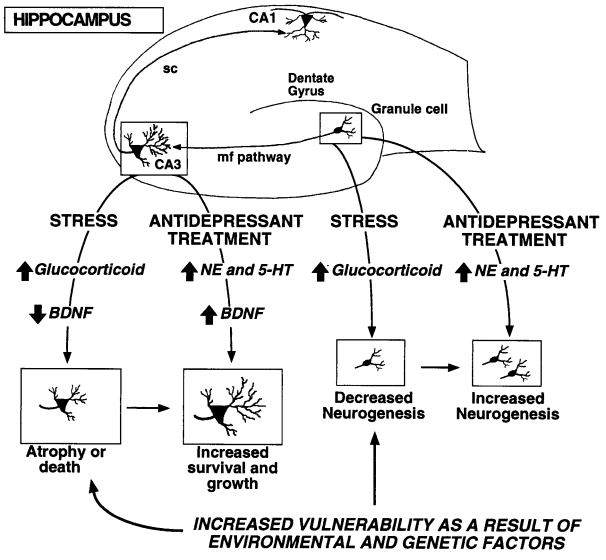

Figure 1. Diagrammatic representation of the actions of stress and antidepressant treatment on hippocampal neurons: A molecular and cellular hypothesis of depression. Stress or glucocorticoid treatments are reported to cause atrophy and, in severe cases, death of CA3 pyramidal neurons and to decrease neurogenesis of dentate gyrus granule cells in the hippocampus. These effects of stress and glucocorticoid treatments on CA3 neuronal atrophy and death are thought to be mediated by reduced glucose transport, increased glutamate and Ca^{2+} excitotoxicity, and decreased expression of BDNF. Glucocorticoids are known to contribute to the stress-induced down-regulation of neurogenesis. It is possible that these effects contribute to the atrophy of the hippocampus observed in depression. Individual vulnerability to stress and depression could result from environmental or genetic factors that influence neuronal atrophy and survival. This model also proposes that antidepressant treatments oppose these actions of stress and either block or reverse hippocampal atrophy and cell death. The mechanisms underlying such actions of antidepressants have not been well defined but may involve up-regulation of BDNF via increased NE and 5-HT signal transduction.

adrenal glucocorticoids (Figure 1) (for core references and complete reviews see Sapolsky 1996a; McEwen 1999). The adverse effects of chronic exposure to stress on these neurons can be subdivided into three areas. First, atrophy of CA3 neurons has been reported in both rodents and nonhuman primates after exposure to repeated restraint stress. This effect is also observed in response to administration of glucocorticoids at a dose that approximates levels that are induced by stress. Atrophy is demonstrated by a decrease in the number and length of branch points of the apical dendrites of CA3 neurons. Second, death of CA3 neurons has been reported to occur in response to severe and long-term stress or glucocorticoid treatment. Finally, neuroendangerment of CA3 neurons by exposure to stress has been demonstrated. In this case, acute exposure to stress or glucocorticoids exacerbates the damage of CA3 neurons caused by other neuronal insults, such as hypoglycemia, hypoxia, or excitotoxins.

Stress Decreases Neurogenesis of Dentate Gyrus Granule Neurons in the Hippocampus of Adult Animals

Although dentate gyrus granule neurons in the hippocampus appear to be relatively resistant to atrophy and death, stress is reported to decrease the birth or neurogenesis of these cells in adult animals (Figure 1). Although the capacity for new cell birth is not observed in most regions of the mature nervous system, the dentate gyrus is one of the few areas where adult neurogenesis has been demonstrated. Neurogenesis is studied by labeling the DNA of dividing cells with radiolabeled thymidine or a thymidine analogue, bromodeoxyuridine (BrdU). Cells can then be visualized by autoradiography or immunohistochemistry, respectively. Adult neurogenesis of granule cells has been reported in rodents and nonhuman primates and more recently in humans (Gould et al 1997, 1998; Ericksson et al 1998; see Greenough et al 1999). Progenitor cells located in the subgranular zone proliferate and migrate into the granule cell layer and hilus. Among the factors demonstrated to influence the rate of neurogenesis are glucocorticoids. Normal rates of neurogenesis, as well as death of granule cells, are dependent on physiological concentrations of glucocorticoids; however, acute stress or exposure to high levels of glucocorticoids decreases neurogenesis of granule cells (Gould et al 1997, 1998).

Although the exact function of neurogenesis has not been determined, increases in the rate of granule cell birth and survival of these neurons are associated with an enriched environment and training in models of learning and memory. Gage and colleagues have reported in two studies that exposure of adult mice to an enriched environment (increased contact with inanimate objects and enhanced social interactions with other mice) increases granule cell neurogenesis (Kempermann et al 1997; van Praag et al 1999). In addition, one study has demonstrated that training of animals in an associative learning task increases the survival of newly formed cells (Gould et al 1999). Although a similar study did not observe an increase of neurogenesis in response to a learning task, this could be related to the experimental design (see Greenough et al 1999). These studies raise the possibility that increased birth and survival of granule cells could contribute to learning, as well as other behavioral and endocrine functions under the control of the hippocampus. Moreover, down-regulation of neurogenesis in response to stress could contribute to deficits in the functional capacity of the hippocampus.

