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Review Article

Signal Transduction and Mood Disorders

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Several theories regarding pathophysiology of mood disorders (depression or bipolar disorders) and the mechanisms of therapeutic agents (antidepressnts or mood stabilizer) have been proposed. Inhibition of monoamine reuptake into nerve endings by antidepressants is one of the cornerstones of the monoamine hypothesis on depression. Many studies have focused on alterations in levels of monoamines and their receptors. More recent studies have been extended to examination of the post-receptor intracellular targets. These include several classes of the guanosine triphosphate-binding proteins that couple receptors and effectors, adenylate cyclase and the inositol phosphate second messenger system. This review summarizes studies on signal transduction and neural plasticity in terms of mood disorders.

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Introduction

Guanine nucleotides binding proteins (G proteins) have been considered as modulators that proliferate signal information from receptors to effectors. G proteins related to membrane signaling have a hetrotrimetric structure (alpha, beta and gamma) and the alpha subunit has a GTP binding site involved in GTPase activity hydrolyzing GTP to GDP. Hence, the G proteins involve a distinctive family of protein molecules implicated in the apparatus of switching signal transduction on and off in the cell.[1] Bipolar disorders are characterized by two episodes of the manic and depressive states, and patients are in general considered to be in remission during the period between the two episodes. Therefore, it is plausible that some difficulty exists in emotional adjustment involving the switching mechanisms which rush or shrink neurotransmission in the brain. Taken together above, one might assume that there is a close link between G protein as the protein molecule in the on-off signal switching and bipolar disorders. Since the amine hypothesis on bipolar disorders was presented, many hypotheses on the etiology of mood disorders have been argued along with the complexity of relations among plural neural transmission systems, which contrast with the increase or decrease in solitary neurotransmission. One of the neurological bases of signal interaction is that different receptors share coupling with the same G protein. Then the signal input from each receptor occurs interactively via the G protein (crosstalk). It is also known that receptor sensitivity is diminished when the release of neurotransmitter is prolonged. In contrast, receptor sensitivity will be raised when the release of neurotransmitters is reduced. Thus, G protein has an important role in regulating receptor affinity to neurotransmitters to keep the temper stable in our social circumstances.

Alterations of biological hypotheses on mood disorders

Table 1 outlines changes in the biological hypotheses on mood disorders. More than 40 years ago, it was established that numerous antidepressants have the capability to inhibit the reuptake of monoamines or monoamine oxidase (MAO). Based on this evidence, the monoamine deficiency theory in depression was proposed. However, there is some disagreement in this theory as below.

(1) Not all antidepressants have the ability to inhibit the reuptake of monoamine or MAO activity.
(2) Not all monoamine reuptake inhibitors have antidepressive effects.
(3) Experiences with antidepressant therapy have revealed that, at clinical doses, it generally takes 2 to 3 weeks for a clinical response to take place, while reuptake inhibition and MAO inhibition can emerge instantly (within minutes) after a single dose of active compounds.

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Table 1. History of biological hypothesis on mood disorders

Period	Hypothesis	Mechanism of therapeutics
1960s	Monoamine deficiency	Monoamine reuptake inhibition
1970s	Receptor supersensitivity NA-ACH imbalance	β, 5HT2 receptor downregulation Muscarinic receptor inhibition
1980s	GABA dysfunction Dopamine dysfunction Second messenger dysregulation	$GABA_B$ receptor upregulation Dopamine receptor stimulation Second messenger effects
1990s	G protein function imbalance	Modulate G protein function
2000s	Neural plasticity Stem cell disorder	Upregulation of camp-CREB-BDNF Neurogenesis

