The Role of Protein Kinase A in Anxiety Behaviors

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Introduction

Anxiety aggregates in families, has both heritable and developmental origins, and is associated with sensitivity to fear. Available data suggest that fear-related disorders are highly complex and polygenic, and despite substantial progress in genetics (and epigenetics), few responsible loci have been identified for these disorders. Recently, molecular genetic approaches, including genomic studies, have been applied to identify pathways that are associated with anxiety risk. There is now ample experimental and preclinical evidence showing that anxiety disorders are associated with abnormal neural processing of threat-related stimuli, which is mediated by the cyclic AMP (cAMP)-protein kinase A (PKA) pathway. In addition, stress in sensitive phases of development may influence structural integrity of specific brain regions and neural processing pathways involved in emotion regulation, consistent with a gene-environment-timing interaction in mood dysregulation [1].

This review focuses on the PKA genetic basis of anxiety disorders for which environmental stressors and stress responses are understood to be central to pathogenesis. We describe the PKA pathway and evidence from preclinical and clinical studies that demonstrate the importance of the role of PKA in anxiety disorders. Finally, we discuss the prospects for clinical translation of PKA genetic pathway findings and future research perspectives.
Overview of the PKA Pathway

PKA is an evolutionarily conserved serine threonine kinase that regulates diverse signal transduction pathways, including cellular development, proliferation, differentiation, apoptosis, and tumorigenesis. PKA is considered the main target for cAMP in the cell, is widely distributed and serves as the principal effector mechanism for G-coupled receptors linked to adenylate cyclase [2]. In the absence of cAMP, PKA is an inactive tetrameric holoenzyme consisting of two catalytic (C) subunits bound to a regulatory (R) subunit dimer, which is compartmentalized to distinct locations in the cell by A-kinase anchoring proteins (AKAPs) [3]. AKAPs contribute to maintaining specificity of PKA signaling, but it is also believed that several isoforms and splice variants of especially the C subunits are important mediators of specificity.

Based on the elution profile on diethylaminoethyl cellulose exchange chromatography, two isoforms of the PKA heterotetrameric enzyme, PKA-I and -II, exist in most cells. The different PKA subtypes (types I and II) have different affinity for cAMP. This is due to the presence of either type I or II R subunits. There are four such subunits in humans (and mice): RIA and RIB, and RIIA and RIIB, coded by the PRKAR1A, PRKAR1B, PRKAR2A, and PRKAR2B genes, respectively [4]. R subunits form a homodimer that binds two C subunits (one each); there are four C subunits in humans (and mice): CA, Cβ, Cy, and protein kinase X gene (PRKX), coded by the PRKACA, PRKACB, PRKACG, and PRKX genes, respectively, in the PKA tetramer (R2C2). When the R and C subunits form a complex, the cAMP-catalytic activity is suppressed. As shown in knockout (KO) mouse studies, these four genes function in a tissue and cell type-specific manner to regulate accurately the activity of the C subunits [5].

The R Subunits

The R1 subunits have much greater affinity for cAMP than the RII subunits. The RIA and RIB isoforms show 80% and the RIIA and RIIB isoforms 68% identity. The RIA-containing holoenzymes are activated at a five-fold lower concentration of cAMP than the RIB-containing holoenzymes [6]. Each R subunit is a separate gene product and has a distinct expression pattern in different tissues [2]. RIA, the subunit that is deficient in primary pigmented nodular adrenocortical disease and Carney complex (CNC; which is associated with psychological disorders including anxiety), is the most abundant and ubiquitously expressed of the four PKA R subunits. RIB is expressed mostly in the central nervous system (CNS), whereas tissue distribution studies suggest that RIA is expressed almost as widely as RIA. RIIB is predominantly expressed in the brain, adipose, and adrenals and may be the principal mediator of cAMP activity in the mammalian CNS since RIIB is widely expressed in the brain but less strongly than RIIA [7].