Clinical Studies Demonstrate Atrophy of Hippocampus in Depressed Patients and Other Mood Disorders

Clinical studies have demonstrated that the size and function of the hippocampus are reduced in patients with depression. Brain imaging studies have reported that the volume of the hippocampus is reduced in patients with depression or PTSD (Sheline et al 1996; Bremner et al 1999; also see Sapolsky 1996b for review). Decreased hippocampal volume is also reported in patients with Cushing's disease, indicating that elevated glucocorticoid levels underlies the reduced volume. This effect has also been shown to be reversible in patients with Cushing's disease, upon normalization of glucocorticoid levels. Studies are currently underway to determine if the volume reduction observed in depression is reversed in patients in remission. Elevation of cortisol levels in elderly patients also correlates with reduced hippocampal volume and is associated with memory deficits (Lupien et al 1998). Studies of hippocampal feedback inhibition of the HPA axis have also demonstrated a functional deficit in patients with depression (Young et al 1991). Glucocorticoid-induced loss of negative feedback could represent a downward cycle of reduced hippocampal function and damage that leads to a further loss of feedback inhibition and elevation of adrenal-glucocorticoids.

Although these clinical studies are consistent with basic research studies, more direct analysis of neuronal atrophy and survival in patients with depression is needed. Postmortem studies of the number of CA3 pyramidal cells and dentate gyrus granule cells in the hippocampus will be necessary to begin to characterize the cellular mechanisms that underlie the reduction in volume and function of the hippocampus.

The Number of Neurons and Glia in Prefrontal Cortex Are Reduced in Patients with Depression

Studies of the prefrontal cortex demonstrate that atrophy and death of neurons also occurs in other brain regions thought to be involved in depression and mood disorders. Brain imaging studies have demonstrated a reduction in blood flow and volume of the prefrontal cortex (Drevets et al 1997). Moreover, two recent studies report that the number of cells in prefrontal cortex are decreased in patients with depression. The first of these studies reports a reduction in the number of glia, but not neurons, in the subgenual prefrontal cortex of patients with major depressive disorder or bipolar disorder (Ongur et al 1998). A second study has reported a decrease in neuronal size and the number of neurons and glia in the prefrontal and rostral orbitofrontal cortex (Rajkowska et al 1999). These findings suggest that atrophy and survival of neurons may also contribute to certain symptoms of depression, such as depressed mood and working memory, that can be attributed to prefrontal cortex. Additional preclinical studies at the molecular and cellular levels are needed to understand how antidepressant agents may influence neuronal systems in the prefrontal cortex.

Molecular Mechanisms Underlying the Actions of Stress

The influence of stress and glucocorticoids on neuronal atrophy and survival could involve multiple complex and overlapping intracellular pathways. Studies of these pathways have identified three major effects, including uptake and metabolism of glucose, increased glutamate and Ca^{2+} excitoxicity, and down-regulation of neurotrophic factors, that could contribute to the actions of stress (for review see Sapolsky et al 1996a). A brief review of these mechanisms is provided here. In addition, it is possible that there is dysregulation of the pathways that control programmed cell death, or apoptosis, in depression. This could also account for loss of neurons and glia observed in prefrontal cortex of patients with depression. There are several reports demonstrating that lithium reduces apoptosis of cerebellar granule neurons in culture (Nonaka et al 1998; Chen and Chuang 1999). This is a potentially interesting area of research for studies of the pathophysiology and treatment of depression that warrants future consideration.

Influence of Stress on Glucose Uptake and Glutamate-Induced Excitoxicity

Glucocorticoids are known to influence glucose uptake and cellular energy in peripheral tissues, and similar effects could contribute to the influence of stress on neuronal atrophy and survival. Glucocorticoids also are reported to decrease the uptake of glucose in fat cells, and a similar effect is observed in primary neuronal cultures (Horner et al 1990). Although the exact mechanisms have not been determined in the brain, studies in fat cells demonstrate that glucocorticoid exposure decreases the expression of the glucose transporter and induces a translocation of the transporter from the cell membrane to an intracellular compartment (see Sapolsky 1996a). Both of these effects would contribute to a reduction in glucose uptake in cells. This could result in decreased cellular metabolism that could eventually cause neurotoxicity or produce a heightened state of neuroendangerment to other types of insult (e.g., excitotoxins, hypoxia, and hypoglycemia).

