Chapter 45
Mechanisms of PACAP in PTSD and Stress-Related Disorders in Humans

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Abstract Pituitary adenylate cyclase-activating polypeptide (PACAP) is a pleiotropic neuropeptide, a neuronal signaling molecule that affects many distinct phenotypic traits. Despite the diversity in PACAP's functioning, by examining PACAP in the typical stress response, we can identify when PACAP levels and signaling become dysregulated. These findings can serve as clues to help identify the mechanisms of psychiatric and clinical disorders. Furthermore, accumulating evidence suggests that PACAP itself may represent a novel treatment target for a variety of disorders. In this chapter, we provide a brief overview of PACAP in the stress response, and review evidence that PACAP levels, signaling, genetic and epigenetic variations may be important mechanisms underlying human illnesses. Posttraumatic stress disorder and other stress-related medical conditions including migraines, Alzheimer's disease, multiple sclerosis, sudden infant death syndrome, and asthma are discussed.

Keywords Alzheimer's disease • Asthma • Migraine • Multiple sclerosis • PACAP • PAC1 • PTSD • Stress • Sudden infant death syndrome

Overview of PACAP and PAC1

Pituitary adenylate cyclase-activating polypeptide (PACAP) is part of the secretin glucagon vasoactive intestinal polypeptide family (VIP) [1]. The two major biologically active forms of PACAP are PACAP-38 and PACAP-27, which are 38 and 27 amino acids long, respectively [1]. PACAP is preferentially released at higher nerve firing rates, which often occur during the experience of a stressor [2, 3]. PACAP is encoded by the adenylate cyclase activating peptide, *ADCYAP1*, gene in humans [4] or the highly conserved rodent equivalent, *Adcyap1*.

Pituitary adenylate cyclase-activating polypeptide type I receptor (PAC1) is a G protein coupled receptor (GPCR), a class of transmembrane receptors that mediate

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cell signaling processes [4]. PAC1 receptors are selective for PACAP [4] (though see evidence that they may be less selective [5]). The PAC1 receptor is encoded by the *ADCYAP1R1* gene or the highly conserved rodent equivalent, *Adcyap1r1* [6].

The General Function of the PACAP Ligand and PACAP-PAC1 Signaling

PACAP and PACAP-PAC1 signaling are involved in diverse biological actions. Overall, PACAP is a neurotrophin, facilitating the development, function, and survival of neurons [3, 7–9]. PACAP's cellular functions also include: (a) synaptic transmission, (b) neuroendocrine signaling, and (c) cytoprotection [8]. PACAP signals both presynaptically and postsynaptically through the PAC1 receptor, and acts as both a neuromodulator, sensitizing cells to incoming signals, and a neurotransmitter, directly conveying signals [10]. PACAP-PAC1 signaling can both facilitate transmission at low doses and depress transmission at higher doses (e.g., in hippocampal CA1 synapses and possibly other brain regions) [11]. Because of this dose-dependent synaptic transmission, PACAP can both facilitate and inhibit neuroendocrine release through a cascade effect [10]. Finally, PACAP improves the likelihood that cells will survive when affronted by stressors such as environmental toxins, hypoxia, and excitotoxins, for example, by reducing oxidative stress [3, 7, 9]. More broadly PACAP-PAC1 signaling is central in development (e.g., high expression during embryogenesis), reproductive functions, thermoregulation, feeding, urinary reflexes, metabolism, and perhaps even the regulation of social interaction [4, 7].

PACAP-PAC1 signaling subserves different functions in the central vs. peripheral nervous system. Within the central nervous system, the PAC1 receptor is highly expressed in the lateral septum, amygdala (central and basolateral), bed nucleus of stria terminalis (BNST), paraventricular nucleus of the hypothalamus, hippocampus, locus coeruleus, and periaqueductal gray, among other regions [1, 4]. PACAP-PAC1 signaling in these neural structures and their associated networks plays a role in learning, memory, behavior, circadian rhythm, and the stress response [4]. In the periphery, PACAP-PAC1 signaling is integral to immune function, the inflammatory response, insulin secretion, and catecholamine release [4].

Assessing PACAP and PAC1 Genetic Variation

PACAP and PAC1 can be measured in a number of ways. Here we briefly overview three ways PACAP can be assessed as they pertain to findings discussed in our chapter. First, genetic variation can be measured, for example, by examining common or rare single nucleotide polymorphisms in a gene. Certain polymorphisms may be "risk" factors for specific disorders. Second, epigenetic variation can be assessed, for example, by measuring the methylation status of a gene. Methylation status is

thought to modulate gene expression dynamically. Third, variation in gene expression can be measured at two different levels (a) directly through mRNA levels or (b) one step removed by detecting protein production, that is, the amount or levels of the PACAP protein or PAC1 receptor in the blood, cerebrospinal fluid, specific tissue (e.g., in the amygdala), or on individual cell bodies.