The C Subunits

It has been shown that Cβ-containing PKA holoenzymes dissociate more easily on binding to cAMP than those containing CA [8]. Tissue distribution studies suggest that Cβ is expressed primarily in the brain [9], whereas CA is ubiquitously expressed. Cy is present only in the testes [10]. Both CA and Cβ subunits exhibit alternative splicing at the first exon, resulting in amino terminal differences between transcript variants, named PKA-Cα1, PKA-Cα2, PKA-Cβ1, and PKA-Cβ2 [3]. These C subunits, as well as the C subunit-binding protein HA95, are involved in pre-messenger RNA (pre-mRNA) splicing, possibly through a cAMP-independent mechanism [11]. There is little known about PRKX.

Guanine Nucleotide-Binding Proteins

Guanine nucleotide-binding proteins (G proteins) occupy a central position and play a critical role in the transduction of extracellular signals to cellular targets [12]. Approximately 80% of the receptors for neurotransmitters, hormones, and neuromodulators elicit their responses through G proteins. More than 16 distinct genes encode the G protein α-subunits, and there is a splice variant in at least two genes [13]. A total of 5 distinct β-subunit genes and 12 γ-subunit genes have also been identified. The β- and γ-subunits bind tightly to each other, and the β-subunit also contains a common binding site for α-subunit recognition. In the inactive state, the α-subunit of the G protein is bound to guanosine diphosphate (GDP) and to βγ-subunits. Both α- and βγ-subunits can interact with effectors. The α-subunit confers receptor-effector specificity to G proteins, whereas the γ-subunit has a G protein-specific recognition site. The binding of agonists to receptors causes an interaction of receptors with G proteins, which, in turn, releases GDP in an exchange with guanosine triphosphate (GTP). The α-subunit binds to GTP, leading to the generation of α-GTP and a βγ-subunit dimer. The α- and βγ-subunits can then activate various effectors to moderate cellular responses [14].
**G Proteins in Modulating PKA**

The binding of neurotransmitter to G protein-coupled receptors leads to the activation of G proteins, which activate adenylyl cyclase, leading to the production of cAMP. Of the various Ga isoforms, Gsα stimulates adenylyl cyclase, whereas Giα mediates the inhibition of adenylyl cyclase. Stress signals lead to the hypothalamic release of corticotropin-releasing hormone (CRH) and vasopressin, inducing pituitary secretion of adrenocorticotropic hormone, which binds its G-protein-coupled receptor (melanocortin receptor 2 or MC2R) in adrenal fasciculate, activating adenylyl cyclase [15].

The activation of adenylyl cyclase catalyzes the conversion of adenosine triphosphate to cAMP (fig. 1). After an increase in intracellular cAMP, cAMP serves as a second messenger, and the PKA R subunits bind to cAMP in a cooperative manner, which results in the conformational change in the R subunits, leading to the disassociation of the tetrameric PKA holoenzyme into an R2-(cAMP)4 dimer and two monomers of catalytically active C kinase. The binding of cAMP to an R subunit lowers its affinity for the C subunit. This causes the R subunits to remain in the cytoplasm, and the free C subunits to either translocate into the nucleus or catalyze the transfer of phosphates from ATP to serine and threonine residues in a diverse number of cytosolic target substrates in the vicinity of the PKA holoenzyme [16].

**PKA Cytosolic Targets**

Activation of PKA in vivo leads to tau hyperphosphorylation at both PKA and non-PKA phosphorylation sites [17]. Thus, PKA phosphorylates targeted intracellular proteins modifying hormonal and neurotransmitter responses, including receptor downregulation or desensitization, alteration of neurotransmitter release, and activation or repression of gene expression. In addition to its role in gene transcription, PKA is capable of phosphorylating many substrates involved in neurotransmitter release; receptor desensitization; cortisol biosynthesis; cell growth, differentiation, and survival, and synaptic plasticity [18].

**PKA Nuclear Targets**

Upon activation, a proportion of the C subunit translocates to the nucleus [19]. The major targets for C subunit phosphorylation in the nucleus are a group of cAMP-responsive nuclear factors [20], which bind and regulate the expression of genes containing cAMP-responsive elements (CREs), called CRE-binding proteins (CREBs) [21]. The understanding of how specificity of the nuclear C is maintained is sparse; however, it is expected that temporal and spatial regulation may involve C subunit targeting to nuclear structures independently of the R subunit. This is supported by recent reports demonstrating that the C subunit associates with the nuclear A-kinase-interacting protein [22] and the chromatin-associated protein HA95 [11, 23].