Another mechanism that is thought to be involved in the actions of stress and glucocorticoids is increased glutamate and calcium excitotoxicity. Stress or glucocorticoid

administration is reported to increase levels of glutamate in extracellular dialysate in the hippocampus (see Sapolsky 1996a). Glutamate, acting via NMDA and non-NMDA ionotropic receptors, increases intracellular levels of Ca^{2+}, and sustained activation of glutamate-induced Ca^{2+} is known to underlie the excitoxic effects of repeated seizures and ischemia. Based on these observations, it has been suggested that enhanced glutamate release could also contribute to glucocorticoid-induced neuroendangerment. Several questions remain to be answered regarding the role of glutamate and calcium in the actions of stress, however. For example, the expression of immediate early genes, such as Fos, that are typically induced in the hippocampus by stimuli that release glutamate (such as seizure or ischemia) are not induced by stress. Second, there is some evidence that elevation of glutamate could occur in response to placement of the dialysis probe (see Lowy et al 1995). In spite of these questions, elevation of glutamate and intracellular Ca^{2+} remains a viable potential mechanism that could contribute to stress- and glucocorticoid-induced endangerment and toxity of hippocampal neurons.

Expression of Brain Derived Neurotrophic Factor (BDNF) Is Down-Regulated by Stress

BDNF belongs to the neurotrophic factor family, which also includes nerve growth factor, neurotrophin-3, and neurotrophin-4/5 (Thoenen 1995). Neurotrophic factors were originally characterized based on their ability to influence the differentiation and development of specific populations of neurons in the nervous system. More recent studies demonstrate that these factors also influence the survival and function of neurons in the mature, adult brain. The expression of BDNF is induced in response to neuronal activity, for example, and has been shown to play a critical role in cellular models of learning and memory (i.e., long-term potentiation or LTP).

Evidence for the involvement of BDNF, and possibly dysfunction of other neurotrophic factor systems, in responses to stress have also been reported. Smith and colleagues have demonstrated that immobilization stress decreases the expression of BDNF in hippocampus (1995). In this report, acute or repeated exposure to immobilization stress decreased levels of BDNF mRNA in the major subfields of hippocampus (CA1 and CA3 pyramidal and dentate gyrus granule cell layers). We have replicated this effect and also found that other types of stress decrease the expression of BDNF (Nibuya et al 1999). Decreased expression of BDNF could contribute to the adverse effects of stress on hippocampal neurons (see below).

The mechanisms underlying the influence of stress on BDNF expression have not been fully characterized. Administration of corticosterone produces a small decrease in levels of BDNF mRNA in the dentate gyrus, but not in CA1 or CA3 pyramidal cell layers (Smith et al 1995). Removal of the adrenal glands does not block the down-regulation of BDNF in dentate gyrus, although the effects in CA1 and CA3 are attenuated. These results suggest that the down-regulation of BDNF can be explained in part by elevation of adrenal-glucocorticoids but suggest that other factors also contribute to this effect of stress. Another possibility is that monoamine systems, such as NE and 5-HT that are activated by stress, could influence the expression of BDNF. We have found that pretreatment with a $5\text{-}HT_{2A}$ antagonist (i.e., ketanserin or MDL 100, 907) reduces by approximately half the down-regulation of BDNF (Vaidya et al 1997, 1999). Although the exact mechanism has not been determined, stress-induced down-regulation of BDNF in the hippocampus by $5\text{-}HT_{2A}$ receptors could occur via activation of GABAergic interneurons that inhibit hippocampal neuronal activity (see Vaidya et al 1997 and 1999 for further discussion). Because some antidepressant drugs exhibit antagonist properties for $5\text{-}HT_{2A}$ receptors, it is interesting to speculate that blockade of BDNF down-regulation could contribute to the action of these drugs. Additional studies will be required to determine if the glucocorticoid and $5\text{-}HT_{2A}$ receptor mechanism mediate the entire stress response or if other factors also contribute to this effect.

Neuronal Plasticity in Response to Antidepressant Treatment

These reports of atrophy and cell death in stress and depression raise the possibility that the action of antidepressants may involve reversal or blockade of these effects or direct regulation of synaptic architecture, dendritic morphology, and survival of neurons. In support of this hypothesis, several studies have reported that antidepressant treatments exert positive actions on these cellular processes. These studies have focused on the hippocampus, and future work will be required to determine the influence of antidepressants on cell survival in prefrontal cortex and other brain regions.

Influence of Antidepressant Treatment on Atrophy of Hippocampal Neurons

The influence of antidepressant treatment on the atrophy of CA3 pyramidal neurons has been examined by McEwen and colleagues (Watanabe et al 1992). Their studies demonstrate that administration of an atypical antidepressant (tianeptine), but not a 5-HT selective reuptake inhibitor (fluoxetine), blocks the stress-induced atrophy of CA3 pyramidal cells. Tianeptine has the unusual property of

enhancing reuptake of 5-HT. Administration of either drug alone did not influence the morphology of the apical dendrites of these neurons. Further studies are required to determine if other classes of antidepressant drugs or different treatment regimens for the 5-HT selective reuptake inhibitors can block the atrophy of hippocampal neurons in response to stress.