Brief Overview of PACAP/PAC1 in the Stress Response

PACAP-PAC1 signaling plays a modulatory role in the function of the autonomic nervous system, and stress systems (e.g., Hypothalamic-Pituitary-Adrenal axis, sympathoadrenomedullary system) [12]. In general, increased PACAP expression is associated with an up-regulated stress response (and vice versa), and decreased PACAP is associated with a down-regulated stress response [3, 8, 12, 13]. In the extreme, dysregulation in PACAP levels and signaling are associated with the development of various psychiatric disorders [8]. First we briefly review PACAP-PAC1 signaling in the healthy stress response, and then we address its mechanisms in a dysregulated stress response associated with Posttraumatic Stress Disorder and other medical conditions (also see Eiden Chap. 41 of this handbook for more details on the PACAPergic circuits underlying responses to stress).

Autonomic Nervous System

PACAP is present in most areas of the brainstem, especially autonomic sensorimotor areas (e.g., locus coeruleus) [1]. Within the autonomic nervous system, injections of PACAP into the cerebrospinal fluid were associated with increased sympathetic nerve activity, blood pressure, and heart rate, and suppressed parasympathetic nerve activity in the mouse [12, 14]. This suggests that part of PACAP's modulatory role is to maintain homeostasis by keeping a balance between sympathetic and parasympathetic nerve activity [12, 14].

Stress Systems

As mentioned earlier, PACAP is present in many limbic brain regions involved in emotional and stress responding (e.g., amygdala, BNST, hypothalamus) [12]. The Hypothalamic-Pituitary-Adrenal (HPA) axis is a cascade of stress hormone release and feedback that occurs between the hypothalamus, pituitary, and adrenal glands once an event or stimulus is consciously or unconsciously categorized as stressful [15]. There is evidence to suggest that PACAP has modulatory control over the HPA axis [4, 8]. PACAP is expressed in neuroanatomical regions associated

with HPA axis potentiation, activity, and control, for example, the hypothalamus, and amygdala [1]. Also, PACAP stimulates *CRH* gene expression [1, 12], promoting the release of corticotropin-releasing hormone, a neurotransmitter in the HPA axis cascade [16].

The sympathoadrenomedullary system is also activated during the stress response. During chronic stress, high frequency sympathetic nerve firing in the adrenal medulla secretes catecholamines recurrently [2, 16]. PACAP may be the primary neurotransmitter regulating the adrenomedullary synapse central to this process, facilitating the ability to release catecholamines in a sustained manner [2, 8, 12].

PACAP's influence on these systems, however, may be context-dependent based on the type of stressor an organism is experiencing [8]. For example, PACAP may only modulate the HPA axis in response to psychogenic stressors (also termed "processive stressors"), that is, those mediated by cortical and limbic structures in the brain and requiring some top-down processing (e.g., in humans, one's reputation is threatened; in rodents, a well-lit environment) [8, 15]. PACAP receptors densely populate the prefrontal cortex and hippocampus, areas related to inhibitory control, top-down regulation of HPA axis stress circuitry, and memory function [10, 15]. This may be part of the reason PACAP modulates HPA axis activity only for top-down psychogenic stressors. In contrast, PACAP may only modulate the sympathoadrenomedullary system in response to systemic stressors [3, 8]. Systemic stressors pose a direct threat to survival, and are relayed directly to the hypothalamus most likely through the brainstem (e.g., in humans and rodents, a predatory attack) [8, 15]. PACAP's context-dependent stress system modulation may provide insight regarding potential mechanisms of dysregulation in psychiatric and medical conditions, and what systems to target for treatment in each context.

Overall, PACAP/PAC1 expression and signaling are necessary for an appropriate acute or chronic stress response, helping to preserve cellular function under stress. Abnormalities in PACAP/PAC1 expression, signaling, or feedback homeostasis can dysregulate the stress response systems, however, and have been associated with the development of psychiatric disorders [4]. Dysfunction in the PACAP system can occur for a myriad of reasons. For example, chronic stress could alter dendritic structure in areas where PACAP is heavily expressed (e.g., BNST) or risk alleles could lead to hyper- or hypo-production of PACAP in response to stressors. Here we focus on variation in PACAP protein blood levels, genetic and epigenetic variation associated with posttraumatic stress disorder (PTSD) and other stress-related conditions.

PACAP and the Dysfunctional Stress Response

Chronic activation of the stress response is a potent disruptor of cognition. It accumulates wear and tear on the body and brain (e.g., allostatic load), and is linked to increased risk for developing psychiatric disorders like anxiety, depression, and schizophrenia [17–19]. The development of these maladaptive cognitive and behavioral patterns appears to be highly PACAP-dependent [8]. An excess of PACAP, for

example, is associated with PTSD; in contrast, a PACAP deficiency is associated with schizophrenia [6, 20]. As illustrated in Fig. 45.1, here we review the association between PACAP and PTSD, and a number of other medical conditions, including migraines, Alzheimer's disease, multiple sclerosis, sudden infant death syndrome, and asthma.

