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Emerging evidence for the role of pituitary adenylate cyclase-activating peptide in neuropsychiatric disorders

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Abstract

Stress activates many brain nuclei and causes acute changes in several physiological and behavioral responses to restore homeostasis in affected organisms. While this response is protective, chronic stress exposure causes the sustained activation of these nuclei, leading to maladaptive physiological changes that underlie pathological mood and affective states. Hence, chronic stress may produce anxiety and mood disorders by promoting neuronal plasticity within these stress-responsive nuclei. A growing body of evidence attributes neuropeptide systems in mediating not only the physiological stress response but also pathological states that develop following chronic stress exposure. Recent preclinical data suggest that pituitary adenylyl cyclase-activating polypeptide (PACAP) and its receptors (PAC₁, VPAC₁, and VPAC₂) play an important role in the behavioral and endocrine responses to stress, as well as in mood and affective disorders. Human studies also point out the significance of the PACAP/PAC₁ receptor system in these disorders. For instance, PACAP through PAC₁ receptor up-regulates the expression of DISC1 (disrupted in schizophrenia 1) and impedes its association with its interacting protein. Interestingly, the DISC1 gene mutation is linked to schizophrenia and depression. Moreover, a link between PACAP blood titer and fear physiology,

post-traumatic stress disorder (PTSD) diagnosis and symptoms has been reported in heavily traumatized female patients. Additionally, in the peripheral blood, methylation of the gene encoding the PAC₁ receptor is also associated with PTSD. This book chapter describes the emerging evidence that entails PACAP in the stress response and stress-mediated neuropsychiatric disorders.

1. Introduction

Pituitary adenylyl cyclase activating polypeptide (PACAP), a pleiotropic polypeptide, is related to the secretin/glucagon/vasoactive intestinal polypeptide (VIP) family and is present in two biologically active forms, PACAP-38 and PACAP-27.¹ PACAP and VIP have a high resemblance in their primary structure, and both peptides exert a variety of functions through activating G-protein coupled receptors. VIP and PACAP share two common receptors (VPAC₁ and VPAC₂ receptors) while only PACAP binds with high affinity to PAC₁ receptors.²

High to moderate expression of PACAP and its selective (PAC₁) receptor have been reported in the hypothalamic and limbic structures and in other brain regions as well as in peripheral tissues.³ The broader distribution of PACAP in and outside the central nervous system suggests its involvement in several biological processes, such as regulation of food intake, energy metabolism, body temperature, neuronal survival, and the neuroendocrine and behavioral response to stress.^{3–7}

Earlier studies showed expression of PACAP and its receptors in the hypothalamus and adrenal glands. Predominantly abundant innervations of hypophysiotropic neurons by nerve fibers comprising of the neuropeptide PACAP suggests a prominent role of this peptide/receptor system in the control of the hypothalamic-pituitary-adrenal (HPA) axis and stress response (reviewed in Ref. 8). Along this line, PACAP increases the expression of CRH in the paraventricular nucleus (PVN) of the hypothalamus^{9–11} and intra-PVN administration of PACAP mimics behavioral changes result from the activation of PVN by CRH or a stressor.¹² Likewise, restraint stress-induced secretion of corticosterone is attenuated in mice lacking PACAP,¹³ suggesting that PACAP plays a functional role in the stress response.

While activation of the stress circuit acutely promotes a “fight or flight” response, the prolonged activation of the HPA axis can lead to maladaptive neuroendocrine and behavioral changes. The negative behavioral consequences of chronic stress result from continuous or inappropriate activation

of the stress circuit and continued secretion of stress mediators, leading to improper activation of the fear circuit and the development of anxiety. These mediators also exert anti-rewarding actions, leading to diminished mood and anhedonia as observed in depressed patients. These mediators even overwhelm the sleep-wake cycle that causes insomnia as well as daytime somnolence which are frequently reported in patients with post-traumatic stress disorder (PTSD). Interruption of executive and cognitive functions also could occur as an outcome of continuous activation of the stress circuit.¹⁴ Considering that stress is one of the leading causes of neuropsychiatric disorders, we first discuss the role of PACAP in the stress response followed by its involvement in the pathophysiology of stress-induced mental illnesses.

