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Elucidating biological risk factors in suicide: role of protein kinase A

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Abstract

Suicide is a major public health concern. Although there have been several studies of suicidal behavior that focused on the roles of psychosocial and sociocultural factors, these factors are of too little predictive value to be clinically useful. Therefore, research on the biological perspective of suicide has gained a stronghold and appears to provide a promising approach to identify biological risk factors associated with suicidal behavior. Recent studies demonstrate that an alteration in synaptic and structural plasticity is key to affective illnesses and suicide. Signal transduction molecules play an important role in such plastic events. Protein kinase A (PKA) is a crucial enzyme in the adenylyl cyclase signal transduction pathway and is involved in regulating gene transcription, cell survival, and plasticity. In this review, we critically and comprehensively discuss the role of PKA in suicidal behavior. Because stress is an important component of suicide, we also discuss whether stress affects PKA and how this may be associated with suicidal behavior. In addition, we also discuss the functional significance of the findings regarding PKA by describing the role of important PKA substrates (i.e., Rap1, cyclic adenosine monophosphate–response element binding protein, and target gene brain-derived neurotrophic factor). These studies suggest the interesting possibility that PKA and related signaling molecules may serve as important neurobiological factors in suicide and may be relevant in target-specific therapeutic interventions for these disorders.

Keywords

Animal models; Depression; G proteins; Human postmortem brain; Protein kinase A; Signal transduction; Suicide

Introduction

Suicide is a major public health concern. Approximately one million people commit suicide worldwide each year, and 30,000 people commit suicide in the United States alone (Goldsmith et al, 2002). The lifetime suicide attempt rate among adults is approximately 10% (Minino and Smith, 2002; Bertolote, 2001); among adolescents, suicide is the third leading cause of death after motor vehicle crashes and homicide (National Center for Health Statistics, 1992). Previously, most of the studies of suicidal behavior were focused on the

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roles of psychosocial and sociocultural factors; however, these factors are of too little predictive value to be clinically useful. Therefore, research on the biological perspective of suicide has gained a stronghold and appears to provide a promising approach for identifying biological risk factors associated with suicidal behavior.

Recently, it has been suggested that the pathogenesis of mood disorders/suicide involves altered plasticity of neuronal pathways (Garcia, 2002). In fact, it has been proposed that mood disorders/suicide result from an inability of the brain to make appropriate adaptive responses to environmental stimuli as a result of impaired synaptic and structural plasticity (Duman et al, 2000; Dwivedi et al, 2005a; Dwivedi, 2010). Support for this idea comes from studies demonstrating altered brain structure during stress and depression (major components of suicidal behavior) and from suicidal patients. The alterations include reductions in cell number, density, cell body size, and neuronal and glial density in frontal cortical or hippocampal brain areas; and decreases in the parahippocampal cortex and cortical laminar thickness (Altshuler et al, 1990; Rajkowska, 1997; Bremner et al, 2000; Rosoklija et al, 2000; Cotter et al, 2002; Rajkowska, 2002). In addition, changes in synaptic circuitry (Aganova and Uranova, 1992), synaptic connectivity (Honer, 1999), number and shape of dendritic spines (Hajszar et al, 2005), synapse formation (McEwen, 2000), neuronal atrophy (Sheline, 2000), and spatial cognition deficits (Sackeim, 2001) have been reported in depression and/or during stress. The cellular mechanisms that underlie such compromised neural plasticity and structural impairments in mood disorders and suicide are not clearly understood; however, recent findings from our own laboratory and from others provide evidence that such changes may be a consequence of disruption in the cellular mechanisms governing structural/neural plasticity and cellular resilience (Young, 2001; Nestler et al, 2002; Dwivedi et al, 2003; Joëls et al, 2004; Dwivedi, 2005; Duman and Monteggia, 2006; Dwivedi et al, 2006a, 2006b; 2009).

A number of studies suggest the involvement of serotonergic, and to some extent, noradrenergic systems in suicide, which include alterations in levels of serotonin and norepinephrine, their metabolites, and the receptors to which these neurotransmitters bind in the brain and peripheral tissues of subjects who have committed suicide (hereafter referred to as “suicide subjects”) (Pandey and Dwivedi, 2006, 2007; Stockmeier, 1997). In addition to these neurotransmitter receptors, downstream signal transduction pathways play a critical role in regulating neural plasticity by integrating various physiological processes, which, in turn, are important for behavioral and environmental adaptations. Given the critical importance of intracellular signaling pathways in such behavioral and environmental adaptations, recent studies have focused on the role of various signaling molecules in the pathophysiology of mood disorders and suicide (Dwivedi, 2005a; Pandey and Dwivedi, 2006).

Of various signaling pathways, adenylyl cyclase–cyclic adenosine monophosphate (cAMP) signaling is one of the best-characterized signal transduction mechanisms in the central nervous system (CNS). In this signaling pathway, protein kinase A (PKA) is a key phosphorylating enzyme, which, on activation by cAMP via G protein/adenylyl cyclase, triggers a wide variety of physiological responses in the brain that are important for cell survival, synaptic plasticity, and activation or repression of gene expression (Borrelli et al, 1992; Nestler and Greengard, 1994). This review focuses on the role of PKA in suicidal behavior. Because stress is an important component in suicidal behavior, we discuss whether stress regulates PKA and whether changes in PKA in suicide subjects are a consequence of the stress response. Briefly, we also discuss molecules that are substrates of PKA or whose expression is regulated by PKA. Specifically, we will discuss PKA substrate Rap1, cAMP response element binding (CREB), and target gene brain-derived neurotrophic factor (BDNF) in the context of their role in suicide.

Adenylyl cyclase–cAMP pathway: Role of G proteins in modulating PKA and their role in suicide

Guanine nucleotide-binding proteins (G proteins) occupy a central position and play a critical role in the transduction of extracellular signals to cellular targets (Neer, 1995; Clapham and Neer, 1997; Hamm, 1998; Freissmuth et al, 1999; Neves et al, 2002). Approximately 80% of the receptors for neurotransmitters, hormones, and neuromodulators elicit their responses through G proteins, which are heterotrimers consisting of three subunits (α , β , and γ), each encoded by a specific gene. The β and γ subunits bind tightly to each other, and the β subunit also contains a common binding site for α subunit recognition. The α subunit binds to guanosine triphosphate (GTP) and confers receptor-effector specificity to G proteins. The γ subunit has been reported to have a G protein-specific recognition site. In the resting state, guanosine diphosphate (GDP) is bound to the α subunit, and the three subunits (α , β , and γ) are associated as a trimer. Receptor-mediated activation of G proteins causes the release of GDP from the α subunit, allowing GTP to bind to and induce the dissociation of the G protein α subunit from the $\beta\gamma$ subunits. The α and $\beta\gamma$ subunits can then activate various effectors to modulate cellular responses (Figure 1). Based on complementary DNA sequencing and the similarity of amino acid sequences, $G\alpha$ subunits have been classified into four major classes: G_s , G_i , G_q , and G_{12} . More than 16 distinct genes encode the G protein α subunits, and there is a splice variant in at least two genes (Gilman, 1987; Simon et al, 1991; Neer, 1995; Hamm, 1998). A total of 5 distinct β subunit genes and 12 γ subunit genes have also been identified (Hildebrandt, 1997). Once activated, both α and $\beta\gamma$ subunits can activate or inhibit multiple effectors to modulate cellular responses. Of the various $G\alpha$ isoforms, $G_s\alpha$ stimulates adenylyl cyclase, whereas $G_i\alpha$ mediates the inhibition of adenylyl cyclase. The activation of adenylyl cyclase catalyzes the conversion of adenosine triphosphate to cAMP. Cyclic AMP serves as a second messenger and activates the phosphorylation enzyme PKA. Once activated, PKA phosphorylates various intracellular proteins and thereby modifies hormonal and neurotransmitter responses, including receptor downregulation or desensitization, alteration of neurotransmitter release, and activation or repression of gene expression (Borrelli et al, 1992; Nestler and Greengard, 1994). The response generated by cAMP can be terminated by the hydrolysis of cAMP into 5'AMP by phosphodiesterases or by removal of the phosphate group by protein phosphatases. The activity of phosphodiesterases can be enhanced through phosphorylation by PKA, thus causing an abrupt termination of the signal (Beebe, 1994).

Because G proteins play a critical role in modulating adenylyl cyclase activity and, therefore, cAMP formation, there have been some studies elucidating the role of G proteins in depression and, to some extent, suicidal behavior. These studies include examination of either expression of G protein subunits or G protein-mediated functional response in peripheral tissues and human postmortem brain. For example, Cowburn et al (1994) reported that basal, GTP γ S-stimulated, or forskolin-stimulated adenylyl cyclase activity is decreased in the cerebral cortex of depressed patients. In another study, Ozawa et al (1993) found that AAGTP binding to $G_{i/o}\alpha$ was decreased in the temporal and parietal cortices of depressed patients. Avissar et al (1997) reported that levels of $G_s\alpha$ and $G_i\alpha$ were significantly decreased in mononuclear leukocytes of depressed patients. This was associated with hypofunctional $G_s\alpha$ and $G_i\alpha$, as measured by β -adrenergic and muscarinic cholinergic agonist-stimulated G protein binding capacity. On the other hand, Karege et al (1998) measured the levels of $G_q\alpha$, $G_i\alpha$, and $G\beta$ and found that levels of all these proteins were elevated in the platelets of depressed patients. To our knowledge, no change in the levels of $G_s\alpha$ or $G_i\alpha$ in the granulocytes (Spleiss et al, 1998) or in mononuclear leukocytes (Young et al, 1993) of depressed patients has been reported.

In a large postmortem brain study, we examined the expression levels of G proteins in suicide. We found a significant decrease in both messenger RNA (mRNA) and protein levels of $G_{12}\alpha$ and a significant increase in levels of $G_s\alpha$ in the prefrontal cortex (PFC) of suicide subjects compared with nonpsychiatric control subjects. These changes were present in all suicide subjects regardless of psychiatric illnesses. Similar results were reported by Pacheco et al (1996), showing a significant increase in $G_s\alpha$ in Brodmann area 10 and a decrease in $G_{12}\alpha$ in Brodmann areas 8 and 9 of depressed suicide subjects. Although the study results in depressed subjects are somewhat inconsistent, it appears that suicide brain exhibit hyperfunctional adenylyl cyclase–cAMP activity.

Because most of the effects of cAMP are mediated by its receptor PKA, and because a number of studies suggest that PKA is regulated by sustained activation of cAMP (Spaulding, 1993; Francis and Corbin, 1999), studying the status of PKA provides direct evidence of altered cAMP signaling. In addition, PKA participates directly in many physiological functions in the CNS; by phosphorylating the components of other signaling cascades, it provides the means for cross talk between the adenylyl cyclase–cAMP and other signaling systems (Beebe, 1994; Bornfeldt and Krebs, 1999; Jordan et al, 2000). Therefore, a number of recent studies have focused on the role of PKA in depression and suicide.