**PKA and CREB**

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. This bifurcation and time-dependent regulation of the cAMP-responsive signaling pathways may enable the cell to endure and/or enforce a cellular response provoked by a cAMP-elevating stimulus [24, 25]. 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 [26].

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PKA and Anxiety

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The Role of Phosphodiesterases

The response generated by cAMP can be terminated by the hydrolysis of cAMP into 5′AMP by phosphodiesterases (PDEs) or by removal of the phosphate group by protein phosphatases (fig. 1). The PDE activity is enhanced through PKA phosphorylation, leading to an abrupt termination of the signal [27]. It has been shown that binding of RIα activates PDE catalysis several fold, demonstrating a dual function of RIα, both as an inhibitor of the PKA C subunit and as an activator for PDEs. Deletion mutagenesis has localized the sites of interaction to one of the cAMP-binding domains of RIα and the catalytic PDE domain of RegA. Binding of RegA facilitates dissociation of cAMP followed by hydrolysis of the released cAMP to 5′AMP [25].

PKA Subunits Compensate for Each Other’s Expression and Function

Studying the R subunit in the murine immune system, Schillace et al. [28] showed that T cells isolated from the RIα mice were stimulated to the same extent as wild-type (WT) mice by anti-CD3/anti-CD28 and by a cocktail of phorbol 12-myristate 13-acetate and ionomycin stimulation and were inhibited to the same extent as WT mice by anti-CD3/anti-CD28 and by a cocktail of phorbol 12-myristate 13-acetate and ionomycin stimulation. No differences were seen in the ability of the T cells to differentiate into Th1 or Th2 cells and produce cAMP. The results suggested either that the RII subunit is not important for this type of cell biology or that other proteins are compensating for RII function. In addition, compensation can occur through a change in the subcellular localization of the protein without increasing the quantity of the protein [28]. Whether RI AKAPs are involved in PKA subunit regulation to compensate for this function remains unknown [28].

RI compensation has been shown in the hippocampus and cerebral cortex of RIα mice, resulting in a PKA holoenzyme with a significantly increased basal activity, probably caused by a lower threshold of activation associated with the RI holoenzyme [29]. Similarly, it has previously been shown that in some cell types, the RIα mouse C subunit of PKA remains colocalized with the L-type Ca2+ channel, suggesting that, despite a lower affinity for AKAP binding, RIα is anchoring the C subunit in the absence of RII [29]. This compensation is possible by RI binding not only to RII AKAPs, but also to dual specificity AKAPs (D-AKAPs), which bind both RI and RII [30, 31].

Pilot studies had shown that cells can compensate for the increased levels of either Ca or Cβ subunits with a corresponding elevation of RI protein, implying that both isoforms of C can interact with RI to form a holoenzyme [32]. Although accounting for just 5–10% of total PKA activity in mouse cells, Cβ also seems to compensate for states of Ca deficiency [33] and in the presence of strain-specific genetic modifiers [34] or dysregulated PKA activity [35]. Thus, in Ca KO mice, compensatory increases in Cβ levels occurred in the brain, whereas many tissues, including skeletal muscle, heart, and sperm, contained less than 10% of the normal PKA activity [36].

The Role of PKA in Anxiety

Fear Learning and Anxiety

PKA activity is affected by various neurotransmitters (i.e. acetylcholine, dopamine, norepinephrine, serotonin, and histamine) that are involved in alertness, anxiety, emotion, or mood indirectly through the stimulation of the G-protein-coupled receptor or adenyl cyclase or directly by cAMP [37]. The CRE is present in many genes and functions as a promoter/enhancer element in many brain areas that respond to environmental stimuli [38]. The observed differences in memory processes that are associated with mood, anxiety, or emotion are likely due to the effects of substances that effect adenyl cyclase activity [39]. For example, dopaminergic D1 and B-noradrenergic receptors enhance, while serotonin receptors inhibit the activity of adenyl cyclase [39].