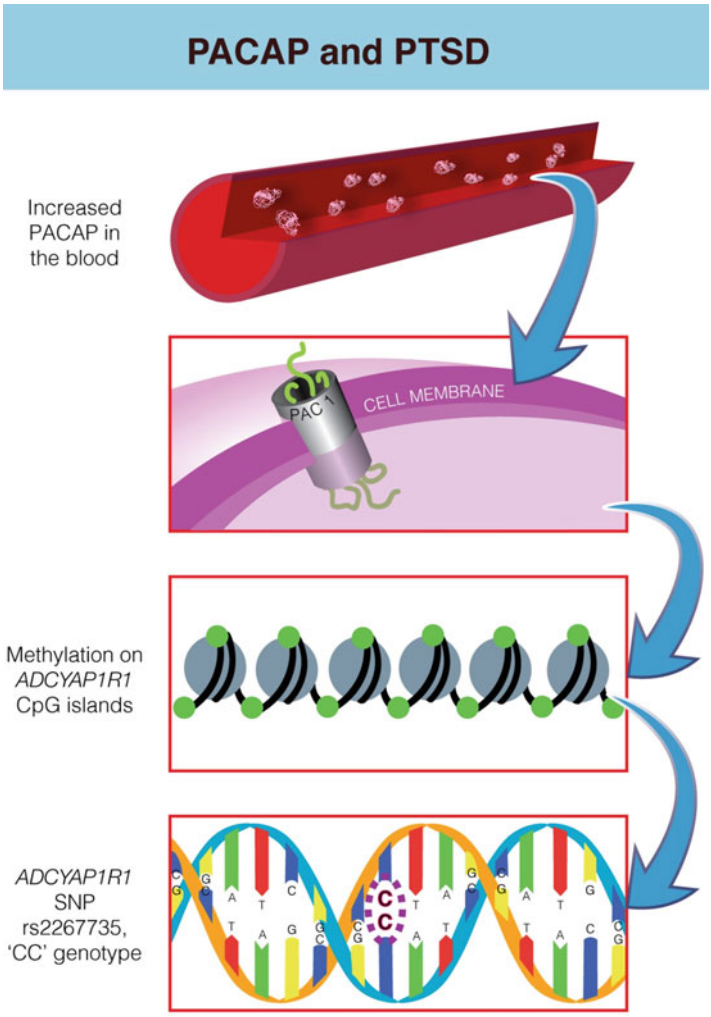

Fig. 45.1 Schematic Overview of PACAP, PAC1, and PTSD. Depiction of the three identified PACAP–PAC1 mechanisms related to posttraumatic stress disorder, including increased PACAP protein levels in the blood, methylated CpG islands on the PAC1 receptor gene (*ADCYAP1R1*), and the rs2267735 SNP CC genotype on *ADCYAP1R1*. The *small circles* in the methylation panel indicate methylation in the PAC1 receptor gene methylation panel. *SNP* single nucleotide polymorphism. Note the schematic depictions are not drawn to scale

Rodent Model Evidence for PACAP-PAC1 Signaling and Stress-Related Symptoms

Evidence from rodent models supports the connection between PACAP, PACAP-PAC1 signaling, activity in the amygdala and BNST, and PTSD-like symptomatology. Our group has found, for example, that there is a correlation between levels of fear learning and *Adcyap1r1* expression as measured by mRNA levels in mouse models of fear conditioning [6]. More specifically, the consolidation of fear memories increased *Adcyap1r1* mRNA in the mouse amygdala with a trend toward similar increases in the medial prefrontal cortex [6]. This suggests that fear memories are associated with PAC1 receptor gene expression in areas of the brain related to processing fear.

In addition, shock-stressed rats injected with PACAP in the central nucleus of the amygdala were more likely to withdraw and freeze compared to control rats, which were more likely to actively bury a shock probe [12, 21]. This suggests that in the context of an intense stressor, a PACAP surge in the central amygdala induced dissociative-like or passive coping compared to active engagement, anxiety-like behaviors.

The bed nucleus of the stria terminalis (BNST) is an anatomical component of the extended amygdala thought to be involved in emotional behavior and to mediate stress responses [6]. A week of chronic stress including forced swim, foot shocks, restraints, oscillation stress, and pedestal stress induced a large increase in BNST PACAP expression (as measured by mRNA), and an exaggerated startle response during light exposure [12, 22, 23]. PACAP injected into rodent BNST also increased anxiogenic responses (e.g., baseline startle amplitude) [12, 22, 23]. Together these findings suggest PACAP signaling in BNST may enhance anxiety-like behaviors. Overtime, chronic stress and PACAP expression may alter the structure of BNST to increasingly promote anxiety-like behaviors [22].