PKA: A critical phosphorylating enzyme in the adenylyl cyclase–cAMP signaling pathway and its role in suicide

Protein kinase A was one of the first protein kinases to be discovered (Walsh et al, 1968). As shown in Figure 1, PKA is a holoenzyme composed of two distinct subunits: regulatory (R) and catalytic (C). These regulatory and catalytic subunits form a tetrameric holoenzyme (R_2C_2). In the absence of cAMP, PKA exists as a stable inactive tetramer. The catalytic activity of cAMP is suppressed when the C subunits form a complex with the R subunits. The C subunit is initially phosphorylated by phosphoinositide-dependent kinase at threonine 197 (Cauthron et al, 1998; Cheng et al, 1998) in the activation loop. This phosphorylation is necessary for the maturation and optimal biological activity of PKA (Adams et al, 1995; Steinberg et al, 1993). After an increase in intracellular cAMP, the regulatory PKA subunits bind to cAMP in a cooperative manner, which results in the conformational change in the regulatory subunits, leading to the disassociation of the holoenzyme into an $R_2(cAMP)_4$ dimer and two monomers of catalytically active C kinase.

The R subunits remain in the cytoplasm, and the free catalytic subunits either translocate into the nucleus (Wolf et al, 1999) or remain in the cytosol. In both locations, PKA C subunits phosphorylate serine and threonine residues of specific substrates (Scott et al, 1990). In the nucleus, PKA regulates the transcription of genes by modulating the activity of cAMP-responsive nuclear factors. One of the most important cAMP-responsive nuclear factors is CREB, which binds to and regulates the expression of genes containing a CREB consensus in their promoter region. Brain-derived neurotrophic factor is one such crucial gene, whose transcription is regulated by PKA/CREB and whose role in depression and suicide has recently been demonstrated. In addition to its role in gene transcription, PKA is capable of phosphorylating many substrates involved in neurotransmitter release; receptor desensitization; cell growth, differentiation, and survival; and synaptic plasticity (Borrelli et al, 1992; Nestler and Greengard, 1994; Lara et al, 2003; Abel and Nguyen, 2008).

Based on the elution profile on diethylaminoethyl cellulose exchange chromatography, two major forms of PKA have been identified (i.e., types I and II). These two types differ in their structure in the regulatory subunits incorporated, termed RI or RII, whereas their catalytic subunits are either identical or very similar. The R subunits are highly dynamic multidomain proteins. Cloning studies have revealed multiple isoforms for each regulatory subunit: two

RI subunits (RI α and RI β) and two RII subunits (RII α and RII β). The RI α and RI β isoforms have 80% homology, whereas the RII α and RII β isoforms show 68% homology. The different PKA subtypes (types I and II) have different affinity for cAMP. The RI subunits have much greater affinity for cAMP than the RII subunits. The RI α -containing holoenzymes are activated at a five-fold lower concentration of cAMP than the RI β -containing holoenzymes (Cadd et al, 1990). Each regulatory subunit is a separate gene product and has a distinct expression pattern in different tissues (Scott et al, 1987; Clegg and McKnight, 1988; Skälhegg and Tasken, 2000). For example, tissue distribution studies suggest that RI α is ubiquitously distributed and that RII α is expressed in brain and testis. Similarly, RI β is ubiquitously distributed and RII β is predominantly expressed in brain, adrenal, and adipose tissues and is the principal mediator of cAMP activity in the mammalian CNS (Sarkar et al, 1984).

Three distinct catalytic subunits have been identified: C α , C β , and C γ . The importance of the presence of the various C subunits is not clear; however, it has been shown that C β -containing PKA holoenzymes dissociate more easily on binding to cAMP than those containing C α (Gamm et al, 1996). Also, these isozymes form either homodimers or heterodimers of the R subunits, yielding holoenzyme complexes of PKA composed of a number of different combinations, including RI α ₂C₂, RI β ₂C₂, RII α ₂C₂, RII β ₂C₂, and RI α RI β C₂. The presence of multiple C subunits adds substantial diversity to the action of PKA. Tissue distribution studies suggest that C β is expressed primarily in the brain (Uhler et al, 1986), whereas C α is ubiquitously expressed. C γ is present only in testis. Both C α and C β subunits exhibit alternative splicing at the first exon, resulting in amino terminal differences between transcript variants.

PKA and Suicide

In a comprehensive study, we examined the role of PKA in suicide. We determined cAMP binding to regulatory subunits of PKA and catalytic activity of PKA in the PFC of adult suicide subjects and well-matched nonpsychiatric healthy control subjects (Dwivedi et al, 2002). We observed that cAMP binding to regulatory subunits of PKA was significantly decreased in the PFC of suicide subjects; however, there were no significant differences in the affinity of cAMP binding to these subunits. In addition, we observed that PKA activity was decreased in the PFC of adult suicide subjects compared with healthy control subjects in both the presence and the absence of cAMP. This suggests that decreased activation of PKA is not dependent on less availability of cAMP but on other factors, such as altered expression of certain catalytic and/or regulatory subunits. To confirm this finding, in a different cohort of brain samples, we studied similar paradigms in postmortem brain of suicide subjects and well-matched healthy control subjects obtained and found similar changes in cAMP binding and PKA activity in the PFC of suicide subjects (Dwivedi et al, 2002) as previously reported (Dwivedi et al, 2004).

To examine if the decreases in PKA activity and cAMP binding in postmortem brain of suicide subjects are associated with changes in the expression of specific catalytic and/or regulatory subunits, we determined the protein and mRNA expression of all the catalytic and regulatory subunits of PKA in the same postmortem brain samples in which we studied cAMP binding and PKA activity (Dwivedi et al, 2004). We observed interesting results in both cohorts of brain samples. For example, there was a significant decrease in mRNA and protein expression of selective regulatory RII β and catalytic C β subunits in the PFC of suicide subjects. No changes were observed in expression without the RI α , RII α , RI β , or C α subunits (Dwivedi et al, 2004). These findings were independently confirmed by Odagaki et al (2001). These results suggest that decreases in cAMP binding and PKA activity could be

due to decreases in the gene expression of $RII\beta$ and $C\beta$, respectively. Changes in the expression of catalytic and regulatory subunits of PKA are quite specific.

Recently, to examine whether the changes in PKA in adult suicide are similar to those in the teenage suicide population, we conducted similar studies in postmortem brain of teenage suicide subjects (Pandey et al, 2005). The rationale behind this determination was that the characteristics and risk factors for teenage suicide may be similar to those of adult suicide in some respects but not in others. One distinct difference is that teenage suicide is driven primarily by impulsive aggressive behavior (Brent et al, 1993, 1996). Similarly, as in the adult population, we found that PKA activity was decreased in the PFC of teenage suicide subjects. However, when we determined the protein and gene expression levels of PKA subunits, a different pattern of changes in expression of PKA emerged. Although the protein and mRNA expression levels of $RII\beta$ and $C\beta$ were decreased in the PFC of adult suicide subjects, the data in the PFC of teenage suicide subjects showed that expression levels of $RI\alpha$ and $RI\beta$ (not $RII\beta$ or $C\beta$) were significantly decreased. This is quite distinct from what we observed in the adult population. When teenage suicide subjects were divided into those with a history of major mental disorders and those with no such history, no significant differences in PKA activity or protein and mRNA expression of any of the subunits were noted between the two groups (Pandey et al, 2005), suggesting that the observed changes were not related to specific mental disorders but to suicide. Certain questions remain. Why are different regulatory and catalytic subunits altered in teenage vs adult populations? Is this related to specific behavioral characteristics, such as affective disorders or impulsive aggressive behavior? There is currently no explanation for such contrasting results in teenage vs adult suicide; overall, there is a decrease in PKA activation in the brain of both these populations. Further molecular studies are required to delineate this issue. Recently, Shelton et al (2009a) reported that PKA activity is decreased in the PFC of depressed subjects. In another study, these investigators show that melancholic depressed subjects show lower PKA $RI\alpha$ and $C\beta$ expression in the frontal cortex, whereas those who died by suicide show lower PKA $RI\alpha$ and Ca expression (Shelton et al, 2009b). Thus, whether a decrease in specific PKA subunits is related to specific clinical phenotype needs to be further examined.

Stress and PKA

Stress is an important component of suicidal behavior, and a number of studies suggest a strong relationship between a hyperactive hypothalamic-pituitary-adrenal axis and suicidal behavior (Clayton, 1985; Monk, 1987; Arato et al, 1989; Lopez et al, 1992; Hiroi et al, 2001; Van Heeringen et al, 2000). Given the role of stress in suicidal behavior, we examined whether stress affects PKA in different animal models.

Effect of Glucocorticoids on PKA

In rat brain, we examined the following effects: (1) increased endogenous glucocorticoids and (2) bilateral adrenalectomy and supplementation with exogenous glucocorticoid on various measures of PKA (Dwivedi and Pandey, 2000). The rats were implanted with corticosterone pellets containing different concentrations of corticosterone (50- and 100-mg pellets releasing 2.38 and 4.76 mg/d of corticosterone, respectively). Rats were sacrificed on day 1, 4, or 14. In a separate experiment, an adrenalectomy was performed and these adrenalectomized rats were implanted with different doses of corticosterone pellets. When the rats were implanted with a 50- or 100-mg corticosterone pellet, the level of corticosterone on day 14 was greater than on day 4 and was dose dependent; the serum level of corticosterone was lower with the 50-mg dose vs the 100-mg dose. We could not detect any endogenous corticosterone in adrenalectomized rats on either day 4 or day 14 after adrenalectomy. However, after simultaneous implantation of corticosterone pellets,

adrenalectomized rats showed a reversal in the level of corticosterone; with the 100-mg dose, the level of corticosterone was almost reversed to the normal level, both after 4 and 14 days of treatment. We observed that short-term treatment of corticosterone (day 1) had no significant effects on any measures of PKA. However, 4 days of corticosterone treatment significantly decreased cAMP binding to the regulatory subunit of PKA and catalytic activity of PKA in the frontal cortex and hippocampus. We observed much more profound effects on cAMP binding to the regulatory subunit of PKA and the catalytic activity of PKA after 14 days of corticosterone treatment. These effects were also dose dependent. A higher dose of corticosterone (100-mg pellet) was much more effective in causing changes in PKA than a lower dose (50-mg pellet). Adrenalectomy produced the opposite results. For example, adrenalectomized rats showed increased PKA activity and cAMP binding in both the frontal cortex and hippocampus in a time-dependent manner. These changes were reversed by corticosterone treatment in a dose-dependent manner. The higher dose of corticosterone completely reversed the observed changes in PKA after adrenalectomy (Dwivedi and Pandey, 2000). Interesting results were noted when we examined the expression levels of regulatory and catalytic subunits: mRNA and protein expressions of RI α , RI β , and C β isoforms were significantly decreased in the cortex and hippocampus after corticosterone treatment. Adrenalectomy increased the expression of these subunits, and corticosterone treatment of adrenalectomized rats reversed the adrenalectomy-induced changes in PKA RI α , RI β , and C β isoforms (Dwivedi and Pandey, 2000).