Abel and Kandel’s [26] seminal work on cellular mechanisms of gill-withdrawal memory formation reflex in Aplysia identified the central role of PKA in the process of memory formation. Since then, the role of PKA in fear memory formation has been characterized in different processes in several species (Aplysia, Drosophila, mouse, chick, and rat) [40–43]. There is now ample evidence to support that fear memories can form quickly and may be difficult to eliminate [44–46]. The mechanisms of PKA involved in fear memory consolidation and neural plasticity include a wide range of cellular processes, including the activation of CREB and other transcription factors involved in the regulation of de novo protein synthesis required for long-term memory formation, and interaction with various intracellular signaling cascades and receptors [26]. Robinson-White and Stratakis [47] described PKA signaling as a ‘central hub’ which interacts with various other signaling pathways in endocrine cells. PKA functions as a mediator and communicator of cAMP effects to mitogen-activated protein kinases, and protein kinase C (PKC) and B pathways [47,
The regulation of the hypothalamic-pituitary-adrenal (HPA) and autonomic nervous system via signal transduction pathways such as PKA and PKC, may be important in the expression of genes that contain cAMP in their promoters, which include key proteins that regulate the stress response in the brain (e.g. brain-derived neurotrophic factor, glucocorticoid receptor) [49]. These kinase pathways have a time-dependent activation profile in relation to the learning process involving fear memories.

Signaling activity in neural circuits before or after stimuli may influence PKA activity and long-term potentiation (LTP), affecting fear learning and memory of the event [40, 50, 51]. PKA has two peaks of activity in the process of long-term memory formation, with the first occurring a few minutes after the event, and the second occurring 2–3 h after the event (requires both transcription and protein synthesis). The PKA pathway is also an important component of short-term memory within the first hour after the event. The phosphorylated form of CREB also increases at these same time periods as PKA and contributes to the synthesis of new proteins that are essential for long-term memory formation [39].

Threat Processing and the Brain

The hippocampus, amygdala, and prefrontal cortex (PFC) play key roles in the formation of memories of stressful stimuli, including fear [46, 52]. Fear and anxiety are evolutionary conserved defensive responses that are essential to survival. Anxiety (state) is a normal response to a potential threat in the environment. Pathological anxiety (trait) occurs when the defensive response is not attenuated when the threat is no longer present or when there is a deficit in the ability to discriminate a threat from safety (i.e. prediction error). Thus, anxiety may be defined as a generalized state of distress elicited by nonspecific cues. It is well established that pathological anxiety is associated with abnormalities in threat detection and fear learning [53–56]. A bias in threat processing has been implicated in the etiology and maintenance of anxiety disorders and posttraumatic stress disorder (PTSD) [57–61]. Also, PTSD has been characterized as a disorder of impaired safety signal learning.

Studies of human and rodent threat response have elucidated the topography and time sequence of neural engagement during threat-processing behavior [61–64]. With threat exposure, the amygdala and attention processes are immediately engaged. The amygdala, which is part of the limbic system, is a group of nuclei located within the anterior medial portion of the temporal lobe.
**Direct/Indirect Inhibition of the PKA Pathway: Lessons from Transgenic Mice**

*The 'Switchboard'*

Animal studies, including transgenic mice, have enriched our knowledge of the role of the cAMP pathway in anxiety. Research with transgenic animals that were unable to express CREB or PKA normally, or with PKA inhibitors, established the essential role of PKA and CREB for memory formation [40, 74–78]. Mice with null mutations in the genes encoding PKA subunits and pharmacologic agents that interfered with the activity of PKA or CREB have helped to elucidate the precise timing of the intervention of cAMP, PKA, or CREB in the process of fear memory formation (e.g. short- or long-term memory) [76, 78–80]. Studies using inhibitors or activators of PKA provide evidence to support that PKA is a crucial intracellular regulator of neuroplasticity in the amygdala [81–83]. The amygdala has been likened to a 'switchboard' for fear/threat processing, with different types of threat stimuli dependent on neural activation of distinct nuclei (cortical, basolateral, basomedial, lateral, and striatal (medial and central) in the amygdala.

**Inhibitors of Protein Synthesis or PKA Activity**

Research with transgenic mouse models with inhibitors of protein synthesis or PKA activity demonstrate that inhibition of PKA activity blocks LTP in the hippocampus and interferes with memory consolidation for fear in the amygdala [41, 84, 85]. Infusion of PKA inhibitors into BLA immediately following fear-conditioning training dose-dependently blocked consolidation of fear memory (24 h after training) but not short-term memory (4 h) [86]. Infusion of inhibitor Rp-cAMPs into the CEA decreased CREB function and decreased neuropeptide Y (NPY) expression and provoked anxiety-like behavior and alcohol intake in nonpreferring rats [87].