Influence of Antidepressant Treatment on Hippocampal Neurogenesis

Another mechanism by which antidepressant treatment could oppose the actions of stress is via up-regulation of the neurogenesis of dentate gyrus granule neurons. Preliminary studies from our laboratory indicate that chronic, but not acute, antidepressant treatment increases neurogenesis of hippocampal granule cells (Duman and Malberg 1998). An increase in the number of BrdU-labeled neurons is observed in response to chronic administration of several different classes of antidepressants, including 5-HT and NE selective reuptake inhibitors, a monoamine oxidase inhibitor, and electroconvulsive seizures (ECS). Increased BrdU labeling in response to fluoxetine has also been observed by another group (Jacobs and Gould, Princeton University, unpublished observation). In a preliminary report, these investigators have also demonstrated that administration of a 5-HT1A agonist increases neurogenesis, suggesting that this receptor subtype could mediate the action of 5-HT (Jacobs et al 1998). Although additional studies are required to determine if up-regulation of BrdU labeling is a result of increased cell birth or survival and to determine if the labeled cells are neurons or glia, elevated neurogenesis provides another mechanism by which antidepressant treatment could oppose the actions of stress.

Influence of Antidepressant Treatment on Sprouting of Hippocampal Neurons

Granule neurons in the hippocampus are also reported to undergo sprouting in response to excitotoxin treatment and kindling paradigms, and we have found that chronic ECS administration also induces sprouting of granule cells (Vaidya et al 1999). This effect is dependent on repeated ECS treatment and is long lasting (e.g., observed up to at least 6 months after the last ECS treatment). Excitotoxin- and kindling-induced sprouting are thought to be, at least in part, adaptations in response to death of target neurons (see Vaidya et al 1999). In contrast, there is no evidence of cell loss or dying neurons in response to chronic ECS. Alternatively, we have reported that ECS-induced sprouting is significantly attenuated in BDNF heterozygous knock-out mice, which express half of the normal level of BDNF. In this study, infusion of BDNF alone was not sufficient to induce sprouting, suggesting that BDNF is necessary but not sufficient to produce sprouting. Chronic administration of antidepressant drugs does not influence sprouting of granule cells, suggesting that this effect may be specific to ECS. Further studies are required to determine the functional significance of increased sprouting in the actions of ECS.

Role of the cAMP Signal Transduction Cascade in the Actions of Antidepressants

One signal transduction cascade implicated in the action of antidepressant treatment is the cAMP pathway. Although early studies demonstrate that β-adrenergic receptor (βAR) coupling to this second messenger system is decreased, more recent studies show that the postreceptor, intracellular components of the cAMP cascade are up-regulated by antidepressant treatment.

Adaptations of the Intracellular Components of the cAMP Pathway

Evidence for up-regulation of the cAMP cascade in response to chronic antidepressant treatment has been demonstrated at several levels (Figure 2). First, one study reported that coupling of the stimulatory G protein, Gs, to adenylyl cyclase is increased by chronic antidepressant treatment (Ozawa et al 1991). Second, levels of cAMP-dependent protein kinase (PKA) in particulate fractions of limbic brain are reported to be up-regulated (Nestler et al 1989; Perez et al 1989). One study has also reported that levels of PKA in the nuclear fractions of cerebral cortex are increased, suggesting that gene expression is regulated by antidepressant treatment (Nestler et al 1989). This is supported by the finding that expression of the cAMP response element binding protein (CREB), a transcription factor that mediates many of the actions of the cAMP system on gene expression, is also up-regulated by chronic, but not acute, antidepressant treatment (Nibuya et al 1996).

The mechanisms underlying increased expression of CREB mRNA and protein have not been elucidated in vivo, but cell culture studies demonstrate that this could occur via activation of the cAMP system (Widnell et al 1994). This implies that there is a positive feed-forward mechanism that regulates the expression and function of CREB. It is also possible that altered expression or function of CREB could contribute to the pathophysiology of depression. A recent postmortem study has demonstrated that the expression of CREB is decreased in the temporal cortex of patients with depression and that antidepressant treatment reverses this effect (Dowlatshahi et al 1998). It is also possible that CREB is reduced in response to the depressive state, however. Additional studies are required to confirm this observation in a larger