Thus, our studies demonstrate that stress modulates PKA, and this effect could be at the transcription level because the expression levels of selective isoforms of PKA regulatory and catalytic subunits are altered. These findings are quite similar to those we observed in human postmortem brain of suicide subjects.

Effect of Learned Helpless Behavior on PKA

Because the ability to cope with stress is critical for humans, parallel studies of the effects of uncontrollable stress have been performed in animals, with the result of proactive interference with the acquisition of escape/avoidance responses (Seligman and Maier, 1967). This phenomenon is termed *learned helplessness* (LH) and has been used extensively as an animal model of stress-induced behavioral depression, which is a common risk factor in suicidal behavior (Petty and Sherman, 1979; Sherman et al, 1982).

By using this model, we explored whether PKA is altered in brain of LH rats and whether the changes are similar or dissimilar to suicide brain. For this, we examined PKA after acute or repeated shock stress. Rats were exposed to inescapable shock either once (on day 1) or twice on days 1 and 7) and were tested for escape latency on days 2 and 4 after day 1 inescapable shock or days 2, 8, and 14 after days 1 and 7 inescapable shock. Our behavioral and analytical findings demonstrate selective and significant changes in PKA in the brain of rats after acute shock stress that persists when the stress is repeated. The higher escape latency was present in LH rats exposed to inescapable shock on day 1 and tested for escape latency on day 2, but not in those rats tested on day 4, suggesting that the behavioral deficit does not persist for 4 days after a single inescapable shock paradigm. Thus, our observations suggest that exposure to a second inescapable shock produces a longer-lasting behavioral deficit. Interesting results emerged when we determined the measures of PKA in the brain of LH rats. The LH rats, given inescapable shock on day 1 and tested on day 2, showed a significant decrease in [3 H]cAMP binding in the frontal cortex and hippocampus. Similarly, both basal and cAMP-stimulated PKA activities were significantly decreased in the frontal cortex and hippocampus of these LH rats. When [3 H]cAMP binding and basal and cAMP-stimulated PKA activity were measured in the frontal cortex and hippocampus of LH rats that were tested on day 4 after inescapable shock, the changes in these measures had disappeared. On the other hand, in LH rats given inescapable shock twice (once on days 1

and 7) and tested on day 14, both [^3H]cAMP binding and basal and cAMP-stimulated PKA activity were significantly decreased; the magnitude of these decreases was even greater than in LH rats given a single inescapable shock and tested on day 2.

To examine whether the changes in catalytic and regulatory activities of PKA are due to changes in the expression of specific catalytic or regulatory subunits, we determined the mRNA and protein levels of the various catalytic and regulatory subunits of PKA in the frontal cortex and hippocampus of LH rats. The expressed protein levels of the various catalytic and regulatory subunits in the frontal cortex and hippocampus of LH rats given inescapable shock once on day 1 or repeated inescapable shock on day 7 showed that the protein levels of RII β , C α , and C β were selectively decreased. These changes were not present in LH rats given inescapable shock once and tested on day 4. The changes in protein levels of RII β , C α , and C β were associated with changes in their respective mRNA levels. As with [^3H]cAMP binding and PKA activity, the magnitude of the changes in mRNA and protein levels of these PKA subunits was greater in LH rats that were exposed to the repeated shock paradigm compared with LH rats that were subjected to a single inescapable shock paradigm. Our results suggest that the changes in [^3H]cAMP binding and PKA activity could be due to the reduced expression of specific PKA regulatory and catalytic subunit isoforms, respectively. As with the corticosterone experiments previously described, it appears that in rats with LH, the expression of selective catalytic and regulatory subunits is controlled transcriptionally.

Overall, the findings for PKA in rats with LH and in rats exposed to corticosterone are similar to those observed in the postmortem brain of suicide subjects. It appears that stress plays an important role in modulating PKA. This is further supported by a finding that PKA activity is decreased in the subtype of depression that is associated with high steroid levels (McEwen, 1999).

Antipsychotics, suicide, and PKA

Suicide is a leading cause of death among patients with psychotic disorders (Altmura et al., 2003; Pinikahana et al., 2003) and about 20–40% of schizophrenia patients attempt suicide (Fenton et al., 1997; Radomsky et al., 1999). Despite that suicidal behavior is common among schizophrenia patients, there are a very few data showing the effectiveness of antipsychotics in preventing suicidal behavior. Typical antipsychotics do not show much effectiveness in reducing the risk of suicide (Winkour and Tsuang, 1975; Khan et al., 2001; Johns et al., 1986; Siris, 2001). On the other hand, atypical antipsychotics have some potential impact on suicidal behavior in schizophrenia patients (Keck et al., 2000; Meltzer, 2001; Meltzer et al., 2003). Several studies suggest that clozapine reduces the risk of suicidal behavior (Meltzer and Okayli, 1995; Walker et al., 1997; Wagstaff and Perry, 2003; Glick et al., 2004). A recent meta analysis by Hennen and Baldessarini (2005) suggest that long-term treatment with clozapine is associated with 3-fold overall reduction of risk of suicidal behavior in schizophrenia patients. Interestingly, in an earlier study, we demonstrated that antipsychotics affect various measures of PKA (Dwivedi et al., 2002). We examined regulatory and catalytic activities of PKA and expression of various isoforms of regulatory and catalytic subunits were in rat brain after single or chronic (21-day) treatment with haloperidol (1 mg/kg) or clozapine (20 mg/kg). It was observed that chronic but not acute treatment of clozapine significantly decreased [^3H]cAMP binding to the regulatory subunit of PKA as well as catalytic activity of PKA in particulate and cytosol fractions of the rat cortex, hippocampus, and striatum. In these fractions, clozapine significantly decreased protein levels of selective RII α -, RII β -, and Cat β -subunit isoforms of PKA. These decreases were accompanied by decreases in their respective mRNA expression. In contrast, chronic but not acute treatment of haloperidol significantly increased [^3H]cAMP

binding and the catalytic activity of PKA in particulate and cytosol fractions of only the striatum brain area. In addition, chronic treatment of haloperidol significantly increased mRNA and protein levels of RII α - and RII β -subunit isoforms in the striatum. None of the antipsychotics caused any change in the expression of the Cat α -, RI α -, or RI β -subunit isoform. These results, thus, suggest that haloperidol and clozapine differentially regulate PKA catalytic and regulatory activities and the expression of selective catalytic and regulatory subunit isoforms of PKA, which may be associated with their mechanisms of action. These studies further suggest that beneficial effect of clozapine on suicidal behavior could be associated with their effectiveness towards PKA.

Functional relevance of altered PKA in suicide brain: downstream targets

The findings that PKA activation is lower in the brain of suicide subjects and similar results in the brain of rats in different stress models provide an interesting possibility that many physiological functions in the brain, mediated by PKA, may be altered during suicide or stress. Although further research should be conducted, our findings that the expression of specific catalytic and regulatory subunits is decreased in suicide subjects and during stress in laboratory animals provide further evidence that there is a defect in the transcription of genes corresponding to certain catalytic and regulatory subunits of PKA. As previously discussed, this defect could be associated with altered levels of glucocorticoids. One question is as follows: What could be the relevance of such changes in the expression of specific PKA regulatory and catalytic subunits with respect to their role in suicide and depression? The specific functions of RII β and C β are not clearly known. However, tissue distribution studies suggest that RII β is predominantly expressed in brain, adrenal, and adipose tissues and is the principal mediator of cAMP activity in the mammalian CNS (Sarkar et al, 1984); whereas C β is expressed primarily in the brain (Uhler et al, 1986). Ludvig et al (1990) showed that RII β immunolabeling is associated with postsynaptic structures, suggesting that this subunit is involved in several postsynaptic neuronal functions. Also, many studies have demonstrated that RII β and C β subunits may be specifically involved in neuronal and behavioral functions. For example, Constantinescu et al (2002) showed that the RII β subunit can translocate to the nucleus and induce phosphorylation of one of the most important substrates of PKA (i.e, CREB). Also, RII β -mutant mice exhibit defective motor behavior (Brandon et al, 1998) and show an increased tendency to consume ethanol (Thiele et al, 2000). The C β -mutant mice, on the other hand, show impaired hippocampal plasticity (Qi et al, 1996). Targeted disruption of the RI β subunit gene results in mice that exhibit defects in long-term depression and depolarization, which suggests a deficit in a learning-related form of synaptic plasticity (Brandon et al, 1998). An RI β deficiency produces selective defects in mossy fiber long-term potentiation (Brandon et al, 1998).

Another important aspect of PKA is its involvement in the cross talk between different signaling mechanisms. For example, PKA interferes at various levels with other signaling pathways, including inactivation of phospholipase C β (Liu and Simon, 1996); phosphorylation of inositol 1,4,5-tris phosphate receptors, thereby modulating Ca²⁺ influx (Bugrim, 1999); phosphorylation of G proteins, with a consequent decrease in phosphoinositide hydrolysis and Ca²⁺ release (Wen et al, 1992); and phosphorylation of calcium-calmodulin kinase (Matsushita and Nairn, 1999). In addition, cAMP stimulates mitogen-activated protein kinase activity in cultured neurons (Villalba et al, 1997; Vossler et al, 1997) and is required for its nuclear translocation (Impey et al, 1998). Furthermore, cross talk between the cAMP/PKA and mitogen-activated protein kinase pathways is necessary to regulate genetic expression (Sengupta et al, 2007).

How PKA contributes to suicide pathogenesis is not clear. However, given the significance of PKA in many biological actions in the brain, together with emerging studies demonstrating specific roles for its catalytic and regulatory subunits in physiological and behavioral manifestations, the observations of decreased catalytic and regulatory activities and the expression of specific regulatory and catalytic subunits in the postmortem brain of suicide subjects suggest that abnormalities in PKA may be critically important in the pathophysiology of suicidal behavior. As previously mentioned, many of the physiological actions of PKA are mediated through phosphorylation of substrates. Some of the crucial substrates include Rap1 and transcription factor CREB. In addition, BDNF is a crucial gene regulated by CREB via PKA activation. In the following sections, we will briefly discuss the most relevant findings of these substrates and their relevance to suicide.