2. PACAP and stress response

Stress can be defined as a condition when homeostasis is threatened, or the organism observes a situation to be threatening. The HPA axis mediates the stress response. Corticotropin releasing factor/hormone (CRF/CRH) has long been known as the key regulator of the HPA axis and plays a crucial role in stress responses.¹⁵ Briefly, activation of the HPA axis by a stressor involves the release of CRH from neurosecretory cells in the PVN of the hypothalamus. CRH travels through the portal circulation to the pituitary and stimulates the release of adrenocorticotropin hormone (ACTH) from the anterior pituitary. ACTH, in turn, reaches its site of action and acts on the adrenal glands to cause the release of glucocorticoids, which not only mediate the stress response but also act as negative feedback to reduce further activation of the HPA axis.¹⁶ The latter is a biologically protective mechanism that promotes a “fight or flight” response to a stressor. However, the prolonged activation of the HPA axis can lead to maladaptive behavioral changes, such as anxiety and/or depression, in which the negative feedback mechanism may be impaired. Thus, the HPA axis can be the target not only for the stressors but also that of endogenous chemicals that serve as regulators of the stress response and could contribute to the pathophysiology of stress-related neuropsychiatric disorders.

PACAP and its receptors are found in the hypothalamus and adrenal glands. Predominantly abundant innervation of hypophysiotropic neurons by nerve fibers comprising of the neuropeptide PACAP suggests a prominent role of this peptide/receptor system in the control of the HPA axis

and stress response (reviewed in Ref. 8). Indeed, PACAP increases the expression of CRH in the PVN^{9–11} and local administration of PACAP into the PVN mimics behavioral changes result from the activation of PVN by CRH or a stressor,¹² showing that PACAP is involved in the stress response. Along this line, restraint stress causes secretion of corticosterone in wild-type mice, a response that is attenuated in mice lacking PACAP.¹³ Moreover, repeated stress (2–3 h per day for 7 days) leads to unhabituated corticosterone secretion and this response is reduced in mice lacking PACAP.¹⁷

Chronic variable stress increase PACAP and PAC₁ receptor transcript in the bed nucleus of stria terminalis (BNST), raising the possibility that PACAP may regulate the stress response and could contribute to the pathophysiology of mood and affective disorders via acting not only on the HPA axis but also on extrahypothalamic brain regions. Indeed, local administration of PACAP into the BNST increases corticosterone secretion^{18,19} and induces anxiety-like behaviors.¹⁸ Furthermore, PACAP injection into the central nucleus of amygdala (CeA) mimics the behavioral and neuroendocrine manifestations of stress.²⁰

Epinephrine and norepinephrine release from the adrenal medulla represent the cardinal feature of acute stress responses. Neuropeptides are active as transmitters in this circuitry. Remarkably, recent findings in the preceding decade have enhanced our understanding of the perception of the process of neuropeptide–catecholamine interactions in the brain and periphery. A new picture of stress circuitry has emerged, in which catecholamine and neuropeptide systems are intimately interconnected, both centrally and peripherally, during responses to both systemic and psychogenic stress. PACAP modulates the HPA axis in response to acute psychogenic but not systemic stressors, through activation of CRH (reviewed in Ref. 8). PACAP facilitates the biosynthesis of catecholamines in the adrenal medulla.²¹ Thus, one other target could be via a local action at the level of the adrenal gland which has not been fully explored and not covered in this chapter.

Recent studies have suggested that action of antidepressants on synaptic plasticity is mediated by their regulatory influence not only upon small-molecule neurotransmitters but also via neuropeptides which may act both as neurotransmitters and as neuromodulators. Neuropeptides and catecholamines act on several circuits in the central and peripheral nervous systems to facilitate systemic and psychological stress responses, as well as enduring adaptive and maladaptive changes to stress. These could lead to survival with resilience, or survival with vulnerability, as manifested in mood and

affective disorders, such as anxiety, depression, PTSD, obesity and eating disorders (reviewed in Ref. 22). A growing body of literature suggests that the PACAP/PAC₁ receptor system may be a potential contributor to neuropsychiatric disorders, which is described below.