In the late 1970s, many researchers paid attention on postsynaptic adaptation to chronic treatment with antidepressants. Downregulation of ß-adrenergic receptors or 5HT2A receptors is observed in many kinds of antidepressant administration for 1-2 weeks. In addition, ß-adrenergic agonist-stimulated cAMP production in rat brain slices decreases as well. These observations parallel the lag time for therapeutic advantage seen in the clinical treatment of antidepressants. Downregulation is believed to happen via the neuron's homeostatic mechanisms, in which prolonged exposure increases monoamine in the synaptic gap by reuptake inhibition or MAO inhibition of the antidepressant action. Conversely, second generation antidepressants, which do not inhibit monoamine reuptake or catabolism, also induce receptor downregulation with chronic administration. In consideration of this receptor downregulation, the receptor supersensitivty hypothesis in depression has been presented. This hypothesis appears to diametrically contradict the original monoamine hypotheses. If the receptor supersensitivty hypothesis is correct, specific blockers of these receptors might be clinically effective. However, few data support this hypothesis. Moreover, it is reported that selective serotonin inhibitors do not produce the receptor downregulation. Therefore, it seems unlikely that a specific neurotransmission system is just enhanced or reduced by the action of antidepressants. Furthermore, various neurochemical agents have been shown to act as potent antidepressants. These include selective serotonin uptake inhibitors, GABAergic agents, ß-adrenergic agonists, phosphodiesterase inhibitors, inositol (second messenger precursor), forskolin analogs (second messenger activators) and omega 3 fatty acids. Tricyclic antidepressants also act as neurotransmitter receptor-effector systems which include dopamine, Ach, GABA and certain peptides.

In addition, the mechanisms of action of antidepressants and the pathology of mood disorders involve receptor-G protein-effector function because G protein is a key element in the interaction of different neurotransmitters and play a critical role in neurotransmission (amplification and deamplication). More recent hypothesis will be fully described later.

Antidepressants and G proteins

General desensitization phenomena caused by agonist exposure involve a two-step reaction, i.e. functional uncoupling of the receptor and G protein in the early stage and receptor internalization in the late stage. However, no one has established that the mechanism of antidepressant-induced desensitization is the same as that of receptor-agonist stimulation. In contradiction to the development and recovery of ß receptor desensitization induced by agonist exposure, antidepressant-induced desensitization is a slow phenomenon. Furthermore, Dibner and Molinoff[2] reported that ß receptors in brain slices treated with desipramine caused further ß receptor downregulation in *in vitro* exposure to an agonist, suggesting that the mechanisms of desensitization phenomena may be different for antidepressant- and receptor-agonist-induced desensitization.

Accordingly, the role of ß receptor function in the human brain and the mechanisms of ß receptor downregulation should be reassessed from the viewpoint of downstream signal transduction.

The first report by Menkes et al.[3] demonstrated that long-term administration of various antidepressants enhances guanine nucleotide activation of adenylyl cyclase in rat cortex and hypothalamus membranes. They suggested that this augmentation of adenylyl cyclase is associated with a facilitated G protein-catalytic moiety of adenylyl cyclase. Their idea was not apparently in agreement with the opinion that antidepressant-induced subsensitivity of noradrenalin elicited cAMP production in rat brain slices. They explained this obvious inconsistency was due to alpha 2 adrenergic inhibition of adenylyl cyclase enhanced through G protein-catalytic moiety interaction, and that thereby the net cyclic AMP accumulation was decreased by noradrenalin. There are several reports that GTP analogs, NaF or forskolin stimulated-adenylyl cyclase activity is enhanced long-term antidepressant treatment of membranes. Interestingly, Newman and Lerer[4] reported bi-directional results for forskolin-simulated cAMP production of membranes and slice preparations, observing an increase in membranes and a decrease in slices. They suggested that forskolin interacted with different components of the adenylyl cyclase in the two preparations, reflecting the components of the adenylyl cyclase system distal from the receptor caused by chronic antidepressant treatment. Their report is suggestive in that many factors induced uninform results. Slice preparations have tighter coupling of receptors and G proteins than membrane preparations, and therefore seem to reflect receptor changes or the agonist receptor-G protein coupling state. In contrast with slice preparations, membrane preparations appear to reflect the G protein-effector state distal from the receptor. Even in membrane preparations there are complicated effects on the receptor-G protein-effector system.