CREB in Suicide

The regulation of gene expression is a fundamental mechanism of brain development, homeostatics, and adaptation to the environment. Transcription factor CREB is a member of the leucine zipper family, which binds to the consensus motif 5'-TGACGTCA-3', found in the promoters of many neuronally expressed genes, and thereby activates or represses the transcription of target genes (Borelli et al, 1992). Phosphorylation of CREB at serine 133 by PKA is the critical step in its activation. In its active form, CREB regulates many aspects of neuronal functioning, including excitation of nerve cells, CNS development, and long-term synaptic plasticity (Marshall and Dragunow, 2000). One of the many important genes whose transcription is mediated through the activation of PKA/CREB is BDNF. To determine if CREB could possibly be involved in suicide, we recently examined the mRNA and protein expression, and the CREB-DNA binding activity (a measure of functional characteristics), of CREB in the PFC and hippocampus of suicide subjects (Dwivedi et al, 2003). The protein expression of CREB was significantly decreased in the nuclear fractions of the PFC and hippocampus obtained from suicide subjects compared with healthy control subjects. This decrease in protein expression levels was associated with a significant decrease in the mRNA levels of CREB, in both the PFC and hippocampus of suicide subjects. We also found decreased functional activity of CREB, such that CRE-DNA binding activity was significantly decreased in the nuclear fractions of both the PFC and hippocampus of suicide subjects. These changes in CREB were present in all suicide subjects irrespective of psychiatric diagnosis. Similar results were noted in the postmortem brain of teenage suicide subjects (Pandey et al, 2006). In addition, recently, Young et al (2004) reported altered levels of phosphorylated CREB in the amygdala of suicide subjects. The results of these studies suggest that the expression of PKA and the expression and functional characteristics of one of the important substrates of PKA (i.e., CREB) are abnormal in the brain of suicide subjects.

These findings may have important clinical implications. For example, all antidepressants activate or upregulate the expression of CREB in rat brain (Nibuya et al, 1996). Similar results have been shown in postmortem brain of depressed patients treated with antidepressants (Dowlathshahi et al, 1998). In contrast, the phosphorylation and expression of CREB are decreased in postmortem brain of depressed patients, which could be associated with a decrease in PKA activity (Yamada et al, 2003). Given the role of CREB in various physiological actions in the brain, the findings of its increased expression by antidepressants and of its decreased expression and functional characteristics in the brain of depressed/suicide subjects are quite important and implicate this gene in depression/suicidal behavior. Recently, Zubenko et al (2002) performed a linkage analysis of six polymorphic markers located in a 15cM region of chromosome 2q33-35 and found a linkage of depression to a 451Kb region of 2q33-34, which contains the *CREB1* gene. This study further suggests that the *CREB1* may be an attractive susceptibility candidate protein for depression.

Rap1 in Suicide

Rap1, another important substrate of PKA, belongs to Ras family of small GTP-binding proteins (Kawata et al, 1988; Pizon et al, 1988) and phosphorylated by PKA at its C-terminal serine 180 residue in a wide variety of cells, including neurons and glia (Dugan et al, 1999; Vossler et al, 1997). The activation of Rap1 leads to its participation in a variety of physiological functions, including cell proliferation, cell survival, cell adhesion, differentiation (Zwartkuis and Bos, 1999; Bos et al, 2001), and plasticity. The activation mechanism of Rap1 involves guanine nucleotide exchange factors that substitute the bound GDP for GTP. The GTP-bound form is active, and the GDP-bound form is inactive. GTPase-activating proteins induce the hydrolysis of the bound GTP to complete the cycle (Bos et al, 2001). To examine whether Rap1 is altered in suicide brain, we examined the activation of Rap1 in the PFC and hippocampus of suicide subjects. We found that activation of Rap1 was significantly decreased in both the PFC and hippocampus of suicide subjects. Because PKA phosphorylates Rap1 at its C-terminal serine 80 residue (Quilliam et al, 1991) and makes it sensitive to Smg GDS to stimulate its GDP/GTP exchange reaction (Hata et al, 1991), it is quite possible that the decrease in Rap1 activation could be due to a decreased PKA level. Recently, Perez et al (1999, 2000) reported significantly increased immunolabeling of Rap1 in the platelets of patients with bipolar disorder but no change in patients with unipolar or bipolar disorder who have psychotic features (Perez et al, 2002). On the other hand, they found a significantly lower protein level of Rap1 in untreated depressed patients compared with untreated euthymic patients and healthy subjects (Perez et al, 2001). These studies indicate that depression/suicide may be associated with lower activation/expression of Rap1.

The functional significance of Rap1 in depression/suicide is yet to be explored; however, interestingly, it has been demonstrated that various physiological functions mediated by Rap1 are through the activation of signaling mechanisms mediated by the extracellular signal-regulated kinase (ERK) pathway (Hattori et al, 2003). In neuronal cells expressing B-Raf, PKA stimulates the ERK pathway via activation of Src-dependent kinases (Qiu et al, 2000). In addition, Ca^{2+} -mediated PKA-dependent activation of Rap1/B-Raf/ERK signaling in hippocampal neurons is an important regulator of activity-dependent neuronal functions (Grewal et al, 2000). Therefore, Rap1 links the PKA and ERK signaling systems through B-Raf; at this level, these two signaling systems cross talk to each other. In addition to synaptic plasticity, ERK signaling is critical in many physiological functions, including neuroprotection, proliferation, and cell survival (Kolkova et al, 2000; Cheung et al, 2004; Sweatt, 2004). Morozov et al (2003) showed that mice with a dominant interfering mutant of Rap1 had a reduced basal level of phosphorylated ERK in the hippocampus, which was associated with deficient learning and memory storage. Recently, we reported significantly decreased activation of ERK-1 and ERK-2 in the PFC and hippocampus of suicide subjects (Dwivedi et al, 2001).

BDNF in Suicide

Brain-derived neurotrophic factor, a critical gene regulated by PKA, participates in a wide range of physiological functions (eg, neurite outgrowth, phenotypic maturation, morphological plasticity, and synthesis of proteins for differentiated functioning of neurons and synapses) (Huang and Reichardt, 2001). Several studies also suggest that BDNF is also involved in nerve regeneration, structural integrity, and maintenance of neuronal plasticity in the adult brain, including activity-dependent regulation of synaptic activity, and in neurotransmitter synthesis (Thoenen, 1995; Altar et al, 1997; Bartrup et al, 1997). Thus, a pathological alteration of the neurotrophic factor system may lead not only to altered neural maintenance and regeneration and, therefore, structural abnormalities in the brain, but also to reduced neural plasticity. Emerging evidence demonstrates that BDNF may play a crucial

role in the pathophysiology of suicide. To our knowledge, we were the first to examine the role of BDNF in postmortem brain tissues of subjects who committed suicide. We determined the mRNA and protein expression of BDNF in the PFC and hippocampus of suicide subjects and healthy control subjects (Dwivedi et al, 2003). We found that the mRNA level of BDNF was approximately 40% to 50% lower in both the PFC and hippocampus of suicide subjects. By using antibody that recognizes mature BDNF, we also found a significantly decreased protein level of BDNF in the PFC and hippocampus of suicide subjects. The mRNA and protein levels of BDNF were well correlated in these brain areas of suicide subjects, suggesting that a decrease in BDNF is not due to unavailability but that there is a defect in the transcription of the *BDNF* gene in suicide brain specimens. When we divided suicide subjects into those who had depression and those who had another psychiatric disorder, we found that the decrease in expression of BDNF was present in all suicide subjects regardless of psychiatric diagnosis. We also found a marginal decrease in BDNF in those suicide subjects who showed positive antidepressant toxicology features. Our findings demonstrate that suicidal behavior is associated with reduced level of BDNF. More recently, Karege et al (2005) examined the expression of BDNF in the PFC, hippocampus, and entorhinal cortex of suicide subjects. Similar to our findings, they found that the level of BDNF was significantly lower in the PFC and hippocampus; however, they did not find any change in the entorhinal cortex, suggesting that a decrease in BDNF may be specific to certain brain areas. In addition, Karege et al (2005) found that suicide subjects who were receiving antidepressant did not show any change in the level of BDNF, suggesting that psychotropic drugs normalize the decreased level of BDNF in these subjects.

In addition to postmortem brain studies, there have been some studies demonstrating that BDNF is altered in peripheral tissues of suicidal patients. Recently, many studies have examined BDNF levels in blood cells of suicidal subjects. Although the significance of BDNF in blood cells is unclear, it has been shown that BDNF may cross the blood-brain barrier and that platelet BDNF shows similar changes postnatally, similar to the brain (Karege et al, 2002), suggesting that there are parallel changes in the blood and brain levels of BDNF. Kim et al (2007) measured plasma BDNF levels in depressed patients who had recently attempted suicide, nonsuicidal depressed patients, and healthy controls. They found that BDNF level was significantly lower in suicidal depressed patients vs nonsuicidal depressed patients or healthy controls. Interestingly, BDNF levels were not different between fatal and nonfatal suicide attempts. Similarly, Lee et al (2007) found that plasma BDNF level was decreased in depressed suicidal patients vs. depressed nonsuicidal patients. These studies suggest that reduction of plasma BDNF level is related to suicidal behavior in those with depression and that BDNF level may be a biological marker of suicidal depression. On the other hand, Deveci et al (2007) reported that serum BDNF level was lower in both the attempted suicide group and the depressed group vs. the control group. Similarly, platelet BDNF level was lower in both nonsuicidal and suicidal depressed patients vs healthy controls (Lee and Kim, 2009). Interestingly, Dawood et al (2007) used direct internal jugular vein blood sampling methods to circumvent the issue of whether BDNF is released from sources other than the brain. They examined the relationship between brain BDNF production and suicide risk in patients with depression who were free of medication. They found that the venoarterial BDNF concentration gradient was significantly reduced in patients at medium to high suicide risk and that there was a significant negative correlation between suicide risk and the internal jugular venous venoarterial BDNF concentration gradient. This study clearly implicates BDNF and suicide risk.

Conclusions

The summary of findings of PKA/CREB/Rap1/BDNF in suicide is provided in Figure 2. From the studies of G proteins in suicide brain, there is an overall increase in adenylyl

cyclase–cAMP signaling in suicide brain; however, at the downstream level at PKA, a reduction in activation of PKA is observed, which appears to be associated with reductions in the expression of specific regulatory and catalytic subunits of PKA. Thus, it appears that although there may be an increase in cAMP formation, the downstream event at the level of PKA is suboptimal. Whether this is because of an adaptive response to increase in cAMP or because some other factor is playing a role is not clear; however, from a stress model and glucocorticoid studies in animals, it can be inferred that both stress and glucocorticoids modulate the transcription of the same PKA subunits, in which changes were found in suicide brain. Whether the changes in PKA are a consequence of stress or inherent to suicide is not clear; at this juncture, further studies will be needed to clarify this issue. Additional behavioral phenotypes will shed some light on this aspect; our laboratory is engaged in delineating various behavioral and molecular aspects of mutations in specific PKA regulatory subunits in mice.