3. PACAP and anxiety disorders

Anxiety disorders are the most common psychiatric disorder in the USA. Anxiety disorders usually are long-lasting and debilitating and characterized by thoughts and feelings of uncontrollable fear and accompanied by somatic, affective, and behavioral symptoms. PACAP is present in most of the brainstem areas including autonomic sensory and motor system related areas, but also in the centers involved in stress adaptation, such as the ventral tegmental area and locus coeruleus.⁷ As stated above, local administration of PACAP in the PVN influences the CRF expression⁹ and mimics behavioral changes result from the activation of PVN by CRH or a stressor.¹² Furthermore, intracerebroventricular PACAP administration produces anxiety- and depressive-like behaviors in rodents, effects produced by CRF.²³ On the other hand, PACAP deficiency seems to be protective against the deleterious effects and preventing the chronic activation of the HPA axis.²⁴

PACAP and PAC₁ receptors are widely expressed in the CeA and BNST, raising the possibility that this peptide/receptor system is not only involved in the stress response but also in anxiety disorders. Interestingly, fear conditioning and estrogen replacement therapy each is shown to increase PAC₁ receptor gene expression.²⁵ Furthermore, local administration of PACAP in the CeA is found to be anxiogenic in rats and that the melanocortin receptor 4 (MC4R) system mediates the anxiogenic effects of PACAP.²⁰ These authors suggested that dysregulation of this neuropeptide system may contribute to the pathophysiology of the anxiety group of disorders.²⁰ Interestingly, chronic variable stress, which leads to anxiety and depression in rodents, increases PACAP and PAC₁ receptor transcript expression in the BNST and local administration of PACAP in the BNST induces anxiety-like behaviors in rats.¹⁸ On the other hand, continuous infusion of PACAP antagonist (PACAP6–38) in the BNST reduces anxiety-like behaviors induced by chronic variable stress,¹⁸ suggesting that PACAP alterations in the BNST may mediate stress-induced BNST neuroplasticity and that this may be the underlying mechanism of some forms of human anxiety disorders. In line with these observations, mice lacking

PACAP spend more time on the open arms of the elevated plus maze compared to their wild-type controls,^{26–28} suggesting that the lack of PACAP is protective against the development of anxiety disorders.

Human studies have also suggested the possible involvement of the PACAP/receptor system in the pathophysiology of anxiety and PTSD.²⁵ Interestingly, a single nucleotide polymorphism in the PAC₁ receptor gene leads to decreases in amygdala and hippocampus connectivity and may contribute to aberrant fear and anxiety disorders.²⁹ Together, these studies assign a significant role to neuropeptide systems in the mediation of the stress response, making them potential drug targets for the treatment of stress and anxiety disorders.

4. PACAP and major depression

Major depressive disorder (MDD) is an enduring and life-threatening psychiatric condition characterized by low mood, psychomotor changes, and marked decreased interest or pleasure in most activities, also known as anhedonia (American Psychiatric Association 2013). Major depression is among the leading causes of disability around the globe according to the World Health Organization (WHO, 2012). The lifetime prevalence of depression in the industrialized population ranges between 15% and 25%.³⁰ It is important to note that patients diagnosed with depression show somewhat different symptoms and the diagnostic criteria themselves are already somewhat contradictory as opposing phenomena might support the same “major depressive disorder” diagnosis (like hyperphagia vs. hypophagia, insomnia vs. hypersomnia or weight gain vs. weight loss). Considering this heterogeneous pathophysiological background, it is not surprising that 30% of patients do not respond to the currently available medications influencing monoaminergic neurotransmission in the brain. The need for new strategies to treat the disease is evident.