Posttraumatic Stress Disorder

PACAP Blood Levels Associated with PTSD in Human Females

Our group has found that PACAP protein levels in the blood are associated with PTSD in females, but not males [6]. That is, higher PACAP-38 blood levels were associated with more PTSD symptoms, and a PTSD diagnosis as measured by (1) the PTSD Symptoms Scale, and (2) a greater startle reflex response during a classic fear conditioning paradigm [6].

PAC1 Genetic Variation Associated with PTSD in Human Females

We have also found that genetic variation is associated with PTSD symptoms on a diverse set of measures. Although PACAP gene (*ADCYAP1*) variation was not associated with PTSD symptomatology, PAC1 receptor gene (*ADCYAP1R1*) variation

was associated with PTSD in females (but not males) [6]. We found that an *ADCYAP1R1* single nucleotide polymorphism, rs2267735, was associated with increased likelihood of PTSD diagnosis and symptoms, spanning the intrusive, hyperarousal, and avoidance/numbing symptom clusters. Specifically, across several studies, the rs2267735 'CC' genotype is associated with higher levels of PTSD hyperarousal symptoms compared to 'G' carries as measured by the (1) PTSD Symptoms Scale, (2) increased amygdala and hippocampal reactivity to threat, (3) decreased functional connectivity between the amygdala and hippocampus, and (4) an increased startle response (regular and dark enhanced) in a classic fear conditioning paradigm [6, 24–26]. Notably, the positive relationship between *ADCYAP1R1*, other PTSD "risk" genotypes (e.g., *FKBP5*, *CRHR1*, *DBH*, *DRD2*, *NPY*, *NTRK2*) and PTSD symptoms was removed if an early exposure intervention was administered in the immediate aftermath of a traumatic experience [27]. These findings suggest that genetic risk for PTSD in the aftermath of trauma may be ameliorated with environmental or therapeutic interventions, preventing the development of trauma-related sequelae.

This association between *ADCYAP1R1* genotype and PTSD symptom severity (as measured by the PTSD Symptoms Scale and Clinician-Administered PTSD Scale) has been replicated, but only when participant trauma load was high or traumas included childhood maltreatment [24, 28]. The association between *ADCYAP1R1* genotype and PTSD symptom severity was not found in those with a lower trauma load [24, 28–30]. Other work with an index trauma of child loss has found *ADCYAP1R1* risk polymorphisms predict PTSD numbing symptoms, but not overall PTSD symptom severity [30]. Together this evidence suggests that differences in types of trauma exposure and trauma load may interact with the relationship between PACAP-PAC1 signaling and PTSD.

The association between *ADCYAP1R1* genotype and PTSD symptom severity as measured by startle response has also been replicated in adult females and both male and female children [31]. The association between PACAP and PTSD symptomatology in both male and female preadolescent children is especially intriguing given adult sex differences in PTSD. We return to this point later on in this chapter.

PAC1 Epigenetic Methylation Status Associated with PTSD in Humans

Similar to the genetic variation results, variation in DNA methylation of the PACAP gene (*ADCYAP1*) was not associated with PTSD symptomatology, but PAC1 receptor gene (*ADCYAP1R1*) methylation variation was associated with PTSD symptoms and diagnosis in males and females [6]. Specifically, methylation on *ADCYAP1R1* CpG islands was associated with more PTSD symptoms and a PTSD diagnosis [6, 25].

Notably, the aforementioned results appear to be relatively specific to PTSD. That is, PACAP protein blood levels and PAC1 receptor genetic and epigenetic variation did not predict other Axis I psychiatric disorders (e.g., depression, schizophrenia) or neurodegenerative diseases (e.g., Alzheimer's disease) within the same cohort [6, 28].

PACAP and PTSD Sex Differences in Adults

Although women are more likely to experience fewer traumatic events over their lifetime, they are nearly two times more likely to receive a PTSD diagnosis compared to men [25, 32]. It is possible that genetic variation contributes to these sex differences. In particular, dynamic PAC1 receptor regulation by the gonadal hormone estrogen may be a central figure in PTSD-related sex differences. There is evidence, for example, that estrogen induces expression of PACAP and PAC1 receptor genes in rats [6]. Specifically, estradiol increased *Adcyap1* and *Adcyap1r1* mRNA levels in the BNST [6].

As mentioned earlier, *ADCYAP1R1* polymorphisms are associated with greater startle responses in a fear conditioning paradigm for both male and female human children, but in adults, the association between *ADCYAP1R1* polymorphisms and fear conditioning is only seen in females, not males [31]. Given greater estrogen levels in adult females compared to preadolescent females (or more similar estrogen levels in female and male children vs. adults) this is further evidence supporting the dynamic relationship between estrogen, fear, trauma, and PACAP-PAC1 signaling. Ongoing work is examining estrogen's stress-dependent mechanistic role in the regulation of *ADCYAP1R1* expression. These data suggest that the rs2267735 polymorphism may exert its effect in females by differentially modulating the ability of estrogen receptors to bind to, and thus properly modulate, an estrogen response element within the *ADCYAP1R1* gene.