If it is true that G protein mediated adenylyl cyclase is enhanced subsequently to long-term antidepressant treatment, which postreceptor components are key targets in the action of antidepressants? In general, adenylyl cyclase activity is based on the balance between stimulatory (Gs) and inhibitory (Gi) GTP binding protein function. We separately measured the stimulation and inhibition of adenylyl cyclase by GppNHp.[5,6] GppNHp dependent-stimulation, but not inhibition of adenylyl cyclase, increased without any change in affinity of guanine nucleotide to G proteins, suggesting that chronic antidepressant administration promotes increased coupling between

Gs and the catalytic unit of adenylyl cyclase.[5,6]

One might also expect that the change in the amount of G protein varies with antidepressant effects. Our reports demonstrated that guanine nucleotide binding is unaffected by chronic antidepressant treatment.[5,6] However, one study[7] reported that some antibodies to G protein alpha subunits measured by ELISA showed a change in the amount of G protein caused by chronic antidepressant treatment. Our study and others did not observe any changes in the quantity of Gs and Gi alpha sununits due to chronic treatment with antidepressants as determined by western blotting.[8,9] These studies do not seem to confirm whether the amounts of G proteins change. However, it is suggestive that the amounts of G proteins do not parallel the amount of mRNA; for example, in the brain there are 10 times more Gi and Go proteins than Gs protein, while mRNA of Gs is abundant. These results indicate that turnover of protein synthesis occurs more quickly in Gs than in Gi and Go. Thus, long-term adaptive action such as chronic antidepressant treatment does not seem to be reasonable for protein synthesis of Gs.

Acute effects of several antidepressants except for MAO-inhibitors, antipsychotics or anxiolytics, have demonstrated to increase [^{3}H]-GTP binding in a homogenate of rat cerebral cortex. These effects were partially inhibited by pertussis toxin, suggesting that target sites of antidepressants involve Gi- and Go-like proteins. Furthermore, the same laboratory also reported that the GTPase activity of purified Go and the ratio of [^{35}S]-GTPγS binding to the purified protein were increased by antidepressants, indicating that the drugs enhance the dissociate reaction of G protein heterotrimer subunits.[10] We also examined the *in vitro* effects of antidepressants on functional photoaffinity by labeling GTP binding protein. Saturation binding studies were performed by incubating membranes with increased concentrations of [^{32}P]-AAGTP, followed by UV irradiation and SDS-PAGE. The specifically bound isotherms for each of the G proteins studied showed characteristics of a one-site model. Scatchard analysis revealed increases in the Bmax and Kd of AAGTP binding for each of the G proteins (especially stimulatory G proteins) with the addition of antidepressants such as amitriptyline, clomipramine, desipramine and mianserin except for MAO inhibitors, antipsychotics or anxiolytics. These results suggested that drugs having antidepressive properties may directly affect G protein, especially Gs protein.[11]

Based on current evidence that cationic amphiphilic compounds, including neuropeptides, antidepressants and polyamine, directly modify G protein functions in a receptor-independent fashion, development of new drugs targeting G protein is under discussion for the treatment of neuropsychiatric diseases. Although it will be necessary to extend this preliminary work to a broad spectrum of psychoactive drugs in the near future, we believe the attempt to observe difference in the specificity and selectivity of action sites in each G protein of psychoactive drugs will contribute to the development of novel psycotherapeutics.