**PDE4 Inhibitors**

PDE4 is an enzyme that catalyzes the hydrolysis of cAMP and has a crucial role in the regulation of its intracellular concentration. PDE4 is highly expressed in brain regions involved in the regulation of memory, anxiety, and depression (hippocampus, amygdala, and nucleus accumbens) [88]. Studies with rodents support the role of PDE4 in CNS processes including depression [89], learning and memory [90], and anxiety [91]. Studies using PDE4 inhibitors (rolipram and etazolate) demonstrate an antidepressant-like effect [92–96], a reversal of memory deficits [97–99] and a reduction in anxiogenic behavior [100–103]. Amplification of the cAMP pathway with type IV-specific PDE inhibitor rolipram [90, 97, 104, 105] increased the long-term but not the short-term memory of contextual fear conditioning and object recognition (table 1).

Also, studies using genetic deletion mutants of specific PDE4 subtypes have shown neurobehavioral effects. For example, PDE4A KO mice display anxiogenic-like behavior [102, 106], which suggests that PDE4B may be involved in the regulation of anxiety. However, studies of PDE4B KO mice vary, with reports of an anxiogenic-like behavioral phenotype [102] or no difference in measures of anxiety-like behavior [107], and an antidepressant effect [95, 108, 109]. Recently, McGirr et al. [110] reported that specific inhibition of PDE4B (a catalytic domain mutant form of PDE4B) resulted in anxiolysis and facilitated memory acquisition.

**Increased PKA Activity**

Studies with transgenic mice expressing the G protein (Gnas) that stimulates adenyl cyclase activity provide evidence to support that increased cAMP signaling is associated with an anxiety-like phenotype and provide indirect evidence that an increase in PKA activity is associated with an increased risk for anxiety [111]. Bourtchouladze et al. [112] reported that transgenic mice that express a constitutively active form of Gsa in the forebrain exhibited impairments in spatial learning and in contextual and cued fear conditioning. There were significant increases in adenyl cyclase activity in the cortex, hippocampus, and striatum and increased cAMP levels in the striatum; however, in the cortex and hippocampus of Gnas mice, the cAMP levels were significantly reduced due to PKA-dependent compensatory upregulation in total cAMP PDE activity [113]. Direct activation of PKA by infusion of 8-bromo-cAMP into BLA immediately after training demonstrated that glucocorticoids (GC) influence the efficacy of noradrenergic stimulation in the BLA on memory consolidation via the β-adrenoreceptor-cAMP cascade [114].

Our research with a mouse model with the loss of one Prkar1a allele showed augmentation of anxiety-like behaviors associated with an increase in PKA activity in the BLA and CEA and an increase in threat bias [115, 116]. Functionally, loss of Prkar1a is associated with excess PKA signaling, and these findings highlight the importance of cAMP/PKA signaling in neural areas relevant to the emotional processing of threat (i.e. amygdala). A recent report of KO mouse with a downregulation in the C subunit found no difference in PKA activity in the amygdala...
Table 1. Preclinical research studies of anxiety or fear memory using stimulation, direct, or indirect inhibition of PKA pathway, protein synthesis and various transgenic mice models

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PKA-Related Targets Associated with Anxiety-Like Behavior

Evidence from preclinical studies demonstrate that various targets in the PKA pathway are involved in the regulation of emotional behavior and alterations in this pathway are associated with anxiety and other comorbid behaviors and are therapeutic targets. For example, mice with targeted ablation of CREB during adulthood in forebrain neurons showed severe anxiety phenotype but unaltered hippocampal-dependent long-term memory in context-dependent fear conditioning. CREB protein during adulthood seems to be pivotal for the regulation of emotional behavior [87, 118–120]. In addition, studies support a role for NPY and several other CREB-related target genes in anxiety and alcohol abuse [87, 121–126]. Pandey et al. [122] reported that alcohol-preferring rats had lower levels of CREB, p-CREB, and NPY in the CEA, an area known to have a crucial role in anxiety behaviors as well as alcohol intake [126–128].