The studies of CREB, Rap1, and BDNF also indicate that PKA and its substrates and target genes are also altered in suicide, although a few study results are contradictory to what we and other investigators have reported in the postmortem brain of suicide subjects. For example, Lowther et al (1997) reported no change in cAMP binding to regulatory subunits in the postmortem brain of depressed suicide subjects, whereas Odagaki et al (2001) reported increased expression of total and phosphorylated CREB (an active form of CREB) in the PFC of depressed suicide subjects. Even a few animal study findings argue against the BDNF hypothesis of depression (Groves, 2007). Thus, although caution is required when interpreting the data, most studies show abnormalities in PKA, CREB, and BDNF in suicide.

Another important issue is whether the changes in PKA, CREB, and BDNF are specific to suicide or are related to mental disorders. Most suicidal patients have one or another form of psychiatric illness. As previously mentioned, some of the findings observed in PKA signaling in suicide have been found in depressed patients. Because many suicidal patients have depression, it is to be expected that such findings would also be present in depressed patients. In fact, in our study population, more than 60% of the subjects had a history of major depression. Therefore, although it appears that alterations in PKA are related to suicide, the possibility that changes in PKA signaling could be associated with a depressed mood or the inability to experience pleasure cannot be excluded.

Overall, in view of the current findings, a novel concept of the neurobiology of suicide is evolving, in which disruptions in PKA/CREB/Rap1/BDNF signaling could be seen as important biological risk factors that may be useful in delineating the etiology of these disorders and could eventually result in site-specific therapeutic interventions. Further studies will be important to investigate whether there is a genetic linkage of these abnormalities to suicide.

Abbreviations

BDNF	brain-derived neurotrophic factor
CREB	cyclic adenosine monophosphate response element binding protein
PFC	prefrontal cortex
PKA	Protein kinase A

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References

- Abel T, Nguyen PV. Regulation of hippocampus-dependent memory by cyclic AMP-dependent protein kinase. *Prog Brain Res*. 2008; 169:97–115. [PubMed: 18394470]
- Adams JA, McGlone ML, Gibson R, Taylor SS. Phosphorylation modulates catalytic function and regulation in the cAMP- dependent protein kinase. *Biochemistry*. 1995; 34:2447–2454. [PubMed: 7873523]
- Aganova EA, Uranova NA. Morphometric analysis of synaptic contacts in the anterior limbic cortex in the endogenous psychoses. *Neurosci Behav Physiol*. 1992; 22:59–65. [PubMed: 1614620]
- Altamura AC, Bassetti R, Bignotti S, Pioli R, Mundo E. Clinical variables related to suicide attempts in schizophrenic patients: a retrospective study. *Schizophr Res*. 2003; 60:47–55. [PubMed: 12505137]
- Altar CA, Cai N, Bliven T, Juhasz M, Conner JM, Acheson AL, Lindsey RM, Wiegand SJ. Anterograde transport of brain-derived neurotrophic factor and its role in the brain. *Nature*. 1997; 389:856–860. [PubMed: 9349818]
- Altshuler LL, Casanova MF, Goldberg TE, Kleinman JE. The hippocampus and parahippocampus in schizophrenia, suicide, and control brains. *Arch Gen Psychiatry*. 1990; 47:1029–1034. [PubMed: 2241505]
- Arato M, Banki CM, Bissette G, Nemeroff CB. Elevated CSF CRF in suicide victims. *Biol Psychiatry*. 1989; 25:355–359. [PubMed: 2536563]
- Avissar S, Nechamkin Y, Roitman G, Schreiber G. Reduced G protein functions and immunoreactive levels in mononuclear leukocytes of patients with depression. *Am J Psychiatry*. 1997; 154:211–217. [PubMed: 9016270]
- Bartrup JT, Moorman JM, Newberry NR. BDNF enhances neuronal growth and synaptic activity in hippocampal cell cultures. *Neuroreport*. 1997; 8:3791–3794. [PubMed: 9427372]
- Beebe SJ. The cAMP-dependent protein kinases and cAMP signal transduction. *Semin Cancer Biol*. 1994; 5:285–294. [PubMed: 7803765]
- Bertolote, JM. Suicide in the world: an epidemiological overview, 1959–2000. In: Wasserman, D., editor. *Suicide: An Unnecessary Death*. London, England: Dunitz; 2001. p. 3-10.
- Bornfeldt KE, Krebs EG. Crosstalk between protein kinase A and growth factor receptor signaling pathways in arterial smooth muscle. *Cell Signal*. 1999; 11:465–477. [PubMed: 10405757]
- Borrelli E, Montmayeur JP, Foulkes NS, Sassone-Corsi P. Signal transduction and gene control: the cAMP pathway. *Crit Rev Oncog*. 1992; 3:321–338. [PubMed: 1329990]
- Bos JL, de Rooij J, Reedquist KA. Rap1 signaling adhering to new models. *Mol Cell Biol*. 2001; 21:369–377.
- Brandon EP, Logue SF, Adams MR, et al. Defective motor behavior and neural gene expression in RII β -protein kinase A mutant mice. *J Neurosci*. 1998; 18:3639–3649. [PubMed: 9570795]
- Bremner JD, Narayan M, Anderson ER, Staib LH, Miller HL, Charney DS. Hippocampal volume reduction in major depression. *Am J Psychiatry*. 2000; 157:115–118. [PubMed: 10618023]
- Brent D, Bridge J, Johnson B, Connolly J. Suicidal behavior runs in families: a controlled family study of adolescent suicide victims. *Arch Gen Psychiatry*. 1996; 53:1145–1149. [PubMed: 8956681]
- Brent D, Kolko D, Wartella M, et al. Adolescent psychiatric inpatients' risk of suicide attempt at 6-month follow-up. *J Am Acad Child Adolesc Psychiatry*. 1993; 32:95–105. [PubMed: 8428891]
- Bugrim AE. Regulation of Ca²⁺ release by cAMP-dependent protein kinase: a mechanism for agonist-specific calcium signaling? *Cell Calcium*. 1999; 25:219–226. [PubMed: 10378083]
- Cadd GG, Uhler MD, McKnight GS. Holoenzymes of cAMP-dependent protein kinase containing the neural form of type I regulatory subunit have an increased sensitivity to cyclic nucleotides. *J Biol Chem*. 1990; 265:19502–19506. [PubMed: 2174040]

- Cauthron RD, Carter KB, Liauw S, Steinberg RA. Physiological phosphorylation of protein kinase A at Thr-197 is by a protein kinase A kinase. *Mol Cell Biol.* 1998; 18:1416–1423. [PubMed: 9488457]
- Cheng X, Ma Y, Moore M, Hemmings BA, Taylor SS. Phosphorylation and activation of cAMP-dependent protein kinase by phosphoinositide-dependent protein kinase. *Proc Natl Acad Sci U S A.* 1998; 95:9849–9854. [PubMed: 9707564]
- Cheung EC, Slack RS. Emerging role for ERK as a key regulator of neuronal apoptosis. *Sci STKE.* 2004; 251:PE45. [PubMed: 15383672]
- Clapham DE, Neer EJ. G protein beta gamma subunits. *Annu Rev Pharmacol Toxicol.* 1997; 37:167–203. [PubMed: 9131251]
- Clayton PJ. Suicide. *Psychiatr Clin North Am.* 1985; 8:203–214. [PubMed: 3895188]
- Clegg CH, McKnight GS. Genetic characterization of a brain-specific form of the type I regulatory subunit of cAMP-dependent protein kinase. *Proc Natl Acad Sci U S A.* 1988; 85:3703–3707. [PubMed: 3375237]
- Constantinescu A, Gordon AS, Diamond I. cAMP-dependent protein kinase types I and II differentially regulate cAMP response element-mediated gene expression. *J Biol Chem.* 2002; 277:18810–18816. [PubMed: 11886856]
- Cotter D, Mackay D, Chana G, Beasley C, Landau S, Everall IP. Reduced neuronal size and glial cell density in area 9 of the dorsolateral prefrontal cortex in subjects with major depressive disorder. *Cereb Cortex.* 2002; 12:386–394. [PubMed: 11884354]
- Cowburn RF, Marcusson JO, Eriksson A, Wiehager B, O'Neill C. Adenylyl cyclase activity and G-protein subunit levels in postmortem frontal cortex of suicide victims. *Brain Res.* 1994; 633:297–304. [PubMed: 8137164]
- Dawood T, Anderson J, Barton D, Lambert E, Esler M, Hotchkin E. Reduced overflow of BDNF from the brain is linked with suicide risk in depressive illness. *Mol Psychiatry.* 2007; 12:981–983. [PubMed: 17957235]
- Deveci A, Aydemir O, Taskin O, Taneli F, Esen-Danaci A. Serum BDNF levels in suicide attempters related to psychosocial stressors: a comparative study with depression. *Neuropsychobiology.* 2007; 56:93–97. [PubMed: 18037819]
- Dowlatsahi D, MacQueen GM, Wang JF, Young LT. Increased temporal cortex CREB concentrations and antidepressant treatment in major depression. *Lancet.* 1998; 352:1754–1755. [PubMed: 9848357]
- Dugan LL, Kim JS, Zhang Y, Bart RD, Sun Y, Holtzman DM, Gutmann DH. Differential effects of cAMP in neurons and astrocytes. *J Biol Psychiatry.* 1999; 274:25842–25848.
- Duman RS, Malberg J, Nakagawa S, D'Sa C. Neuronal plasticity and survival in mood disorders. *Biol Psychiatry.* 2000; 48:732–739. [PubMed: 11063970]
- Duman RS, Monteggia LM. A neurotrophic model for stress-related mood disorders. *Biol Psychiatry.* 2006; 59:1116–1127. [PubMed: 16631126]
- Dwivedi Y, Conley RR, Roberts RC, Tamminga CA, Pandey GN. [³H]cAMP binding sites and protein kinase A activity in the prefrontal cortex of suicide victims. *Am J Psychiatry.* 2002; 159:66–73. [PubMed: 11772692]
- Dwivedi Y, Mondal AC, Rizavi HS, Conley RR. Suicide brain is associated with decreased expression of neurotrophins. *Biol Psychiatry.* 2005; 58:315–324. [PubMed: 15939410]
- Dwivedi Y, Pandey GN. Adrenal glucocorticoids modulate [³H]cyclic AMP binding to protein kinase A (PKA), cyclic AMP-dependent PKA activity, and protein levels of selective regulatory and catalytic subunit isoforms of PKA in rat brain. *J Pharmacol Exp Ther.* 2000; 294:103–116. [PubMed: 10871301]
- Dwivedi Y, Rao JS, Rizavi HS, et al. Abnormal expression and functional characteristics of cyclic adenosine monophosphate response element-binding protein in postmortem brain of suicide subjects. *Arch Gen Psychiatry.* 2003; 60:273–282. [PubMed: 12622660]
- Dwivedi Y, Rizavi H, Zhang H, Mondal A, Roberts RC, Conley RR, Pandey GN. Neurotrophin receptor activation and expression in human postmortem brain: effect of suicide. *Biol Psychiatry.* 2009; 65:319–328. [PubMed: 18930453]