Human brain study in mood disorders

Our previous study reported that the quantity of various G protein subunits in postmortem brain samples from the parietal and temporal cortices was same in controls and depressive patients as demonstrated by western blotting.[12] However, photoaffinity GTP labeling of Gi/oα, but not Gsα, was significantly augmented in depressives in both cortex regions. In addition to basal activity, guanine nucleotide-, forskolin- and manganese-stimulated adenylyl cyclase activities were decreased. Besides, an increase in phosphodieseterase binding sites (PDE TYPE 4) was observed, suggesting that degradation of cAMP is enhanced in depression and suicide. Cowburn et al.[13] found that brains of suicide with depressive symptoms revealed a significant decrease to the same extent in basal, guanine nucleotide-, forskolin- and manganese-stimulated adenylyl cyclase activities despite an increase in Gs immunoreactivity. This finding agreed with the results of our study on unipolar depressive disorders, indicating reduced Gi in depressive brains. On the other hand, Young et al.[14] reported that the amounts of Gs- and forskolin-mediated adenylyl cyclase were enhanced in the postmortem human brain of bipolar disorders. These findings indicate that cAMP production through G protein and adenylyl cyclase plays a key role in the process of formation of the manic and depressive conditions, and in the difference between unipolar and bipolar mood disorders.

Imbalance hypothesis of G protein function in mood disorders

Many of the past hypotheses on manic-depressive illness finally have looked for causes of biological dysregulation of brain signaling which controls human emotion. Imbalances in the second-messenger system have been hypothesized in the pathophysiology of mood disorders.[15,16] Instability in the second-messenger signaling of emotions has suggested that mood disorders may be caused by functional disproportion of the two major second signaling systems, with depression resulting from hypofunction of cAMP pathways with absolute or relative dominance of the inositol phosphate (IPs) pathways, and mania resulting from contrasting conditions for depression, hypothesized from the mechanisms of action of therapeutic agents and peripheral tissue studies in mood disorders.[15,16]

On the basis of our and other pharmacological and human brain studies, we proposed the imbalance hypothesis of G protein function in mood disorders (Figure 1). cAMP production by adenylyl cyclase is regulated by the balance between Gs and Gi functions. In addition, Gi and Go-like proteins, which are ADP-ribosylated substrates of pertussis toxin, are presumed to activate phosphoinositide metabolism by Phospholipase C. Hence, our results denote that in proportion to Gs and Gi/o in depressives a state was observed, which occurred during hypofunction of the cAMP system and hyperfunction of the IPs system through distorted equilibrium of G protein functions. Antidepressants may have increased Gs function to normalize this imbalance. Since lithium has the potency to inhibit

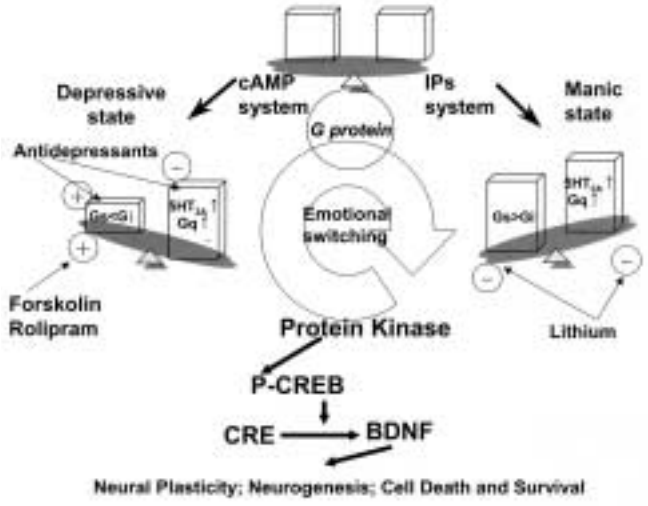

Figure 1. Illustration of the imbalance hypothesis of G protein function in mood disorders.

Gs as well as Gi, this compound suppresses both the manic and depressive states. On the other hand, antidepressants may induce an imbalance in Gs and Gi functions because they promote Gs function rather than other functions of G protein. Therefore, this may cause depression turn to mania and easily induce rapid-cycle formation. A recent postmortem brain study in bipolar mood disorders [17] suggested that there is an imbalance, compared to matched controls, between the activities of the phosphoinositide system via decreasing Gq function and the adenylyl cyclase system via increasing Gs function. Enhanced postsynaptic $5HT_{2A}$ receptor function in depressive patients was reported in the studies of postmortem brain and peripheral tissue. Moreover, it has been reported that chronic treatment with several antidepressants or SSRI can induce downregulation of $5HT_{2A}$ receptor function. Consequently, we hypothesize that the proportion of Gs and Gi/o is altered in the manic state that occurs during hyperfunction of the cAMP system and hypofunction of the IPs system via $5HT_{2A}$ receptor.