Other PKA-related neural receptors involved in anxiety-like behavior include the glutamate receptor GLuR1 [129–133], calmodulin signaling [134], corticotropin-releasing factor (CRF)-1 receptors within medial PFC [135], and cAMP-regulated phosphoprotein 32 kDa [136–139]. Also, CRH has been shown to modulate anxiety-related behavior in rodents [140–144] independent of HPA axis activation [142, 145]. In addition, alterations in cholecystokinin [146], and substance P [147], GABA_A receptor function [148, 149], and 5-HT neurotransmission [150, 151] are associated with anxiety-like behavior.

Clinical and Pharmacokinetic Studies of the PKA Pathway

Fear and Anxiety

Anxiety is the most commonly reported psychiatric conditions. Although anxiety is an adaptive response to potential threats, its pathogenesis is poorly understood. Two factors associated with anxiety are behavioral inhibition and anxiety sensitivity. The expression of anxiety can range from normative to pathological behavior according to the frequency, intensity, duration and/or interference in functioning. Therefore, anxiety disorders may lie at the extreme end of a continuum, rather than involve symptoms that are exclusive to pathological conditions. As such, anxiety disorders would represent a variation in degree but not in kind [152]. This would imply a view of normal and pathological anxiety as falling along a dimension, with diagnostic thresholds reflecting clinical and societal burden rather than discontinuous pathophysiological states.

The response to an environmental stressor involves the individual’s interpretation of the threat, which is regulated by the brain. The brain and nervous system demonstrate adaptive plasticity through local neurotransmitters and systemic hormones, which interact to produce structural and functional changes [52]. The brain is also a target for the actions of stress hormones, in particular GC. With stress exposure, the paraventricular nucleus in the hypothalamus releases CRH and arginine vasopressin, which stimulate the anterior pituitary to release adrenocorticotropic hormone, which in turn stimulates the adrenal cortex to release GC. GC exert a negative feedback to the hypothalamus and anterior pituitary to down-regulate the stress response. GC receptors in the brain are found in high density in the hippocampus, amygdala, and PFC. Their location facilitates the formation of memories associated with strong emotions particularly during stress. Recent studies suggest that the neuropeptide CRF plays a tonic anxiogenic-like role at CRF-1 receptors within the medial PFC, since their blockade per se attenuated anxiety indices and that the anxiogenic-like effects following CRF-1 receptor activation depend on cAMP/ PKA cascade activation in this limbic forebrain area [153]. In mice, adenosine administration increased anxiety-like behaviors in WT mice, whereas caspase-1 KO mice were resistant to adenosine-induced anxiety-like behaviors [154].

Affective Disorders

Recent human and animal neuroimaging studies suggest that dramatic changes in the PFC during adolescence are involved in anxiety, depression, and suicide (diagnoses that have a higher incidence in this age group). A link of suicide to upregulation in the PFC of various protein kinases implicated in the development of fear memory and stress has been suggested [14]. Studies also report abnormalities in G protein subunits or G protein-mediated functional response in patients with depression and suicidal behavior [155, 156], suggesting abnormal (hyperfunction) adenyl cyclase-cAMP activity [49]. Several studies report brain-specific alterations in the protein and mRNA levels of PKA related to teenage suicide victims [157–159]. Thus, in the PFC of suicide victims with major
depression, a region-specific alteration of G-protein-induced activation of the phosphoinosside signal transduction system and of the levels of G-protein α-subunits involved in cAMP synthesis has been demonstrated [160].

Although there is substantial evidence to support the role of the cAMP-PKA pathway in mood disorders, there is a paucity of data about PKA activity in brain areas other than the PFC from postmortem brains of depressed and suicidal patients. The PFC has been a primary focus of investigation in suicide related to its top-down regulatory function of behavior. The hyperfunctional cAMP activity reported in the PFC of depressed and suicidal subjects is consistent with what is known about the association of perturbations in the PFC-amygdala-hippocampal circuitry and anxiety disorders [14, 49]. However, there is substantial evidence from animal studies to support that alterations in the hippocampal-amygdala-prefrontal connectivity as well as alterations in synaptic and structural plasticity are associated with affective illnesses, including generalized anxiety disorder, depression, and suicide.

