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Pituitary adenylate cyclase activating polypeptide in stress-related disorders: data convergence from animal and human studies

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Abstract

The maladaptive expression and function of several stress-associated hormones have been implicated in pathological stress- and anxiety-related disorders. Among these, recent evidence has suggested that pituitary adenylate cyclase activating polypeptide (PACAP) has critical roles in central neurocircuits mediating stress-related emotional behaviors. We describe the PACAPergic systems, the data implicating PACAP in stress biology and how altered PACAP expression and signaling may result in psychopathologies. We include our work implicating PACAP signaling within the bed nucleus of the stria terminalis (BNST) in mediating the consequences of stressor exposure and relatedly, describe more recent studies suggesting that PACAP in the central nucleus of the amygdala (CeA) may impact the emotional aspects of chronic pain states. In aggregate, these results are consistent with data suggesting that PACAP dysregulation is associated with post-traumatic stress disorder (PTSD) in humans.

Keywords

Anxiety; Fear; Pain; Bed Nucleus of the Stria Terminalis; Amygdala; Parabrachial Nucleus

Several decades of research have implicated corticotropin-releasing hormone (CRH) as a critical stress-related peptide since CRH in the hypothalamic paraventricular nucleus (PVN) plays a crucial role in regulating sympathetic and endocrine responses to stressor exposure (see (1–3) for review). Moreover, extrahypothalamic CRH expression is high in many brain regions that respond to stressful stimuli such as the central nucleus of the amygdala (CeA)

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and bed nucleus of the stria terminalis (BNST), and locus coeruleus (LC), and CRH activity in these structures has been argued to mediate behavioral responses to stressful stimuli (4). Overactive CRH activity has been associated with post-traumatic stress disorder (PTSD) and a single nucleotide polymorphism in the CRF 1 receptor gene (rs12944712) has been associated with PTSD onset in traumatized pediatric patients (5). While CRH systems have received considerable attention in normal stress responses and stress-related psychopathology, recent evidence has implicated other peptides in these functions. Of these, pituitary adenylate cyclase activating polypeptide (PACAP) has emerged as a key regulator of stressor responding (6–9) that may be upstream of CRH in stress-related circuits, and PACAP dysregulation has been associated with PTSD (10, see below).

Pituitary adenylate cyclase activating polypeptide (PACAP)

PACAP was identified from hypothalamic extracts based its ability to stimulate anterior pituitary adenylyl cyclase activity (11). From molecular and biochemical analyses, the alternative endoproteolytic processing of the PACAP precursor can generate bioactive α-amidated PACAP38 or PACAP27 (38 or 27 amino acids, respectively) but PACAP38 appears approximately 10 - to 100-fold more abundant in most tissues including the central nervous system (CNS; (12, 13). PACAP is highly conserved among species and as the peptide is expressed in primitive chordates, the peptide is phylogenetically very old and recognized as the ancestral molecule to the glucagon/secretin/VIP superfamily of peptides (14, 15).

From PACAP isolation, the three G protein coupled PACAP receptor subtypes were identified in rapid succession. The PAC1 receptors bind the two forms of PACAP with high affinity and selectivity; VPAC1 and VPAC2 receptors bind both PACAP and VIP with similar affinities (see (16). Uniquely, there are multiple isoforms of the PAC1 receptors from alternative splicing in domains corresponding to the N-terminal extracellular region (short variants) and the third cytoplasmic loop (Hip and/or Hop variants) that can potentially impact ligand binding and intracellular signaling, respectively. The various PACAP receptor subtypes are expressed in specific regions of the CNS (15, 17, 18) and the differential PAC1 receptor isoform transduction of Gαs and/or Gαq can variably engage adenylyl cyclase and phospholipase C (PLC), respectively, to activate signaling pathways resulting in enhanced calcium mobilization, membrane depolarization, action potential frequency and neurotransmitter synthesis and release (16, 19, 20). These signaling mechanisms coupled with those following receptor internalization in signaling endosomes can also engage MEK/ERK and PI3K/Akt pathways, which appear important for trophic signaling during neurodevelopment, survival, repair and regeneration following injury and neuroplasticity following physiological challenges (21). These trophic responses are further amplified by PACAP/PAC1 receptor abilities to enhance expression of other growth factor mechanisms including BDNF and TrkB (22–24). While the neurotrophic and neuroplasticity functions of PACAP/PAC1 receptor signaling are important attributes under physiological adversities, the same mechanisms may participate in the development of pathological anxiety states associated with stressor exposure. Stressor exposure has been shown to enhance indices of neuroplasticity in anxiety-associated brain regions such as the BNST (25–28), and we have observed similar changes concurrent with increases in BNST PACAP signaling

(unpublished observation). But whether stress-induced BNST PACAP signaling drives the maladaptive neuroplasticity in the BNST leading to anxiety-related disorders remains to be investigated.