- Dwivedi Y, Rizavi HS, Conley RR, Pandey GN. ERK MAP kinase signaling in post-mortem brain of suicide subjects: differential regulation of upstream Raf kinases Raf-1 and B-Raf. *Mol Psychiatry*. 2006a; 11:86–98. [PubMed: 16172610]
- Dwivedi Y, Rizavi HS, Conley RR, Roberts RC, Tamminga CA, Pandey GN. Altered gene expression of brain-derived neurotrophic factor and receptor tyrosine kinase B in postmortem brain of suicide subjects. *Arch Gen Psychiatry*. 2003; 60:804–815. [PubMed: 12912764]
- Dwivedi Y, Rizavi HS, Pandey GN. Differential effects of haloperidol and clozapine on [(3)H]cAMP binding, protein kinase A (PKA) activity, and mRNA and protein expression of selective regulatory and catalytic subunit isoforms of PKA in rat brain. *J Pharmacol Exp Ther*. 2002; 301:197–209. [PubMed: 11907174]
- Dwivedi Y, Rizavi HS, Roberts RC, Conley RC, Tamminga CA, Pandey GN. Reduced activation and expression of ERK1/2 MAP kinase in the post-mortem brain of depressed suicide subjects. *J Neurochem*. 2001; 77:916–928. [PubMed: 11331420]
- Dwivedi Y, Rizavi HS, Shukla PK, Lyons J, Faludi G, Palkovits M, Sarosi A, Conley RR, Roberts RC, Tamminga CA, Pandey GN. Protein kinase A in postmortem brain of depressed suicide victims: altered expression of specific regulatory and catalytic subunits. *Biol Psychiatry*. 2004; 55:234–243. [PubMed: 14744463]
- Dwivedi Y. BDNF in suicide pathogenesis. *Ann Med*. 2010; 42:87–96. [PubMed: 20166812]
- Dwivedi Y. The concept of dysregulated signal transduction and gene expression in the pathophysiology of mood disorders. *Curr Psychiatry Rev*. 2005; 1:227–254.
- Dwivedi Y. The concept of dysregulated signal transduction and gene expression in the pathophysiology of mood disorders. *Curr Psychiatry Rev*. 2006b; 1:227–257.
- Fenton WS, McGlashan TH, Victor BJ, Blyler CR. Symptoms, subtype, and suicidality in patients with schizophrenia spectrum disorders. *Am J Psychiatry*. 1997; 154:199–204. [PubMed: 9016268]
- Francis SH, Corbin JD. Cyclic nucleotide-dependent protein kinases: intracellular receptors for cAMP and cGMP action. *Crit Rev Clin Lab Sci*. 1999; 36:275–328. [PubMed: 10486703]
- Freissmuth M, Waldhoer M, Bofill-Cardoba E, Nanoff C. G protein antagonists. *Trends Pharmacol Sci*. 1999; 20:237–245. [PubMed: 10366866]
- Gamm DM, Baude EJ, Uhler MD. The major catalytic subunit isoforms of cAMP-dependent protein kinase have distinct biochemical properties in vitro and in vivo. *J Biol Chem*. 1996; 271:15736–15742. [PubMed: 8662989]
- Garcia R. Stress, synaptic plasticity, and psychopathology. *Rev Neurosci*. 2002; 13:195–208. [PubMed: 12405224]
- Gilman AG. G proteins: transducers of receptor-generated signals. *Annu Rev Biochem*. 1987; 56:615–649. [PubMed: 3113327]
- Glick ID, Zaninelli R, Hsu C, Young FK, Weiss L, Gunay I, Kumar V. Patterns of concomitant psychotropic medication use during a 2-year study comparing clozapine and olanzapine for the prevention of suicidal behavior. *J Clin Psychiatry*. 2004; 65:679–685. [PubMed: 15163255]
- Goldsmith, SK.; Pellmar, TC.; Kleinman, AM.; Bunney, WE., editors. Committee on Pathophysiology and Prevention of Adolescent and Adult Suicide, Board on Neuroscience and Behavioral Health, Institute of Medicine of the National Academies. *Reducing Suicide, A National Imperative*. Washington, DC: National Academies Press; 2002.
- Grewal SS, Fass DM, Yao H, Ellig CL, Goodman RH, Stork PJ. Calcium and cAMP signals differentially regulate cAMP-responsive element-binding protein function via a Rap1-extracellular signal-regulated kinase pathway. *J Biol Chem*. 2000; 275:34433–34441. [PubMed: 10950954]
- Groves JO. Is it time to reassess the BDNF hypothesis of depression? *Mol Psychiatry*. 2007; 12:1079–1088. [PubMed: 17700574]
- Hajsz T, MacLusky NJ, Leran C. Short-term treatment with the antidepressant fluoxetine triggers pyramidal dendritic spine synapse formation in rat hippocampus. *Eur J Neurosci*. 2005; 21:1299–1303. [PubMed: 15813939]
- Hamm HE. The many faces of G protein signaling. *J Biol Chem*. 1998; 273:669–672. [PubMed: 9422713]

- Hata Y, Kaibuchi K, Kawamura S, Hiroyoshi M, Shirataki H, Takai Y. Enhancement of the actions of smg p21 GDP/GTP exchange protein by the protein kinase A-catalyzed phosphorylation of smg p21. *J Biol Chem*. 1991; 266:6571–6577. [PubMed: 1901063]
- Hattori M, Minato N. Rap1 GTPase: functions, regulation, and malignancy. *J Biochem*. 2003; 134:479–484. [PubMed: 14607972]
- Hennen J, Baldessarini RJ. Suicidal risk during treatment with clozapine: a meta-analysis. *Schizophr Res*. 2005; 73:139–145. [PubMed: 15653256]
- Hildebrandt JD. Role of subunit diversity in signaling by heterotrimeric G proteins. *Biochem Pharmacol*. 1997; 54:325–339. [PubMed: 9278091]
- Hiroi N, Wong ML, Licinio J. Expression of corticotropin releasing hormone receptors type I and type II mRNA in suicide victims and controls. *Mol Psychiatry*. 2001; 6:540–546. [PubMed: 11526468]
- Honer WG. Assessing the machinery of mind: synapses in neuropsychiatric disorders. *J Psychiatry Neurosci*. 1999; 24:116–121. [PubMed: 10212553]
- Huang E, Reichardt LF. Neurotrophins: roles in neuronal development and function. *Annu Rev Neurosci*. 2001; 24:677–736. [PubMed: 11520916]
- Impey S, Obrietan K, Wong ST, Poser S, Yano S, Wayman G, Deloulme JC, Chan G, Storm DR. Cross talk between ERK and PKA is required for Ca²⁺ stimulation of CREB-dependent transcription and ERK nuclear translocation. *Neuron*. 1998; 21:869–883. [PubMed: 9808472]
- Joëls M, Karst H, Alfarez D, Heine VM, Qin Y, van Riel E, Verkuyl M, Lucassen PJ, Krugers HJ. Effects of chronic stress on structure and cell function in rat hippocampus and hypothalamus. *Stress*. 2004; 7:221–231. [PubMed: 16019587]
- Johns CA, Stanley M, Stanley R. Suicide in schizophrenia. *Ann N Y Acad Sci*. 1986; 487:294–300. [PubMed: 2882719]
- Jordan JD, Landau EM, Iyengar R. Signaling networks: the origins of cellular multitasking. *Cell*. 2000; 103:193–200. [PubMed: 11057893]
- Karege F, Bovier P, Stepanian R, Malafosse A. The effect of clinical outcome on platelet G proteins of major depressed patients. *Eur Neuropsychopharmacol*. 1998; 8:89–94. [PubMed: 9619686]
- Karege F, Schwald M, Cisse M. Postnatal developmental profile of brain-derived neurotrophic factor in rat brain and platelets. *Neurosci Lett*. 2002; 328:261–264. [PubMed: 12147321]
- Karege F, Vaudan G, Schwald M, Perroud N, La Harpe R. Neurotrophin levels in postmortem brains of suicide victims and the effects of antemortem diagnosis and psychotropic drugs. *Brain Res Mol Brain Res*. 2005; 136:29–37. [PubMed: 15893584]
- Kawata M, Matsui Y, Kondo J, Hishida T, Teranishi Y, Takai Y. A novel small molecular weight GTP-binding protein with the same putative effector domain as the ras proteins in bovine brain membranes: purification, determination of primary structure, and characterization. *J Biol Chem*. 1988; 263:18965–18971. [PubMed: 3143720]
- Keck PE Jr, Strakowski SM, McElroy SL. The efficacy of atypical antipsychotics in the treatment of depressive symptoms, hostility, and suicidality in patients with schizophrenia. *J Clin Psychiatry*. 2000; 61 Suppl 3:4–9. [PubMed: 10724127]
- Montout C, Casadebaig F, Lagnaoui R, et al. Neuroleptics and mortality in schizophrenia: prospective analysis of deaths in a French cohort of schizophrenic patients. *Schizophr Res*. 2002; 57:147–156. [PubMed: 12223245]
- Khan A, Khan SR, Leventhal RM, Brown WA. Symptom reduction and suicide risk among patients treated with placebo in antipsychotic clinical trials: an analysis of the Food and Drug Administration database. *Am J Psychiatry*. 2001; 158:1449–1454. [PubMed: 11532730]
- Kim YK, Lee HP, Won SD, Park EY, Lee HY, Lee BH. Low plasma BDNF is associated with suicidal behavior in depression. *Prog Neuropsychopharmacol Biol Psychiatry*. 2007; 31:578–579. [PubMed: 17134810]
- Kolkova K, Novitskaya V, Pedersen N, Berzin V, Bock E. Neural cell adhesion molecule-stimulated neurite outgrowth depends on activation of protein kinase C and the Ras/mitogen-activated protein kinase pathway. *J Neurosci*. 2000; 20:2238–2246. [PubMed: 10704499]
- Lara J, Kusano K, House S, Gainer H. Interactions of cyclic adenosine monophosphate, brain-derived neurotrophic factor, and glial cell line-derived neurotrophic factor treatment on the survival and