PACAP and Other Stress-Related Medical Conditions

PACAP dysregulation and genetic variation have also been linked to many other conditions including migraines, multiple sclerosis, Alzheimer's disease, sudden infant death syndrome, and asthma. PACAP's involvement in the ability to mount an adaptive stress response and maintain homeostasis may be the common mechanism linking PACAP and these conditions in which there is some stress-related dysregulation. Migraines, for example, can be triggered by stress, and are associated with increased PACAP levels. In contrast, Multiple sclerosis and Alzheimer's disease are associated with massive neuroinflammation, and deficits in PACAP levels. Sudden infant death syndrome and asthma are associated with childhood stress, and linked to genetic variation in the PACAP and PAC1 receptor genes. Below we address each of these in turn.

Migraines Associated with Increased PACAP Levels

Migraines are a neurological disorder characterized by moderate to severe headaches, which can be associated with nausea, light or sound sensitivity [33]. In a sample of individuals who experienced migraines, "migraineurs," PACAP levels were

increased in the plasma during migraine attacks compared to levels when no migraine occurred [34]. Interestingly, migraineurs' migraine-related PACAP levels were comparable to control participants' non-migraine PACAP levels [33, 34]. The injection of PACAP also induced headaches and migraines in both healthy controls and migraineurs [35]. Migraineurs were more likely to develop a migraine after PACAP injection compared to healthy controls, however [35]. Although the exact mechanism by which PACAP facilitates migraines is unclear, at the very least PACAP is part of a signaling pathway in migraines, and is a possible target in migraine therapy [33, 36]. This topic is addressed further in Edvinsson Chap. 35 of this handbook.

Alzheimer's Disease Associated with PACAP Deficits

Alzheimer's disease (AD) is a neurodegenerative disease associated with neuroinflammation and severe deficits in cognitive functioning [37]. Recent rodent and postmortem human research has linked PACAP deficits to AD pathology. Rodent AD models and postmortem human AD patient brains have shown that reduced PACAP protein levels are associated with greater Alzheimer's pathology in the form of β-amyloid and tau protein levels [9]. Human postmortem brains with late-onset AD compared to cognitively healthy controls had reduced expression of the PACAP gene, *ADCYAP1*, throughout the brain, and more specifically, in middle temporal gyrus, superior frontal gyrus, and primary visual cortex (as measured by RNA levels) [38]. The levels of the PACAP protein in these aforementioned regions in addition to entorhinal cortex and cerebrospinal fluid were also reduced [38]. The PACAP deficit in entorhinal cortex and superior frontal gyrus, regions associated with memory and executive functioning [39, 40], was correlated with higher AD neuropathology (e.g., β-amyloid plaque density and neurofibrillary tangles) [38]. Notably, it is the entorhinal-hippocampal afferents that are the earliest to degenerate in AD [41]. In addition, reductions in cerebrospinal fluid PACAP protein levels were correlated with lower scores on a battery of cognitive functioning tasks (Mattis Dementia Rating Scale–Revised scores) [38]. Taken together, these findings demonstrate PACAP dysregulation is associated with AD neuropathology and lower cognitive functioning.

PACAP protein deficits have also been replicated in brains of patients with Mild Cognitive Impairment that also exhibited AD pathology [42]. Interestingly, PAC1 receptor levels were increased in the superior frontal gyrus of these Mild Cognitive Impairment brains compared to control and full blown AD brains, suggesting a compensatory mechanism in which the presence of PAC1 receptors are increased to counteract reduced PACAP levels or pathology interfering with PACAP functioning (or both) [42]. In addition, cortical neuronal cultures prepared from wild-type mouse brains incubated with PACAP and β-amyloid peptides were protected against β-amyloid toxicity (i.e., AD pathology) [9]. Together these findings suggest that PACAP's neurotrophic functioning is protective against AD pathology.

The mechanism for PACAP's protective effects is unclear, however. Some research suggests that PACAP stimulates alpha-secretase activity, which is in a cascade of

activity that can reduce AD pathology (e.g., formation of β-amyloid peptides, and plaque) [43]. It may be that certain risk alleles are associated with lower PACAP levels and those individuals are at greater risk for developing Alzheimer's pathology or AD overwhelms the protective PACAP functioning or any number of causational combinations. More research is needed to address how PACAP deficits contribute to AD pathology, and how PACAP could be targeted as a treatment mechanism for this disease, particularly given its neurotrophic function and localization in circuitry central to AD (e.g., entorhinal cortex) [9, 38].