PACAP has been a critical neurotransmitter/neuropeptide mediating activation of the HPA and hormonal sympathetic adrenal axes. CRH not only initiates activation of the HPA axis via the release of ACTH from the pituitary but also is released from hypothalamic and extrahypothalamic neurons to feedback on noradrenergic systems driving central to peripheral stress “executive” programs (reviewed in Ref. 22). PACAP and its receptor PAC₁ are widely expressed in several nuclei of the hypothalamus as well as in

various extra-hypothalamic regions including the amygdala, hippocampus, and nucleus accumbens.^{7,31,32} Given the importance of these brain regions in motivated behaviors, reward, and the pathophysiology of mental illnesses, PACAP and its receptors may be involved in the pathogenesis of depressive disorders and other neuropsychiatric disorders. Indeed, there is accumulating evidence that the PACAP-ergic signaling plays a vital role in the behavioral and endocrine responses to stress, as well as in synaptic plasticity and neuroprotection.^{24,28,33–35}

Intracranial self-stimulation (ICSS) is a useful approach in which electrical activation of specific brain regions is associated with reward. In rodents, it is achieved by the animal performing an operant response, such as lever pressing. An increase in the ICSS threshold is considered as a reflection of deficits in brain reward circuitries, used as a sign of anhedonia, that is a core symptom of major depression and other mood disorders (American Psychiatric Association 2013). Chronic treatment with antidepressant drugs attenuate increases in the ICSS threshold induced by cocaine withdrawal.³⁶ PACAP, on the other hand, increases ICSS threshold, suggesting that PACAP promotes depressive-like behaviors in rodents.³⁷ PACAP administration also reduces social interaction and preference for a saccharine solution, which is commonly used as a model of anhedonia. However, it fails to alter immobility time in the forced swim test, an animal model of despair.³⁷ The changes observed are blocked by the PACAP receptor antagonist, PACAP6–38, suggesting that the PACAP/PAC₁ receptor system may play a functional role in the pathogenesis of depression, particularly the anhedonia and social dysfunction associated with the disease.³⁷

While exogenous PACAP appears to promote depression-like behaviors in rodents, conflicting results are reported regarding the role of endogenous PACAP in these behaviors in mice lacking PACAP. Hattori and colleagues²⁷ reported a slight reduction in immobility time in the forced swim test in mice lacking PACAP, indicating attenuation of depressive-like behaviors in the absence of endogenous PACAP. However, other groups show that mice lacking PACAP express increased immobility time in the forced swim test,^{34,38–40} suggesting that endogenous PACAP may serve to protect against the development of depressive-like behaviors in rodents.

Endogenous PACAP, however, promotes depressive-like behaviors in subjects exposed to chronic stress. Thus, early life stress (180-min maternal deprivation) in combination with chronic variable stress induces depressive-like behaviors in wild-type mice, and this behavioral change is found to be

reduced in mice lacking PACAP.⁴¹ Additionally, chronic variable stress is also found to cause reduced depressive-like behaviors in adult mice lacking PACAP compared to their wild-type controls.⁴² Moreover, Lehmann and colleagues²⁴ demonstrate that a 14-day social defeat stress exposure caused a significant increase in PVN α -fos expression as well as plasma corticosterone secretion and depression-like behaviors in wild-type mice. These stress-mediated changes are found to be reduced in mice lacking PACAP, suggesting that prolonged activation of the PACAP/PAC₁ receptor system in response to chronic stress may lead to the enduring changes observed in depression.

Human studies underpin the significance of the PACAP/PAC₁ receptor system in mood disorders. For instance, PACAP through PAC₁ receptor up-regulates the expression of the protein DISC1 (disrupted in schizophrenia 1) and radically but momentarily interferes with the interaction of DISC1 with its interacting protein.⁴³ Interestingly, the DISC1 gene mutation is linked not only to schizophrenia⁴⁴ but also to depression.^{45,46} Furthermore, Hashimoto and colleagues⁴⁰ report on a possible linkage between the PACAP gene and MDD, in which they compare 637 MDD patients versus 967 healthy controls and find that a variant in the PACAP gene is associated with MDD. Additionally, a SNP in the PAC₁ receptor gene leads to a significant interaction between this variant and neighborhood crime for patients suffering from major depression.⁴⁷ Interestingly, the PACAP variant gene (rs2856966) is found to be associated with a better treatment outcome to venlafaxine.⁴⁸ Taken together, the above studies suggest that the PACAP system may represent a potential target for the development of novel pharmacotherapies to treat depression and possibly other stress-mediated neuropsychiatric disorders.