- growth of postnatal mesencephalic dopamine neurons in vitro. *Exp Neurol*. 2003; 180:32–45. [PubMed: 12668147]
- Lee BH, Kim H, Park SH, Kim YK. Decreased plasma BDNF level in depressive patients. *J Affect Disord*. 2007; 101:239–244. [PubMed: 17173978]
- Lee BH, Kim YK. Reduced platelet BDNF level in patients with depression. *Prog Neuropsychopharmacol Biol Psychiatry*. 2009; 33:849–853. [PubMed: 19371767]
- Liu M, Simon MI. Regulation by cAMP-dependent protein kinase of a G-protein-mediated phospholipase C. *Nature*. 1996; 382:83–87. [PubMed: 8657310]
- Lopez JF, Palkovits M, Arato M, Mansour A, Akil H, Watson SJ. Localization and quantification of pro-opiomelanocortin mRNA and glucocorticoid receptor mRNA in pituitaries of suicide victims. *Neuroendocrinology*. 1992; 56:491–501. [PubMed: 1335553]
- Lowther S, Katona CL, Crompton MR, Horton RW. Brain [³H]cAMP binding sites are unaltered in depressed suicides, but decreased by antidepressants. *Brain Res*. 1997; 758:223–228. [PubMed: 9203552]
- Ludvig N, Ribak CE, Scott JD, Rubin CS. Immunocytochemical localization of the neuralspecific regulatory subunit of the type II cyclic AMP-dependent protein kinase to postsynaptic structures in the rat brain. *Brain Res*. 1990; 520:90–102. [PubMed: 2207649]
- Marshall R, Dragunow M. Is CREB a key to neuronal survival? *Trends Neurosci*. 2000; 23:48–53. [PubMed: 10652539]
- Matsushita M, Nairn AC. Inhibition of the Ca²⁺/calmodulin-dependent protein kinase I cascade by cAMP-dependent protein kinase. *J Biol Chem*. 1999; 274:10086–10093. [PubMed: 10187789]
- McEwen BS. Effects of adverse experiences for brain structure and function. *Biol Psychiatry*. 2000; 48:713–714. [PubMed: 11063967]
- McEwen BS. Stress and hippocampal plasticity. *Annu Rev Neurosci*. 1999; 22:105–122. [PubMed: 10202533]
- Meltzer HY. Decreasing suicide in schizophrenia. *Psychiatr Times*. 2001; 18:1–5.
- Meltzer HY, Alphs L, Green AI, Altamura AC, Anand R, Bertoldi A, Bourgeois M, Chouinard G, Islam MZ, Kane J, Krishnan R, Lindenmayer JP, Potkin S. International Suicide Prevention Trial Study Group. Clozapine treatment for suicidality in schizophrenia: International Suicide Prevention Trial (InterSePT). *Arch Gen Psychiatry*. 2003; 60:82–91. [PubMed: 12511175]
- Meltzer HY, Okayli G. Reduction of suicidality during clozapine treatment of neuroleptic-resistant schizophrenia: impact on risk-benefit assessment. *Am J Psychiatry*. 1995; 152:183–190. [PubMed: 7840350]
- Minino AM, Smith BL. Deaths: preliminary data for 2000. *Natl Vital Stat Rep*. 2002; 49:1–40.
- Monk M. Epidemiology of suicide. *Epidemiol Rev*. 1987; 9:51–69. [PubMed: 2445596]
- Morozov A, Muzzio IA, Bourtholadze R, Van-Strien N, Lapidus K, Yin DQ. Rap1 couples cAMP signaling to a distinct pool of p42/44 MAPK regulating excitability, synaptic plasticity, learning, and memory. *Cell*. 2003; 39:309–325.
- National Center for Health Statistics. Advance report of final mortality statistics. *NCHS Monthly Vital Stat Rep*. 1992:40.
- Neer EJ. Heterotrimeric G proteins: organizers of transmembrane signals. *Cell*. 1995; 80:249–257. [PubMed: 7834744]
- Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM. Neurobiology of depression. *Neuron*. 2002; 34:13–25. [PubMed: 11931738]
- Nestler, EJ.; Greengard, P. Protein phosphorylation and the regulation of neuronal function. In: Siegel, GJ.; Albers, RW.; Agranoff, BW.; Molinoff, P., editors. *Basic Neurochemistry: Molecular, Cellular, and Medical Aspects*. Boston, MA: Little Brown Press; 1994. p. 449-474.
- Neves SR, Ram PT, Iyengar R. G protein pathways. *Science*. 2002; 296:1636–1639. [PubMed: 12040175]
- Nibuya M, Nestler EJ, Duman RS. Chronic antidepressant administration increases the expression of cAMP response element-binding protein (CREB) in rat hippocampus. *J Neurosci*. 1996; 16:2365–2372. [PubMed: 8601816]

- Odagaki Y, Garcia-Sevilla JA, Huguelet P, La Harpe R, Koyama T, Guimon J. Cyclic AMP-mediated signaling components are upregulated in the prefrontal cortex of depressed suicide victims. *Brain Res.* 2001; 898:224–231. [PubMed: 11306008]
- Ozawa H, Gsell W, Frolich L, et al. Imbalance of the G_s and $G_{i/o}$ function in postmortem human brain of depressed patients. *J Neural Transm Gen Sect.* 1993; 94:63–69. [PubMed: 8129882]
- Pacheco MA, Stockmeier C, Meltzer HY, Overholser JC, Dilley GE, Jope RS. Alterations in phosphoinositide signaling and G-protein levels in depressed suicide brain. *Brain Res.* 1996; 723:37–45. [PubMed: 8813380]
- Pandey GN, Dwivedi Y, Ren X, et al. Brain region specific alterations in the protein and mRNA levels of protein kinase A subunits in the postmortem brain of teenage suicide victims. *Neuropsychopharmacology.* 2005; 30:1548–1556. [PubMed: 15920506]
- Pandey GN, Dwivedi Y, Ren X, Rizavi HS, Roberts RC, Conley RR. Cyclic AMP response element-binding protein in postmortem brain of teenage suicide victims: specific decrease in the prefrontal cortex but not the hippocampus. *Int J Neuropsychopharmacol.* 2006; 18:1–9.
- Pandey GN, Dwivedi Y. Monoamine receptors and signal transduction mechanisms in suicide. *Curr Psychiatry Rev.* 2006; 2:51–75.
- Pandey GN, Dwivedi Y. Noradrenergic function in suicide. *Arch Suicide Res.* 2007; 11:235–246. [PubMed: 17558608]
- Perez J, Tardito D, Mori S, Racagni G, Smeraldi E, Zanardi R. Abnormalities of cyclic adenosine monophosphate signaling in platelets from untreated patients with bipolar disorder. *Arch Gen Psychiatry.* 1999; 56:248–253. [PubMed: 10078502]
- Perez J, Tardito D, Mori S, Racagni G, Smeraldi E, Zanardi R. Altered Rap1 endogenous phosphorylation and levels in platelets from patients with bipolar disorder. *J Psychiatr Res.* 2000; 34:99–104. [PubMed: 10758250]
- Perez J, Tardito D, Mori S, Racagni G, Smeraldi E, Zanardi R. Protein kinase A and Rap1 levels in platelets of untreated patients with major depression. *Mol Psychiatry.* 2001; 6:44–49. [PubMed: 11244484]
- Perez J, Tardito D, Racagni G, Smeraldi E, Zanardi R. cAMP signaling pathway in depressed patients with psychotic features. *Mol Psychiatry.* 2002; 7:208–212. [PubMed: 11840314]
- Petty F, Sherman AD. Reversal of learned helplessness by imipramine. *Commun Psychopharmacol.* 1979; 3:371–373. [PubMed: 575771]
- Pinikahana J, Happell B, Keks NA. Suicide and schizophrenia: a review of literature for the decade (1990–1999) and implications for mental health nursing. *Issues Ment Health Nurs.* 2003; 24:27–43. [PubMed: 12735073]
- Pizon V, Lerosey I, Chardin P, Tavitian A. Nucleotide sequence of a human cDNA encoding a ras-related protein (rap1B). *Nucleic Acids Res.* 1988; 16:7719. [PubMed: 3137530]
- Qi M, Zhuo M, Skolhegg BS, et al. Impaired hippocampal plasticity in mice lacking the C β catalytic subunit of cAMP-dependent protein kinase. *Proc Natl Acad Sci U S A.* 1996; 93:1571–1576. [PubMed: 8643673]
- Qiu W, Zhuang S, von Lintig FC, Boss GR, Pilz RB. Cell type-specific regulation of B-Raf kinase by cAMP and 14-3-3 proteins. *J Biol Chem.* 2000; 275:31921–31929. [PubMed: 10931830]
- Quilliam LA, Mueller H, Bohl BP, Prossnitz V, Sklar LA, Der CJ, Bokoch GM. Rap1A is a substrate for cyclic AMP-dependent protein kinase in human. *J Immun.* 1991; 147:1628–1635. [PubMed: 1908879]
- Radomsky ED, Haas GL, Mann JJ, Sweeney JA. Suicidal behavior in patients with schizophrenia and other psychotic disorders. *Am J Psychiatry.* 1999; 156:1590–1595. [PubMed: 10518171]
- Rajkowska G. Cell pathology in mood disorders. *Semin Clin Neuropsychiatry.* 2002; 7:281–292. [PubMed: 12382210]
- Rajkowska G. Morphometric methods for studying the prefrontal cortex in suicide victims and psychiatric patients. *Ann NY Acad Sci U S A.* 1997; 836:253–268.
- Rosoklija G, Toomayan G, Ellis SP, Keilp J, Mann JJ, Latov N. Structural abnormalities of subicular dendrites in subjects with schizophrenia and mood disorders: preliminary findings. *Arch Gen Psychiatry.* 2000; 57:349–356. [PubMed: 10768696]