This hypothesis is based on the facilitation of Gi function in depressive and manic conditions. In the mammalian brain, Gi (inhibition of adenylyl cyclase) exists 10 times more than does Gs (stimulation of enzyme). As G protein level changes in the developmental period, persons who have a predisposition to absolute or relative dominance of Gi function compared to Gs function may be affected by some environmental or constitutional impacts during a critical period. Such persons may have a tendency to be altered symmetry of G protein function and they may suffer from mood disorders caused by emotional stress.

Transcription factor regulation in mood disorders

In recent times, it was reported that the cAMP response element binding protein (CREB), a transcription factor that mediates many of the actions of the cAMP cascade on gene expression, is upregulated by antidepressant treatment. These findings suggest that CREB is probably a common downstream target of antidepressant treatment.[18]

Dowlatshahi et al.[19,20] suggested that the CREB level in the postmortem temporal cortex was lower in antidepressant-free patients with major depressive disorder than in both antidepressant-treated patients with major depressive disorder and nonpsychiatric control subjects. Furthermore, it is known that chronic antidepressant administration increases the expression of CREB in rat hippocampus.[21] Thome et al.[22] demonstrated that chronic antidepressant administration increases CRE-mediated gene expression and CREB phosphorylation in mice brains. These studies show that the CREB level in the brain is decreased in the depressive state and is increased by chronic antidepressant administration. These previous findings and the present study suggest that alteration in CREB levels may be related to the pathophysiology of major depressive disorder. Meyer et al.[23] suggested that the expression of the CREB gene is positively autoregulated by CREB itself. The present report suggests that the reduced phosphorylation of CREB induces a decline of the transcriptional transactivational activities of CREB, leading to decreased transcription of CREB. Therefore, the decreased level of immunoreactivity of phosphorylated CREB found in postmortem brains of patients with major depressive disorder could lead to a decline in protein levels of CREB, inducing a continuous reduction in CREB-dependent transcription. More recently, we examined immunoreactivities of CREB and phosphorylated CREB in orbitofrontal cortices of human postmortem brains and compared antidepressant-free patients with major depressive disorder and nonpsychiatric control subjects. This was demonstrated by our observations that showed a significant decrease in the level of CREB and phosphorylated CREB in depressive patients. Furthermore, the decreased ratio of phosphorylated CREB immunoreactivity to CREB immunoreactivity in depressive subjects suggests that phosphorylation of CREB is probably impaired in brains of patients with the disease.[24]

A recent report[25] of increased hippocampal BDNF immunoreactivity in postmortem brains of subjects treated with antidepressant medication suggests that BDNF as well as CREB is involved in the pathophysiology of major depressive disorder and in the mechanism of antidepressant action. Yamada et al.[26,27] identified in rat brain a novel gene induced after chronic antidepressant treatment with the RING-H2 finger motif and cysteine string protein. These data may demonstrate a possible role of the altered gene expression in the mechanism of antidepressant action and may imply that antidepressants induce alteration in the neuronal network at the molecular level.

Figure 1 illustrates the imbalance hypothesis of G protein function in mood disorders explained in the last two chapters.

Conclusion

Despite the four decades of research, the molecular basis of mood disorders and the mechanisms of antidepressants are still not clear. However, it has been demonstrated that antidepressants, lithium,

ECT and others modify the coupling receptor-G protein-effector system, causing reorchestration of neurotransmitter dysregulation and neural circuits via upregulation of cAMP-CREB-BDNF signaling in mood disorders. In the future, there will likely be breakthroughs in genetic research in the components of signal transduction and in pharmacological studies on new agents which act directly on such components.

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