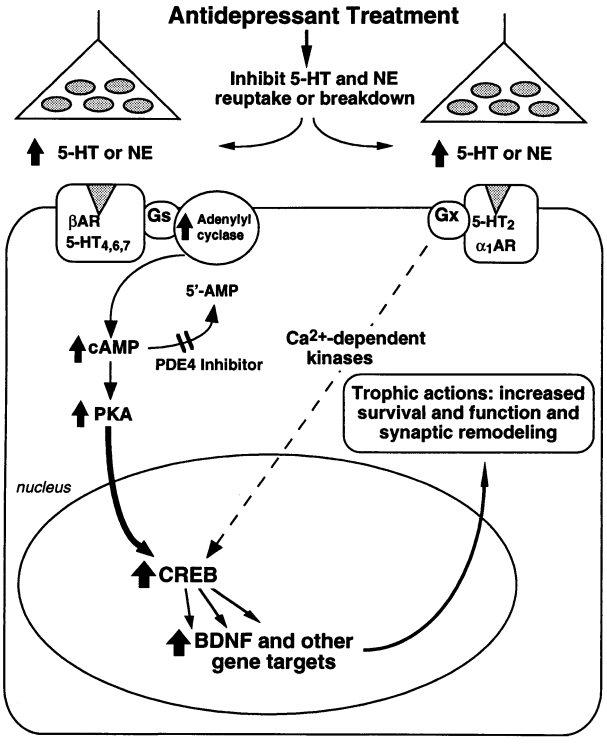

Figure 2. A model depicting the actions of antidepressant treatment on the cAMP signal transduction cascade. Chronic administration of several different classes of antidepressant is reported to up-regulate the cAMP system at several levels, including increased coupling of Gs and adenylyl cyclase, increased levels of cAMP-dependent protein kinase (PKA), and increased expression of cAMP response element binding protein (CREB). These findings suggest that the cAMP system and CREB may be a common postreceptor target of antidepressant treatment. In addition to activation of CREB by the cAMP system and receptors coupled to this pathway (e.g., βAR and 5-$HT_{4,6,7}$), CREB can also be activated by Ca^{2+}-dependent protein kinases and receptors linked to these pathways (e.g., 5-HT_2 and β_1AR). These findings suggest that up-regulation of the cAMP system may contribute to the action of antidepressant treatment. This possibility is supported by studies demonstrating that inhibitors of cAMP phosphodiesterase (PDE4), the enzymes responsible for breakdown of cAMP, have antidepressant efficacy in behavioral models and clinical trials. Up-regulation of CREB also suggests that antidepressant treatment influences the expression of specific target genes, one of which is brain derived neurotrophic factor (BDNF).

sample size, to determine if a similar effect is observed in other brain regions, such as the hippocampus, and to determine if decreased CREB is a state or trait effect.

Is CREB a Common Postreceptor Target of Antidepressant-Induced Neural Plasticity?

Up-regulation of CREB occurs in response to chronic administration of different classes of antidepressants, including both NE and 5-HT selective reuptake inhibitors, supporting the possibility that CREB could be a common postreceptor target of antidepressant treatment (Nibuya et al 1996). CREB is a particularly interesting and viable common target because it can be activated by several different signal transduction pathways. The transcriptional activity of CREB is increased when it is phosphorylated at Ser133. In addition to phosphorylation by PKA, which could occur via NE (βAR) or 5-HT (5-$HT_{4,6,7}$) receptors, CREB can be phosphorylated and activated by Ca^{2+}-dependent protein kinases, including both Ca^{2+}/calmodulin-dependent protein kinase and protein kinase C (Figure 2). These kinases could be regulated by NE and 5-HT receptors, such as the α_1-adrenergic or 5-HT_2 receptors coupled to the phosphatidylinositol system or by other receptors or ion channels that influence Ca^{2+} signaling. In addition, CREB can be phosphorylated by another kinase, referred to as rsk or CREB kinase, that is activated by the MAP kinase pathway (see below).

Although the studies cited support a role for the cAMP system and CREB in the action of antidepressant treatment, there are several points that must be addressed. First, it has been difficult, due to technical problems, to characterize the phosphorylation state of CREB in response to chronic antidepressant treatment. This is a critical point because it is possible that the expression of CREB is increased but that the phosphorylation state is reduced or unaltered. Studies are currently being conducted to address this point using CRE-lacZ transgenic mice that express a reporter gene under the control of a promoter-containing tandem CRE elements (Thome et al 1998). Preliminary studies demonstrate that chronic antidepressant treatment increases reporter gene expression in the CRE-lacZ mice, indicating that the phosphorylation and function of CREB are increased. A related question is how does an increase in the expression of CREB influence neuronal function and behavior. We are addressing this point by creating inducible and region-specific transgenic mice where the transgene is CREB or a dominant negative mutant of CREB (Chen et al 1998). This will allow us to regulate the expression of CREB or its negative mutant inhibitor in specific brain regions in adult animals and then test the functional consequences of altered CREB. These approaches will provide additional evidence to test the role of CREB in the actions of antidepressant treatment.

Activation of the cAMP Cascade as a Target of Antidepressant Treatment

The results of these studies indicate that activation of cAMP signaling and CREB could produce an antidepressant response. Support for this hypothesis has been provided by clinical studies with inhibitors of cAMP

phosphodiesterase (PDE4), the enzyme responsible for the breakdown of cAMP (Figure 2) (see Duman 1998 for review of basic and clinical studies). Administration of rolipram, a relatively selective cAMP PDE inhibitor, has been reported to have antidepressant efficacy in clinical trials, although this compound has not been developed because of its side effects. There are multiple isoforms of PDE4 that are inhibited by rolipram (see Conti and Jin 1999), however, and it is possible that selective inhibition of one of these could result in antidepressant actions without the side effects. We and others have provided evidence supporting a role of PDE4A and PDE4B (Ye et al 1997; Suda et al 1998; Takahashi et al 1999), although further studies are required to test the validity of these isozymes as targets for novel antidepressant medications. Another possibility for activating the cAMP cascade is via agonists for receptors directly coupled to this pathway, such as 5-$HT_{4,6,7}$ receptors. Stimulatory receptors, as well as intracellular sites, may represent novel targets for antidepressant drugs.