- Sackeim HA. Functional brain circuits in depression and remission. *Arch Gen Psychiatry*. 2001; 58:649–650. [PubMed: 11448370]
- Sarkar D, Erlichman J, Rubin CS. Identification of a calmodulin-binding protein that co-purifies with the regulatory subunit of brain protein kinase II. *J Biol Chem*. 1984; 259:9840–9846. [PubMed: 6086653]
- Scott JD, Glaccum MB, Zoller MJ, Uhler MD, Helfman DM, McKnight GS, Krebs EG. The molecular cloning of a type II regulatory subunit of the cAMP-dependent protein kinase from rat skeletal muscle and mouse brain. *Proc Natl Acad Sci U S A*. 1987; 84:5192–5196. [PubMed: 3037538]
- Scott JD, Stofko RE, McDonald JR, Comer JD, Vitalis EA, Mangili JA. Type II regulatory subunit dimerization determines the subcellular localization of the cAMP-dependent protein kinase. *J Biol Chem*. 1990; 265:21561–21566. [PubMed: 2147685]
- Seligman ME, Maier SF. Failure to escape traumatic shock. *J Exp Psychol*. 1967; 74:1–9. [PubMed: 6032570]
- Sengupta N, Vinod PK, Venkatesh KV. Crosstalk between cAMP-PKA and MAP kinase pathways is a key regulatory design necessary to regulate FLO11 expression. *Biophys Chem*. 2007; 125:59–71. [PubMed: 16863676]
- Sheline YI. 3D MRI studies of neuroanatomic changes in unipolar depression: the role of stress and medical comorbidity. *Biol Psychiatry*. 2000; 48:791–800. [PubMed: 11063975]
- Shelton RC, Hal Manier D, Lewis DA. Protein kinases A and C in post-mortem prefrontal cortex from persons with major depression and normal controls. *Int J Neuropsychopharmacol*. 2009b; 12:1223–1232. [PubMed: 19573263]
- Shelton RC, Sanders-Bush E, Manier DH, Lewis DA. Elevated 5-HT 2A receptors in postmortem prefrontal cortex in major depression is associated with reduced activity of protein kinase A. *Neuroscience*. 2009a; 158:1406–1415. [PubMed: 19111907]
- Sherman AD, Sacquitne JL, Petty F. Specificity of the learned helplessness model of depression. *Pharmacol Biochem Behav*. 1982; 16:449–454. [PubMed: 7200610]
- Simon MI, Strathmann MP, Gautam N. Diversity of G proteins in signal transduction. *Science*. 1991; 252:802–808. [PubMed: 1902986]
- Siris SG. Suicide and schizophrenia. *J Psychopharmacol*. 2001; 15:127–135. [PubMed: 11448086]
- Skalhegg BS, Tasken K. Specificity in the cAMP/PKA signaling pathway: differential expression, regulation, and subcellular localization of subunits of PKA. *Front Biosci*. 2000; 5:D678–D693. [PubMed: 10922298]
- Spaulding SW. The ways in which hormones change cyclic adenosine 3'5'-monophosphate-dependent protein kinase subunits, and how such changes affect cell behavior. *Endocr Rev*. 1993; 14:632–650. [PubMed: 8262010]
- Spleiss O, van Calker D, Scharer L, Adamovic K, Berger M, Gebicke-Haerter PJ. Abnormal G protein alpha(s)- and alpha (i2)-subunit mRNA expression in bipolar affective disorder. *Mol Psychiatry*. 1998; 3:512–520. [PubMed: 9857977]
- Steinberg RA, Cauthron RD, Symcox MM, Shuntoh H. Autoactivation of catalytic (C alpha) subunit of cyclic AMP-dependent protein kinase by phosphorylation of threonine 197. *Mol Cell Biol*. 1993; 13:2332–2341. [PubMed: 8455615]
- Stockmeier CA. Neurobiology of serotonin in depression and suicide. *Ann N Y Acad Sci*. 1997; 836:220–232. [PubMed: 9616801]
- Sweatt JD. Mitogen-activated protein kinases in synaptic plasticity and memory. *Curr Opin Neurobiol*. 2004; 14:311–317. [PubMed: 15194111]
- Thiele TE, Willis B, Stadler J, Reynolds JG, Bernstein IL, McKnight GS. High ethanol consumption and low sensitivity to ethanol-induced sedation in protein kinase A-mutant mice. *J Neurosci*. 2000; 20:RC75. [PubMed: 10783399]
- Thoenen H. Neurotrophins and neuronal plasticity. *Science*. 1995; 270:593–598. [PubMed: 7570017]
- Uhler MD, Chrivia JC, McKnight GS. Evidence for a second isoform of the catalytic subunit of cAMP-dependent protein kinase. *J Biol Chem*. 1986; 261:15360–15363. [PubMed: 3023318]
- Van Heeringen K, Audenaert K, Van de Wiele L, Verstraete A. Cortisol in violent suicide attempters: association with monoamines and personality. *J Affect Disord*. 2000; 60:181–189. [PubMed: 11074106]

- Villalba M, Bockaert J, Journot L. Pituitary adenylate cyclase-activating polypeptide (PACAP-38) protects cerebellar granule neurons from apoptosis by activating the mitogen-activated protein kinase (MAP kinase) pathway. *J Neurosci.* 1997; 17:83–90. [PubMed: 8987738]
- Vossler MR, Yao H, York RD, Pan MG, Rim CS, Stork PJ. cAMP activates MAP kinase and Elk-1 through a B-Raf- and Rap1-dependent pathway. *Cell.* 1997; 89:73–82. [PubMed: 9094716]
- Vossler MR, Yao H, York RD, Pan M-G, Rim CS, Stork PJS. cAMP activates MAP kinase and Elk-1 through a B-Raf- and Rap1-dependent pathway. *Cell.* 1997; 89:73–82. [PubMed: 9094716]
- Wagstaff A, Perry C. Clozapine: in prevention of suicide in patients with schizophrenia or schizoaffective disorder. *CNS Drugs.* 2003; 17:273–280. [PubMed: 12665398]
- Wahlbeck K, Cheine M, Essali A, Adams C. Evidence of clozapine's effectiveness in schizophrenia: a systematic review and meta-analysis of randomized trials. *Am J Psychiatry.* 1999; 156:990–999. [PubMed: 10401441]
- Walker AM, Lanza LL, Arellano F, Rothman KJ. Mortality in current and former users of clozapine. *Epidemiology.* 1997; 8:671–677. [PubMed: 9345668]
- Walsh DA, Perkins JP, Krebs EG. An adenosine 3',5'-monophosphate-dependant protein kinase from rabbit skeletal muscle. *J Biol Chem.* 1968; 243:3763–3765. [PubMed: 4298072]
- Wen Y, Anwer K, Singh SP, Sanborn BM. Protein kinase A inhibits phospholipase C activity and alters protein phosphorylation in rat myometrial plasma membranes. *Endocrinology.* 1992; 131:1377–1382. [PubMed: 1324160]
- Winokur G, Tsuang M. The Iowa 500: suicide in mania, depression and schizophrenia. *Am J Psychiatry.* 1975; 132:650–651. [PubMed: 1124812]
- Wolf S, Martinez C, Majzoub JA. Inducible binding of cyclic adenosine 3',5'-monophosphate (cAMP)-responsive element binding protein (CREB) to a cAMP-responsive promoter in vivo. *Mol Endocrinol.* 1999; 13:659–669. [PubMed: 10319317]
- Yamada S, Yamamoto M, Ozawa H, Piederer P, Saito T. Reduced phosphorylation of cyclic AMP responsive element-binding protein in the postmortem orbitofrontal cortex of patients with major depressive disorder. *J Neural Transm.* 2003; 110:671–680. [PubMed: 12768362]
- Young LT, Bezchlibnyk YB, Chen B, Wang J-F, MacQueen GM. Amygdala cyclic adenosine monophosphate response element-binding protein phosphorylation in patients with mood disorders: effects of diagnosis, suicide, and drug treatment. *Biol Psychiatry.* 2004; 55:570–577. [PubMed: 15013825]
- Young LT, Li PP, Kish SJ, et al. Cerebral cortex G_s alpha protein levels and forskolin-stimulated cyclic AMP formation are increased in bipolar affective disorder. *J Neurochem.* 1993; 61:890–898. [PubMed: 8395565]
- Young LT. Postreceptor pathways for signal transduction in depression and bipolar disorder. *J Psychiatry Neurosci.* 2001; 26 suppl:S17–S22. [PubMed: 11590965]
- Zubenko GS, Hughes HB III, Maher BS, Stiffler JS, Zubenko WN, Marazita ML. Genetic linkage of region containing the CREB1 gene to depressive disorders in women from families with recurrent, early-onset, major depression. *Am J Med Genet.* 2002; 114:980–987. [PubMed: 12457397]
- Zwartkruis FJT, Bos JL. Ras and Rap1: two highly related small GTPases with distinct function. *Exp Cell Res.* 1999; 253:157–165. [PubMed: 10579920]

Figure 1.

Overview of protein kinase A signaling. In this signaling pathway, the binding of neurotransmitter (NT) to G protein-coupled receptors leads to the activation of G proteins, which, in turn, activate adenylyl cyclase, leading to the production of cyclic adenosine monophosphate (cAMP). In the inactive state, the α subunit of G protein is bound to guanosine diphosphate (GDP) and to $\beta\gamma$ subunits. The binding of agonists to receptors causes an interaction of receptors with G proteins, which, in turn, releases GDP in exchange with guanosine triphosphate (GTP). This leads to generation of α -GTP and a $\beta\gamma$ subunit dimer. Both α and $\beta\gamma$ subunits can interact with effectors. Protein kinase A (PKA) is a holoenzyme composed of two subunits: regulatory (R) and catalytic (C). These regulatory and catalytic subunits form a tetrameric holoenzyme (R_2C_2). In the absence of cAMP, PKA exists as a stable inactive tetramer. The catalytic activity of cAMP is suppressed when the C subunits form a complex with the R subunits. Cyclic AMP, generated in response to adenylyl cyclase activation, binds to the R subunits of tetrameric PKA holoenzymes. The binding of cAMP to an R subunit lowers its affinity for the C subunit. This causes the release of free C subunits. The C subunit can catalyze phosphorylation of substrates in the cytoplasm or, after translocation of the C subunits, in the nucleus. Protein kinase A is also anchored with A kinase-anchoring proteins, causes compartmentalized localization of PKA, and initiates phosphorylation of substrates in a localized fashion. One of the substrates of PKA is transcription factor cAMP response element binding (CREB) protein, which regulates the transcription of many neuronally expressed genes, including brain-derived neurotrophic factor (BDNF). The exchange protein activated by cAMP (Epac) is stimulated by cAMP to exchange GDP with GTP on Rap1. Rap1 can also be regulated by phosphorylation by a C subunit of PKA. Deactivation of PKA is achieved through degradation of cAMP to 5' AMP by cAMP-specific phosphodiesterases (PDEs).

Figure 2.

Changes reported in the activation and/or expression of specific catalytic (C) and regulatory (R) subunit isoforms of protein kinase A (PKA), cyclic adenosine monophosphate response element binding protein (CREB), Rap1, and brain-derived neurotrophic factor (BDNF) in suicide brain or during stress. (↓) denotes decrease; and (↑), increase.