5. PACAP and post-traumatic stress disorder (PTSD)

Post-traumatic stress disorder (PTSD) is a pervasive and well-known of all the trauma- and stress-related disorders with a twofold greater prevalence in women than men. It affects about 5–10% of the US population and is sparked by exposure to an acute or recurring traumatic event(s). Patients with PTSD, in comparison to trauma-exposed controls, not only exhibit psychological symptoms but also abnormally high conditioned fear responses. This high level of fear may result from a lack of ability to habituate to aversive stimuli, to extinguish or inhibit fear memories, and/or possibly strengthening of the original fear memory.^{49–51}

The cardinal feature of PTSD is the dysregulation of the HPA axis, and that the system is altered and hyper-responsive to cortisol feedback. Recent research in PTSD suggests that increased activity of CRH-containing circuits is involved in the pathophysiology of the disease.⁵² The neuropeptide PACAP is involved in regulating CRH, a key mediator of the HPA axis,⁹ raising the possibility that the PACAP system may be involved in the pathophysiology of PTSD and other stress-related neuropsychiatric disorders.

Available genetic data suggest that PACAP/PAC₁ receptor expression and signaling may be integrally involved in regulating the psychological and physiological responses to traumatic stress and variants of PACAP or PAC₁ receptor gene may be potential contributors of PTSD. For example, in highly traumatized female but not male subjects, there seems to be a strong sex-specific association of PACAP blood levels and PAC₁ receptor gene variants with fear discrimination, PTSD diagnosis, and symptoms.²⁵ The sex-specific association occurs within a single-nucleotide polymorphism (rs2267735) that is located on the so-called estrogen response element within the PAC₁ receptor gene that mediates the regulation of PAC₁ receptor gene by the sex hormones.²⁵ In line with these human data, Mercer and colleagues report that fear conditioning and estradiol additively increase the expression of the PAC₁ receptor gene in mice.⁵³ In line with this, in women with low levels of estradiol, the CC allele is associated with reduced PAC₁ receptor gene expression which is linked to higher signs and symptoms of PTSD.⁵³ These data suggest that perturbations in the PACAP/PAC₁ receptor system are regulated by estrogen and are involved in abnormal fear responses underlying PTSD. Additionally, these sex-related differences in PACAP signaling during emotional learning could provide novel targets for the treatment of PTSD.⁵⁴

Functional magnetic resonance imaging of patients with moderate to high trauma revealed a higher reactivity of limbic structures, such as the amygdala and hippocampus, in patients with this genotype. Likewise, there was decreased connectivity of the amygdala and hippocampus in these individuals,⁵⁵ suggesting that this gene alteration may contribute to aberrant fear, which is a hallmark of PTSD and anxiety disorders.²⁹ Moreover, decreased hippocampal activity is observed in female subjects carrying the PAC₁ variant.⁵⁶ Considering that genetic risk factors may account for up to 30–40% of the heritability of PTSD, the above findings suggest that perturbations in the PACAP/PAC₁ receptor system are regulated by estrogen and are involved in abnormal fear responses underlying PTSD. Additionally, these sex-related differences in PACAP signaling during emotional learning could provide novel targets for the treatment of PTSD.⁵⁴

6. PACAP and schizophrenia

Schizophrenia is a severe and debilitating neuropsychiatric brain disorder that affects 1% of the population. It is equally prevalent across people irrespective of ethnicity, race, and nationality across the globe and is equally prevalent in men and women. Its cause is due to the interaction of several abnormal genes with environmental factors. The course of schizophrenia is variable, with some individuals experiencing exacerbations and others remaining stable but chronically ill.⁵⁷ Schizophrenia is not only a neurodevelopmental disorder but also neurodegenerative disorder.⁵⁸ There have been several reviews recently of various aspects of PACAP as well as its role in neurodevelopmental disorders.⁵⁹ It has been proposed that PACAP may play a role in mental diseases and that regulation of the PACAPergic signals could be a potential treatment for schizophrenia considering schizophrenia is not only neurodegenerative but also neurodegenerative disorder.⁶⁰ PAC₁ receptor gene alternative splicing could contribute to the risk of developing schizophrenia, not only by affecting embryonic neurogenesis but also by impairing proliferation and differentiation of adult stem cells.⁶¹ Accordingly, a reduction in the amount of hippocampal neural stem cells is reported in post-mortem schizophrenic brains,⁶² raising the possibility that this could explain the decrease in hippocampal volume and the correlation with several cognitive deficits observed in schizophrenic patients.