PACAP and the stress response

PACAP and PACAP receptors have been identified in classical HPA and autonomic stress pathways. The roles of PACAP in stress have been reviewed recently and will only be discussed briefly here (7, 8). PACAP-positive terminals can form synapses with hypothalamic PVN CRH-expressing neurons (29) and PACAP can stimulate CRH production and secretion (30). In contrast to wildtype mice, PVN CRH mRNA is not upregulated following restraint in PACAP null animals (31) suggesting that PACAP signaling is upstream of CRH function. However, PACAP may also have direct actions on anterior pituitary corticotrophs for ACTH release and facilitate posterior pituitary vasopressin secretion (11, 32); interestingly, the adrenal glomerulosa does not express PACAP/VIP receptors suggesting that PACAP-induced glucocorticoid release is driven predominantly by enhanced HPA activity. In addition to enhanced glucocorticoid release (33), central PACAP administration can also lead to sympathetic activation (30, 34) suggesting PACAP can regulate central autonomic pathways. In addition, PACAP is found in preganglionic sympathetic neurons in the intermediolateral cell column of the thoracolumbar spinal cord and accordingly, PACAPergic fibers densely innervate sympathetic ganglia. Postganglionic sympathetic neurons selectively express PAC1 receptors at high levels and PACAP potently stimulates sympathetic NPY and catecholamine release, which has implicated PACAP peptides as one of the noncholinergic regulators of sympathetic function (21, 35). Congruently, PACAP is colocalized with acetylcholine in abdominal preganglionic splanchnic nerve terminal terminals innervating the adrenal medulla for epinephrine release. PACAP application increases the excitability of adrenal chromaffin cells (36), and enhances the synthesis and release of adrenal catacholamines (9, 37, 38). Conversely, PACAP knockout mice exhibit disrupted catecholamine release patterns from splanchnic nerve activation and during chronic stress exposure (31, 37), which in aggregate suggest that PACAP is widely distributed throughout the HPA and sympathetic nervous system and is one of the principal facilitators of the endocrine and neuronal responses to stressful challenges. The development of PACAP and PAC1 receptor transgenic animal models have been invaluable in deciphering the roles of the PACAPergic system in behavior and endocrine function, and the observations have been largely consistent with those following central PACAP administration (see Tables 1 and 2).

PACAP and the BNST

Herman and colleagues have shown that PVN activity is regulated by multiple CNS pathways whose activation patterns depend on the nature of the stressor. Notably many of these pathways modulate PVN activity via a relay within the BNST, and the ventral portion of the anterolateral BNST has been shown to regulate PVN activity following activation of the hippocampal ventral subiculum or medial prefrontal cortex (39). Congruent with its roles in regulating HPA function, BNST stimulation has been shown increase plasma corticosterone, and sympathetic arousal (40).

Consistent with a role in regulating physiological responses to stressor exposure, BNST activity has been heavily implicated in mediating anxiety-like behavioral responding, and many anxiogenic pharmacological agents increase the expression of activation markers, such as fos, in the BNST (41). Davis and colleagues (1997) initially found that lesions of BNST cell bodies blocked the enhanced startle responding observed after central administration of CRH, whereas CeA lesions did not (42). Other BNST-mediate anxiety-like behaviors include startle responding enhanced by bright light (43), fear responding to long-duration conditioned stimuli (44), and the anxiogenic behavioral changes observed after uncontrollable stress (45) among many others. BNST activity has been correlated with anxiety-like behavior in nonhuman primates (46) and humans (47). Waddell et al. (2006) argued that BNST activity mediates anxiety-like responding to cues that predicted temporally distant threats (44), while Walker, Davis and colleagues suggested that BNST activity mediates anxiety-like responding when the responses must be sustained for a long period of time (48). In both cases, BNST-dependent responding was associated with a behavioral state more representative of “anxiety” in humans as distinct from “fear”. From these studies many investigators have suggested that maladaptive responding of the BNST may underlie some forms of anxiety disorders in humans, including PTSD (6, 49–52).

Consistent with a role for altered BNST function in stress-related psychopathology, chronic exposure to stressors and/or pharmacological treatments have been shown to enhance BNST plasticity. Repeated exposure to stressors, stress hormones, or drugs of abuse have been shown to increase BNST CRH expression (26), dendritic branching and length (27, 28), BNST volume (27), and synaptic efficacy (25). These changes could be associated with increases in anxiety-like behavior (27). Hence, chronic exposure to stressors facilitates BNST function by changing neurochemistry, morphology and physiology within this structure, which have been associated with increases in fear- and anxiety-like behavior. These results strongly implicate factors with neurotrophic properties in the BNST as integral mediators of the anxiogenic effects of chronic stress; as noted above, PACAP is highly expressed in the BNST, is neurotrophic, and can enhance several indices of neuronal excitability. Hence, BNST PACAP may represent a key system in stress-induced anxiety.