Multiple Sclerosis Associated with Reduced PACAP Levels

Multiple sclerosis (MS) is characterized by chronic neuroinflammatory and neurodegenerative pathology [44]. PACAP's documented immunomodulatory and neurotrophic functions suggest that it could be a therapeutic target for both the inflammatory and degenerative aspects of MS [44, 45]. Indeed, recent evidence demonstrated reduced PACAP-38 levels in the cerebrospinal fluid of individuals with MS compared to healthy controls [46].

Sudden Infant Death Syndrome Associated with Reduced PACAP and PACAP Genetic Variation

Sudden infant death syndrome (SIDS) is the unexpected death of an infant without an identifiable explanation [47]. Despite its unidentifiable cause, SIDS risk factors have been recognized, including infant vulnerability related to: (a) in utero stressors (e.g., maternal smoking), (b) developmental stage, and (c) environmental conditions (e.g., increased heat) [47]. Growing evidence suggests PACAP's role in the development of healthy breathing and an adaptive stress response may in part underlie these vulnerabilities [48]. For example, mice lacking PACAP were more likely to die suddenly within two weeks after birth, mirroring SIDS in humans [48, 49]. Genetic tests in humans reveal an association between PACAP polymorphisms and SIDS. Specifically, a single nucleotide polymorphism in the coding region of PACAP was associated with SIDS [49]. This association was present for African Americans, but not for Caucasians [49], which may be part of the reason why there is a higher incidence of SIDS in African American infants [50]. Together this evidence suggests PACAP dysfunction may be an underlying cause of SIDS, and "risk" alleles may increase the likelihood of SIDS.

Asthma Associated with PAC1 Genetic Variation

Asthma is chronic inflammation of the lungs associated with difficulty breathing [51]. Psychosocial stress is linked to the occurrence of asthma in children and adults [52, 53]. Recent research demonstrates variation in the PAC1 receptor gene,

ADCYAP1R1, is associated with asthma. That is, the single nucleotide polymorphism rs2267735 'CC' genotype is associated with increased odds of developing asthma compared to 'G' carriers [52]. Recent evidence also shows that high childhood stress and a different single nucleotide polymorphism within the *ADCYAP1R1* gene are associated with reduced bronchodilator response, an objective measure of lung function, in children with asthma [54]. Together, these data suggest an important link between childhood stress and asthma may be mediated by genetic differences in PAC1 signaling.

General Conclusions

PACAP is a protein necessary for healthy development and an adaptive stress response. Dysregulation in PACAP/PAC1 levels or their signaling is associated with stress-related pathology in psychiatric and medical conditions. This dysregulation can occur at many levels—from existing genetic variation to epigenetic changes associated with life experiences, each in turn causing cascades of physiological alterations in the functioning of stress systems and neural networks. With continued characterization of PACAP/PAC1 mechanisms in these disorders we hope to better understand how to identify pathology, enable early intervention, and develop novel treatment strategies.

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References

1. Kormos V, Gaszner B. Role of neuropeptides in anxiety, stress, and depression: from animals to humans. Neuropeptides. 2013;47:401–19.
2. Smith CB, Eiden LE. Is PACAP the major neurotransmitter for stress transduction at the adrenomedullary synapse? J Mol Neurosci. 2012;48:403–12.
3. Stroth N, Holighaus Y, Ait-Ali D, Eiden LE. PACAP: a master regulator of neuroendocrine stress circuits and the cellular stress response. Ann N Y Acad Sci. 2011;1220:49–59.
4. Shen S, Gehlert DR, Collier DA. PACAP and PAC1 receptor in brain development and behavior. Neuropeptides. 2013;47:421–30.
5. Dautzenberg FM, Mevenkamp G, Wille S, Hauger RL. N-Terminal splice variants of the type I PACAP receptor: isolation, characterization and ligand binding/selectivity determinants. J Neuroendocrinol. 1999;11:941–9.
6. Ressler KJ, Mercer KB, Bradley B, Jovanovic T, Mahan A, Kerley K, et al. Post-traumatic stress disorder is associated with PACAP and the PAC1 receptor. Nature. 2011;470:492–7.
7. Mester L, Kovacs K, Racz B, Solti I, Atlasz T, Szabadfi K, et al. Pituitary adenylate cyclase-activating polypeptide is protective against oxidative stress in human retinal pigment epithelial cells. J Mol Neurosci. 2011;43:35–43.