Adaptation of βAR-Stimulated cAMP Production

Although these more recent studies demonstrate up-regulation of the cAMP cascade, one of the first reported adaptations to antidepressants was down-regulation of βAR and the ability of these receptors to stimulate cAMP formation. This resulted in the βAR subsensitivity hypothesis, which stated that depression was a consequence of excess βAR and that antidepressants alleviate depression by reducing the number of receptors. In addition to the reports of an up-regulated cAMP cascade cited above, further evidence against this hypothesis has accumulated (see Duman et al 1997 for review). First, using more specific ligands, receptor binding studies have demonstrated that the down-regulation of βAR occurs much more rapidly (i.e., 1–3 days) than the therapeutic action of antidepressant treatment. Second, based on the subsensitivity hypothesis, antagonists of the βAR would be predicted to have antidepressant efficacy, but this has not been reported. In fact, there is evidence that βAR-antagonist treatment may actually produce depression in some patients. In contrast, there is evidence that increased expression and function of βAR may result in an antidepressant response. Administration of thyroid hormone, a treatment reported to increase the expression of βAR, is reported to have antidepressant efficacy.

Up-Regulated cAMP Cascade and Decreased βAR Coupling: Paradoxal Effects or Related Adaptative Responses?

The discovery that the cAMP cascade is up-regulated but that βAR-stimulated production of this second messenger is decreased appears to be contradictory; however, one problem with the βAR-stimulated cAMP assays is that they typically measure levels of receptor activation of the second messenger in an in vitro brain slice or homogenate. In these in vitro systems, the ongoing action of the antidepressant drug on levels of NE cannot be taken into account. In fact, endogenous NE is washed out of the preparations, and the influence of an exogenous agonist is determined. This is a critical point because it is possible that in vivo, even though levels of βAR are decreased by chronic antidepressant treatment, there are sufficient levels of the receptor remaining to produce an elevated intracellular response to the increased levels of NE (Figure 3). This could be the case when compared to the control condition, where there is a normal complement of receptor but where levels of NE are lower than under the antidepressant condition. Assuming this is the case, the responsiveness of the βAR-coupled cAMP system may be reduced relative to that observed in the presence of a maximum concentration of NE as reported in vitro, but in vivo the response would be higher than that observed in the absence of antidepressant treatment. Although there are no in vivo brain studies that address this hypothesis,

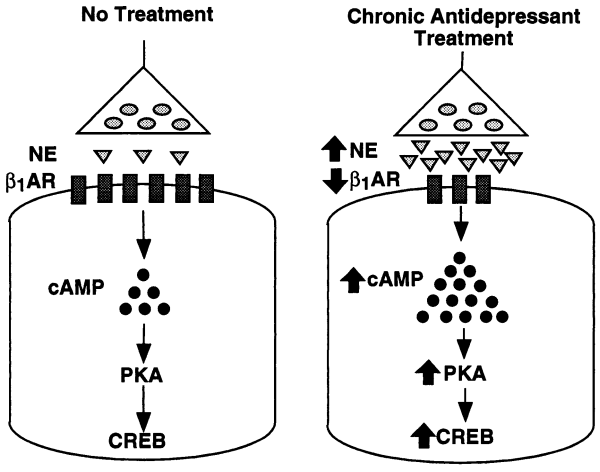

Figure 3. A model explaining the paradoxical up-regulation of the cAMP system and down-regulation of βAR binding sites in response to antidepressant treatment. In the absence of treatment, levels of NE and βAR-stimulated cAMP are relatively low. Chronic antidepressant treatment results in increased synaptic levels of NE and activation of βAR. This leads to down-regulation of βAR, although the receptors are not completely eliminated from the membrane. The model proposes that in the presence of elevated NE, there is sustained activation of the remaining receptors and increased levels cAMP relative to that observed in the absence of treatment. This is a hypothetical model that requires further testing, but it is consistent with results of studies demonstrating that the cAMP cascade is increased even though levels of βAR are decreased by antidepressant treatment.

reports on the influence of antidepressants on heart function are consistent with this idea. Basal heart rate is reported to be increased by chronic antidepressant treatment, even though the response to exogenous isoproterenol is decreased (Rosenstein and Nelson 1991; Roose et al 1998).

The notion that the cellular consequences of receptor activation cannot be predicted based on levels of receptor number is an important concept that could apply to many receptor systems. It is critical to remember that the cellular responses to receptor activation are also dependent on the in vivo levels of the neurotransmitter acting at the receptor, as well as the intracellular pathways that mediate the action of the receptor. These factors could apply to the interpretation of receptor binding studies in preclinical studies, as well as for human brain imaging studies of receptors.