Recent studies using fMRI demonstrate the relevance of data on topography and chronometry of fear circuitry function in rodents, a process known to be dependent on PKA and protein synthesis [161–166]. For example, Monk et al. [167, 168] report that the magnitude of amygdala engagement correlated with both anxiety severity and attention bias, while activity in the ventromedial PFC correlated negatively with activity in the amygdala during brief threat exposure. It is generally accepted that anxiety is associated with an attention bias to threat and there are abundant data that elucidate the neural circuitry engaged in this process, which shows strong conservation across phylogeny. The process involves cued and context conditioning, consolidation, and extinction, processes that are
dependent on the PKA-cAMP pathway. Future research holds promise that neuroimaging technologies such as fMRI, PET scan, diffusion tension imaging, and optogenetics will serve to translate preclinical research to clinical science and inform therapeutics (fig. 2).

Pharmacologic agents currently used to treat anxiety primarily target various neurotransmitters in the brain (e.g. 5-HT receptor, GABA, calcium channel receptor, CRF receptor, cholecystokinin, NPY). Recently, compounds that specifically inhibit cyclic nucleotide PDEs were noted to have CNS effects and are being evaluated for the treatment of mood disorders (i.e. anxiety and depression) and also as cognitive enhancers. The neuropsychiatric effect is achieved via alteration of an intracellular secondary messenger system (cAMP-PKA) rather than antagonism of neurotransmitter receptors [96, 169–171].

Genetic Syndromes

Various genetic syndromes have identified distinct and consistent behavior patterns due to the underlying genetic defect(s). Results of many studies support the finding that alterations in PKA and some of its substrates are associated with various psychiatric disorders, including anxiety, depression, obsessive compulsive and bipolar disorders, schizophrenia, and panic disorder [172–177]. CNC is a rare multiple endocrine neoplasia syndrome that was first described by Dr. Carney (Mayo Clinic, USA) in 1985 as a complex of myxomas (cardiac, skin, cutaneous), spotty skin pigmentation, and endocrine overactivity [178]. CNC (OMIM 160980) is an autosomal dominant multiple endocrine neoplasia syndrome caused by loss-of-function mutations in PRKAR1A in most CNC patients studied to date [179]. It is likely that whereas the loss of R1a leads to the full CNC phenotype, the gain of function in Ca leads to adrenal tumors and Cushinge syndrome only, and the amplification of Cβ is associated with non-adrenal manifestations of CNC, such as skin pigmentation, acromegaly, and myxomas [180]. The long-term implications of these findings, including the consideration that manipulation of the PKA system could be used for therapeutic effect, may bear further investigation.

The behavioral phenotype in patients affected by CNC is difficult to characterize due to the number, type, and onset of endocrine tumors, as well as somatization, which is commonly seen in chronic illness. A recent study of adults and children reported significant differences in diagnosis of psychiatric disorders between adults and children with CNC and PRKAR1A mutation compared to adults and children with CNC and negative PRKAR1A mutation [181]. The most frequent psychiatric diagnoses in adults with PRKAR1A mutation were anxiety, depression, and bipolar disorder (in that order). The most frequent psychiatric diagnosis in children with PRKAR1A mutation were attention-deficit/hyperactivity disorder, anxiety, and depression (in that order).

Conclusions and Future Directions

Mood disorders, such as anxiety and depression, are prevalent psychiatric illnesses. In addition, anxiety disorders may lie at the extreme end of a continuum, rather than involve symptoms that are exclusive to pathological conditions. In this review, we discussed the association of anxiety disorders with an abnormal neural processing of threat-related stimuli, a substantial part of which is influenced by the cAMP-PKA pathway, among others. These data provide both a cellular locus and signaling framework for the development of new therapeutics for the treatment of neuropsychiatric diseases, including anxiety. While animal models used to elucidate the molecular pathways important to anxiety have been useful, they have limitations, so cautious interpretation is appropriate. How PKA, an evolutionarily conserved kinase that regulates diverse signal transduction pathways, interacts with other neurotransmitter systems that are involved with alertness, emotion, or mood will certainly continue to captivate scientists interested in anxiety disorders.

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