8. Mustafa T. Pituitary adenylate cyclase-activating polypeptide (PACAP): a master regulator in central and peripheral stress responses. Adv Pharmacol. 2013;68:445–57.
9. Han P, Tang Z, Yin J, Maalouf M, Beach TG, Reiman EM, et al. Pituitary adenylate cyclase-activating polypeptide protects against β-amyloid toxicity. Neurobiol Aging. 2014;35:2064–71.
10. Vaudry D, Falluel-Morel A, Bourgault S, Basille M, Burel D, Wurtz O, et al. Pituitary adenylate cyclase-activating polypeptide and its receptors: 20 years after the discovery. Pharmacol Rev. 2009;61:283–357.
11. Roberto M, Scuri R, Brunelli M. Differential effects of PACAP-38 on synaptic responses in rat hippocampal CA1 region. Learn Mem. 2001;8:265–71.
12. Hashimoto H, Shintani N, Tanida M, Hayata A, Hashimoto R, Baba A. PACAP is implicated in the stress axes. Curr Pharm Des. 2011;17:985–9.
13. Lehmann ML, Mustafa T, Eiden AM, Herkenham M, Eiden LE. PACAP-deficient mice show attenuated corticosterone secretion and fail to develop depressive behavior during chronic social defeat stress. Psychoneuroendocrinology. 2013;38:702–15.
14. Tanida M, Shintani N, Morita Y, Tsukiyama N, Hatanaka M, Hashimoto H, et al. Regulation of autonomic nerve activities by central pituitary adenylate cyclase-activating polypeptide. Regul Pept. 2010;161:73–80.
15. Herman JP, Figueiredo H, Mueller NK, Ulrich-Lai Y, Ostrander MM, Choi DC, et al. Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. Front Neuroendocrinol. 2003;24:151–80.
16. Eiden LE. Neuropeptide-catecholamine interactions in stress. Adv Pharmacol. 2013;68: 399–404.
17. Ganzel BL, Morris PA, Wethington E. Allostasis and the human brain: integrating models of stress from the social and life sciences. Psychol Rev. 2010;117:134–74.
18. Juster RP, McEwen BS, Lupien SJ. Allostatic load biomarkers of chronic stress and impact on health and cognition. Neurosci Biobehav Rev. 2010;35:2–16.
19. Rodrigues SM, LeDoux JE, Sapolsky RM. The influence of stress hormones on fear circuitry. Annu Rev Neurosci. 2009;32:289–313.
20. Matsuzaki S, Tohyama M. Regulation of pituitary adenylyl cyclase-activating polypeptide (PACAP, ADCYAP1: adenylyl cyclase-activating polypeptide 1) in the treatment of schizophrenia. Expert Opin Ther Targets. 2008;12:1097–108.
21. Legradi G, Das M, Giunta B, Hirani K, Mitchell EA, Diamond DM. Microinfusion of pituitary adenylate cyclase-activating polypeptide into the central nucleus of amygdala of the rat produces a shift from an active to passive mode of coping in the shock-probe fear/defensive burying test. Neural Plast. 2007;2007:79102.
22. Hammack S, Cheung J, Rhodes KM, Schutz KC, Falls WA, Braas KM, et al. Chronic stress increases pituitary adenylate cyclase-activating peptide (PACAP) and brain-derived neurotrophic factor (BDNF) mRNA expression in the bed nucleus of the stria terminalis (BNST): roles for PACAP in anxiety-like behavior. Psychoneuroendocrinology. 2009;34:833–43.
23. Hammack S, Roman CW, Lezak KR, Kocho-Shellenberg M, Grimmig B, Falls WA, et al. Roles for pituitary adenylate cyclase-activating peptide (PACAP) expression and signaling in the bed nucleus of the stria terminalis (BNST) in mediating the behavioral consequences of chronic stress. J Mol Neurosci. 2010;42:327–40.
24. Almli LM, Mercer KB, Kerley K, Feng H, Bradley B, Conneely KN, et al. ADCYAP1R1 genotype associates with post-traumatic stress symptoms in highly traumatized African-American females. Am J Med Genet. 2013;162:262–72.
25. Dias BG, Ressler KJ. PACAP and the PAC1 receptor in post-traumatic stress disorder. Neuropsychopharmacology. 2013;38:245–6.
26. Stevens JS, Almli LM, Fani N, Gutman DA, Bradley B, Norrholm SD, et al. PACAP receptor gene polymorphism impacts fear responses in the amygdala and hippocampus. Proc Natl Acad Sci U S A. 2013;111:3158–63.
27. Rothbaum BO, Kearns MC, Reiser E, Davis JS, Kerley KA, Rothbaum AO, et al. Early intervention following trauma may mitigate genetic risk for PTSD in civilians. J Clin Psychiatry. 2014;1380–7.