Role of BDNF in the Action of Antidepressant Treatment

Up-regulation of the cAMP system and CREB suggests that antidepressant treatments regulate the expression of specific target genes. One gene target of interest is BDNF. Recent studies have provided evidence that up-regulation of BDNF may be involved in the actions of antidepressant treatment and that decreased expression of this neurotrophic factor could contribute to the negative influence of stress on certain neuronal systems.

Neurotrophic Factor Signal Transduction Cascade

The intracellular signal transduction pathways that mediate the actions of neurotrophic factors are fundamentally different from those for G protein coupled receptor-second messengers (Figure 4) (see Russell et al 1995). Neurotrophic factors act on receptors, referred to as Trks, that contain an extracellular binding domain and an intracellular tyrosinse kinase domain. There are at least three different Trk receptors that display some selectivity for the neurotrophic factors: TrkA for NGF, TrkB for BDNF, and TrkC for NT-3/NT-4/5. Binding of two molecules of neurotrophic factor results in receptor dimerization and activation of the intracellular tyrosine kinase domain of the receptor, resulting in phosphorylation of the receptor itself as well as other cellular effector proteins. Depending on the cell type, different effector pathways may be activated. These include phosphatidylinositol-3 kinase, phospholipase C-γ, and the MAP kinase signal transduction pathway. Activation of the MAP kinase pathway involves regulation of several intermediate steps. This includes tyrosine phosphorylation of adaptor proteins, Shc and Gab, and in conjunction with another adaptor protein, Grb, recruitment of Sos. Sos is a guanine nucleotide exchange factor for Ras, which leads to activation of Raf and the MAP kinases, also referred to as ERK1 and ERK2.

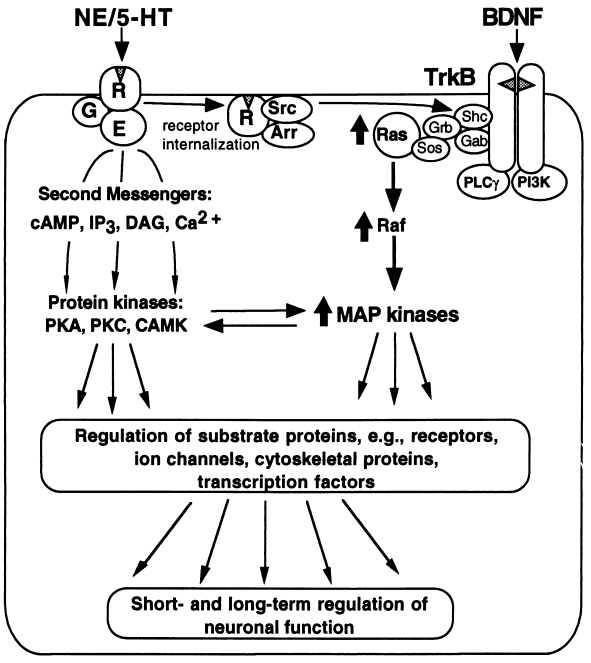

Figure 4. A model demonstrating the signal transduction pathways for neurotrophic factors. Neurotrophic factors, such as BDNF, utilize signaling pathways that are different from the second messenger-dependent systems (i.e., cAMP, IP_3, DAG, Ca^{2+}). Two molecules of BDNF bind to the TrkB receptor, inducing dimerization and tyrosine phosphorylation of the receptor itself as well as effector proteins. These include phosphatidylinositol-3 kinase (PI3K), phospholipase C-γ (PLCγ), and the MAP kinase pathway. Activation of MAP kinase occurs via several intermediate steps, including phosphorylation of Shc and Gab, then recruitment of the adapter protein Grb, and finally Sos. Sos is a guanine nucleotide exchange factor for Ras, which leads to activation of Raf and the MAP kinases. Internalization of βAR or other G protein coupled receptors can also lead to activation of the MAP kinase pathway. Internalization of the βAR is accompanied by binding of β-arresting (Arr), which acts as an adapter protein for a soluble tyrosine kinase, Src. Src is then able to substitute for TrkB and phosphorylate Shc and Gab, thereby activating the MAP kinase pathway. Antidepressant-induced internalization of βAR could lead to activation of the MAP kinase pathway, independent of the cAMP cascade.