PAC₁ is a G-protein-coupled receptor encoded by *Adcyap1*, which is a potential schizophrenia susceptibility gene in rodents and humans. Variations of the PACAP and/or PACAP receptor genes may be a genetic risk factor for psychiatric disorders including schizophrenia-like phenotype in mice.⁶³ Studies have shown that PACAP-deficient mice exhibit behavioral and neurophysiological abnormalities including emotional lability, depression-like behaviors, and memory impairments that are ameliorated with atypical antipsychotic drugs. For example, Ago and colleagues³⁸ show that mice lacking PACAP display reduced performance in the object recognition test. Likewise, PACAP heterozygous mice exhibit deficits in sensorimotor gating, as measured in the prepulse inhibition paradigm following the administration of an aversive stimulus, i.e., administration of a 5HT₂ receptor agonist.⁶⁴ However, an earlier study reports no change in prepulse inhibition in mice lacking PACAP compared to their wild-type controls.²⁷ These authors also show more social interaction, unlike previous studies, with a mild performance deficit in working memory in mice lacking PACAP.²⁷

Human studies, however, have implicated the PACAP/PAC₁ receptor system in schizophrenia. For instance, the PACAP gene allele that is over-expressed in patients with schizophrenia is found to be associated with reduced hippocampal volume and poorer memory performance.⁶³ Likewise, PACAP through PAC₁ receptor up-regulates the production of the protein DISC1 and drastically but briefly reduces interaction of DISC1 with its interacting protein⁴³ and may alter neurite outgrowth. Interestingly, DISC1 gene mutation was linked to schizophrenia.⁴⁴ Likewise, stathmin1, which induces abnormal axonal arborization, may be a linkage for the involvement of PACAP in schizophrenia, as the expression of stathmin 1 is upregulated in the brain of mice lacking PACAP as well as in patients with schizophrenia (reviewed in Ref. 60). These findings suggest that it is possible that neural development via these two proteins is disturbed in schizophrenic patients and PACAP may be involved in this process.

Not only the PACAP/PAC₁ receptor system but also the PACAP/VPAC₂ signaling is suggested to be associated with mental illnesses. Indeed, a duplication of the chromosome that encodes the VPAC₂ receptor is found to be linked to schizophrenia.⁶⁵ Thus, restoring the changes induced by the PACAP/PAC₁/VPAC₂ signaling may be a novel approach to treat schizophrenia.

7. Concluding remarks

The PACAP/PAC₁ receptor system has been implicated in several neuropsychiatric disorders. Previous studies have shown alterations in anxiety and depression in mice lacking PACAP or its receptor compared to their wild-type controls. However, considering that compensatory developmental changes may occur in knockout mice, further studies using pharmacological tools and other approaches are needed to fully characterize the role of the PACAP/PAC₁ receptor system in these disorders. Currently, the lack of selective small-molecule PAC₁ receptor antagonists represents a challenge. However, attempts have been made to develop such small molecules.⁶⁶

PACAP has the potential to be neuroprotective and regulate mood and affective states via multiple mechanisms. Earlier studies have demonstrated that PACAP is associated with the expression of tyrosine hydroxylase, which is the rate-limiting enzyme in the catecholamine synthesis. This can be significant in the monoamine theory of depression. Likewise, studies have shown that PACAP can increase the expression of brain-derived neurotrophic factor (BDNF), which is involved in neurogenesis and neuronal survival.

PACAP is thus well positioned to alter mood and be involved in the pathophysiology of depression and related disorders. Furthermore, studies have shown that PACAP receptors are also found on macrophages and immune cells. Thus, PACAP has the potential to impact neurological and immunological pathways via altering the function of these cells. Future studies can be directed to address these unexplored areas of research.

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