28. Uddin M, Chang SC, Zhang C, Ressler K, Mercer KB, Galea S, et al. Adcyap1r1 genotype, posttraumatic stress disorder, and depression among women exposed to childhood maltreatment. Depress Anxiety. 2013;30:251–8.
29. Chang SC, Xie P, Anton RF, De Vivo I, Farrer LA, Kranzler HR, et al. No association between ADCYAP1R1 and post-traumatic stress disorder in two independent samples. Mol Psychiatry. 2012;17:239–41.
30. Wang L, Cao C, Wang R, Qing Y, Zhang J, Zhang XY. PAC1 receptor (ADCYAP1R1) genotype is associated with PTSD's emotional numbing symptoms in Chinese earthquake survivors. J Affect Disord. 2013;150:156–9.
31. Jovanovic T, Norrholm SD, Davis J, Mercer KB, Almli L, Nelson A, et al. PAC1 receptor (ADCYAP1R1) genotype is associated with dark-enhanced startle in children. Mol Psychiatry. 2012;18:742–3.
32. Tolin DF, Foa EB. Sex differences in trauma and posttraumatic stress disorder: a quantitative review of 25 years of research. Psychol Bull. 2006;132:959–92.
33. Kaiser EA, Russo AF. CGRP and migraine: could PACAP play a role too? Neuropeptides. 2013;47:451–61.
34. Tuka B, Helyes Z, Markovics A, Bagoly T, Szolcsanyi J, Szabo N, et al. Alterations in PACAP-38-like immunoreactivity in the plasma during ictal and interictal periods of migraine patients. Cephalalgia. 2013;33:1085–95.
35. Schytz HW, Birk S, Wienecke T, Kruuse C, Olesen J, Ashina M. PACAP38 induces migraine-like attacks in patients with migraine without aura. Brain. 2009;132:16–25.
36. Schytz HW, Olesen J, Ashina M. The PACAP receptor: a novel target for migraine treatment. Neurotherapeutics. 2010;7:191–6.
37. Perry RJ, Hodges JR. Attention and executive deficits in Alzheimer's disease: a critical review. Brain. 1999;122:383–404.
38. Han P, Liang W, Baxter LC, Yin J, Tang Z, Beach TG, et al. Pituitary adenylate cyclase-activating polypeptide is reduced in Alzheimer disease. Neurology. 2014;82:1724–8.
39. Hafting T, Fyhn M, Molden S, Moser MB, Moser EI. Microstructure of a spatial map in the entorhinal cortex. Nature. 2005;436:801–6.
40. du Boisgueheneuc F, Levy R, Volle E, Seassau M, Duffau H, Kinkingnehun S, et al. Functions of the left superior frontal gyrus in humans: a lesion study. Brain. 2006;129:3315–28.
41. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol. 1991;82:239–59.
42. Han P, Caselli RJ, Baxter L, Serrano G, Yin J, Beach TG, et al. Association of pituitary adenylate cyclase-activating polypeptide with cognitive decline in mild cognitive impairment due to Alzheimer disease. JAMA Neurol. 2015;72:333–9.
43. Kojro E, Postina R, Buro C, Meiringer C, Gehrig-Burger K, Fahrenholz F. The neuropeptide PACAP promotes the alpha-secretase pathway for processing the Alzheimer amyloid precursor protein. FASEB J. 2006;20:512–4.
44. Tan Y, Waschek JA. Targeting VIP and PACAP receptor signalling: new therapeutic strategies in multiple sclerosis. ASN Neuro. 2011;3.
45. Abad C, Waschek JA. Immunomodulatory roles of VIP and PACAP in models of multiple sclerosis. Curr Pharm Des. 2011;17:1025–35.
46. Baranowska-Bik A, Kochanowski J, Uchman D, Wolinska-Witort E, Kalisz M, Martynska L, et al. Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase activating polypeptide (PACAP) in humans with multiple sclerosis. J Neuroimmunol. 2013;263:159–61.
47. Fleming PJ, Blair PS, Pease A. Sudden unexpected death in infancy: aetiology, pathophysiology, epidemiology and prevention in 2015. Arch Dis Child. 2015;100(10):984–8.
48. Wilson RJA, Cumming KJ. Pituitary adenylate cyclase-activating polypeptide is vital for neonatal survival and the neuronal control of breathing. Respir Physiol Neurobiol. 2008;164:168–78.
49. Cummings KJ, Klotz C, Liu W-Q, Weese-Mayer DE, Marazita ML, Cooper ME, et al. Sudden infant death syndrome (SIDS) in African Americans: polymorphisms in the gene encoding the stress peptide pituitary adenylate cyclase-activating polypeptide (PACAP). Acta Paediatr. 2009;98:482–9.

50. Black L, David RJ, Brouillette RT, Hunt CE. Effects of birth weight and ethnicity on incidence of sudden infant death syndrome. J Pediatr. 1986;108:209–14.
51. What is asthma? 2014. http://www.nhlbi.nih.gov/health/health-topics/topics/asthma. Accessed 2015.
52. Chen W, Boutaoui N, Brehm JM, Han YY, Schmitz C, Cressley A, et al. ADCYAP1R1 and asthma in Puerto Rican children. Am J Respir Crit Care Med. 2013;187:584–8.
53. Yii AC, Koh MS. A review of psychological dysfunction in asthma: affective, behavioral and cognitive factors. J Asthma. 2013;50:915–21.
54. Brehm J, Ramratnam SK, Tse SM, Croteau-Chonka DC, Pino-Yanes M, Rosas-Salazar C, et al. Stress and bronchodilator response in children with asthma. Am J Respir Crit Care Med. 2015;192:47–56.