The MAP Kinase System Can Also Be Directly Activated by Internalization of βAR and Other G Protein Coupled Receptors

In addition to the induction of BDNF expression via activation of cAMP and Ca^{2+} dependent pathways, βAR and other G protein coupled receptors are reported to activate the MAP kinases by a pathway that is independent of these second messenger systems (Figure 4) (see Luttrell

et al 1999). This alternate pathway is dependent on internalization of the βAR and recruitment of a soluble tyrosine kinase (Src) that directly phosphorylates the adaptor proteins (Shc and Gab) that lead to activation of Ras and subsequently MAP kinases. Internalization of βAR leads to binding of β-arrestin, which inhibits further G protein activation of the receptor. Recent studies demonstrate that β-arrestin also functions as an adaptor protein that binds both βAR and Src. This pathway could represent an alternate mechanism by which chronic antidepressant treatment, which results in down-regulation of βAR and other monoamine receptors, leads to activation of the MAP kinase pathway. Recent studies demonstrate that 5-HT_{1A} receptors, which are regulated by antidepressant treatment, activate the MAP kinase pathway, possibly via this mechanism (Mendez et al 1999). This could explain how 5-HT_{1A} receptors, which are negatively coupled to the cAMP system, could bypass the cAMP cascade and up-regulate the MAP kinase cascade. It should also be noted that regulation of MAP kinase via internalization of G protein coupled receptors may not be observed in all cases, indicating that there is receptor and/or cellular specificity in the control of this pathway.

Characterization of BDNF Gene Expression

The expression of BDNF in cultured cells is reported to be up-regulated by activation of the cAMP system (Nibuya et al 1995; Duman et al 1997). In addition, neuronal depolarization and activation of voltage sensitive Ca^{2+} channels are also reported to result in robust activation of BDNF expression, which could contribute to the synaptic alterations underlying the influence of BDNF on LTP. Recent studies have begun to characterize the promoter region of the BDNF gene responsible for cAMP and Ca^{2+} regulation (Shieh et al 1998; Tao et al 1998). BDNF is a complex gene of over 40 kb, which contains 5 exons. The first 4 exons contain putative promoter elements that control the expression of BDNF, and the last exon contains the entire coding region for BDNF protein. These studies demonstrate the presence of a CRE in the promoter region of exon III, which is reported to be the most highly regulated exon in response to neuronal depolarization. Activation of this element is mediated by CREB or a CREB-like protein. An additional element has also been identified that is thought to interact with CREB and thereby contribute to full and sustained activation of BDNF. The identity of this transcription factor has not been determined.

Expression of BDNF Is Up-Regulated by Antidepressant Treatment

A role for BDNF in the action of antidepressant treatment is supported by several lines of evidence. First, we have found that chronic administration of different classes of antidepressants increases the expression of BDNF in limbic brain regions, particularly the hippocampus (Nibuya et al 1995, 1996). These studies also demonstrate that antidepressant pretreatment blocks the down-regulation of BDNF in response to stress. Second, direct application of BDNF into the midbrain of rats is reported to have antidepressant effects in behavioral models of depression, including the forced swim and learned helplessness paradigms (Siuciak et al 1996). Third, BDNF is reported to be a potent neurotrophic factor for both the NE and 5-HT neurotransmitter systems (Sklair-Tavron and Nestler 1995; Mamounas et al 1995). These findings demonstrate that BDNF is a target of the cAMP system and antidepressant treatment and that BDNF is sufficient to produce an antidepressant response. Moreover, the results suggest that BDNF could influence monoamine systems via actions at either presynaptic sites (e.g., increased function of monoamine neurons) or postsynaptic sites (e.g., increased output of target neurons).

As discussed for CREB, additional studies will be required to further test the hypothesis that BDNF is an important and relevant target of antidepressant treatment. We are currently testing BDNF knock-out mice and inducible BDNF transgenic mice in neurochemical and behavioral paradigms of depression.

Summary and Conclusions

Preclinical and clinical studies of stress, depression, and action of antidepressant treatment have resulted in a molecular and cellular hypothesis of depression in which neural plasticity plays a major role (see Figure 1). These studies suggest that atrophy and death of neurons in the hippocampus, as well as prefrontal cortex and possibly other brain regions, could contribute to the pathophysiology of depression. This model also provides an explanation for individual vulnerability to stress and depression. Individual variation could result from genetic (e.g., altered expression or mutation of CREB or BDNF) or environmental factors (exposure to subtle neuronal insults, e.g., hypoxia, hypoglycemia, neurotoxins) that alone are not sufficient to cause neuronal damage and depression but that create a state of increased vulnerability to subsequent exposure to stress or other precipitating factors. Chronic antidepressant treatment, via up-regulation of CREB and BDNF, protect these neurons from further damage or possibly even reverse the atrophy and damage that has occurred. Further brain imaging and postmortem studies are needed to test this hypothesis.

The molecular mechanisms that underlie the actions of stress and antidepressant treatments are also being elucidated and could provide novel targets for therapeutic intervention. In addition to the cAMP cascade, the cellular

components of the TrkB-MAP kinase cascade could also be potential targets. The mechanisms that contribute to the actions of stress, including regulatory pathways for glucose uptake and metabolism and glutamate-induced excitotoxicity, have not been explored. It is also likely that the pathways described in this review represent only a rudimentary understanding of the intricate cellular mechanisms that underlie the etiology and treatment of depression. Continued characterization of these complex regulatory pathways and the genes they control are the long-term goals of future studies.

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