In an effort to investigate the role of PACAP in mediating the effects of repeated stressor exposure, we exposed rats to one of several stressors each day for 7 days. The stressors used (restraint, swim, footshock, pedestal, oscillation) were alternated to reduce habituation, and we have reported that this procedures produces and anxiety-like behavioral phenotype (53), anorexia and weight loss, and sensitized stress responding (54). 24-hr after the last stressor exposure (restraint), rats were quickly euthanized, and micropunched tissues from 11 CNS regions were harvested and processed using quantitative polymerase chain reaction (QPCR) for stress-induced changes in PACAP, PAC1 and VPAC receptor transcripts. We found a selective and significant increase in both PACAP and PAC1 receptor transcript in the dorsal aspect of the anterolateral BNST and PVN, and the increases in BNST PACAP transcript were 12–14 fold compared to those from control (non-stressed) animals (53). These data corroborated prior studies implicating PVN PACAP signaling in the response to stressors, and also suggested that BNST PACAP signaling may play a critical role. Moreover, the BNST is one region with high levels of extrahypothalamic CRH expression that has been implicated as a key regulator of PVN activity (39) and a key structure mediating the

behavioral effects of chronic stress (6, 27, 28). Our data suggest that PACAP may be a key regulator of BNST activity and BNST-related behavioral and physiological responses. We found that BNST PACAP transcript increases were not observed following a single acute stressor exposure, suggesting that BNST PACAP-signaling either requires significant time or multiple exposures prior to stress-induced upregulation (55). Interestingly, when we tried to mimic the effects of repeated stressor exposure by repeatedly injecting stress levels of corticosterone, we were unable to produce the same increase in PACAP transcript (55), nor did we observed these increases following repeated treatment with a ghrelin agonist (unpublished data), using a paradigm that has previously been associated with chronic stress effects (56). Together, these data suggest that increases in BNST PACAP may lie upstream of the many physiological outputs of stressor exposure.

From in situ hybridization (ISH) and immunocytochemical analyses the increases in BNST PACAP transcript and protein were localized to the oval nucleus. PACAP expression is rich in this region, although like many peptides PACAP staining was most pronounced in fiber structures rather than cell bodies. The QPCR and ISH results suggest that stress-induced increases in PACAP derive in part from endogenous BNST neuronal induction; however, much of the PACAPergic fibers may represent projection terminals from other nuclei including the PBN (see below). Although CRH is also highly expressed in the BNST oval nucleus, the PACAP-expressing fibers appear distinct but in close proximity to CRH neurons in the BNST and CeA (Kozicz et al., 1997; Missig et al., 2014) suggesting that PACAP and CRH may interact in the regulation of stress and emotional responding.

To examine the roles of BNST PACAP signaling, direct PACAP38 infusions into the BNST elicited anxiety-like responses in the absence of stressor exposure. Bilateral BNST PACAP38 infusions into the BNST increased acoustic startle responding (53), was anxiogenic on an elevated-plus maze (54), produced anorexia and weight loss (57), and increased circulating corticosterone (54, 58). These BNST effects were dose-dependent, not mediated by ventricular leakage, and were observed in both male and female rats. These effects were likely mediated by BNST PAC1 receptor activation, as the anxiogenic and anorexic effects of PACAP were mimicked by BNST infusion of the PAC1 receptor selective agonist maxadilan, and not by VIP activation of VPAC receptors (54).

Although BNST PACAP was sufficient to produce a stress- and anxiety-like phenotype in rats, whether endogenous BNST PACAP signaling participated in the behavioral and endocrine consequences of chronic stress was unclear. In addressing this question the BNST of rats were cannulated bilaterally for chronic delivery of the PAC1/VPAC2 antagonist PACAP(6–38) during the week of repeated stressor exposure (as described above). Importantly, PACAP(6–38) was able to block chronic variate stress-induced weight loss and anxiety-like behavior (54). Moreover, chronic stress sensitized the corticosterone response in response to a subsequent novel stress environment, and in this case acute BNST PACAP(6–38) administered immediately before exposure to the novel environment attenuated the elevated sensitized portion of the corticosterone response to levels prior to the history of stressor exposure (54). These results appear consistent with previous interpretations in PACAP-mediated stress responses (31). PACAP(6–38) may have actions outside of the PACAP system (59). But as BNST PACAP/PAC1 receptor expression was

elevated by chronic stressor exposure, exogenous BNST PACAP application mimicked many of the consequences of chronic stress, and the consequences of chronic stress were blocked by an antagonist at PAC1/VPAC2 receptors, the data in aggregate suggest that BNST PACAP signaling at PAC1 and/or VPAC2 receptors is necessary in mediating the behavioral and endocrine consequences of chronic stress.

PACAP and the CeA

Whereas the BNST has been argued to mediate behavioral states akin to anxiety, the CeA has been studied extensively in the expression of conditioned fear responding; hence, Davis and colleagues have argued that the CeA mediates phasic responses to predictable threat (48). However, CeA activation has also been examined for its role in mediating the emotional component of pain perception. Nociceptive stimuli can increase several markers of CeA activation (60) and the regulation of CeA activity can modify nociceptive thresholds (61). There is an extensive literature describing high rates of comorbidity between pain states and PTSD or related anxiety/mood disorders (62–65) which may reflect maladaptations in CeA responding (66). Painful stimuli can increase a fMRI blood oxygen-level dependent (BOLD) signals in the amygdala (67), and altered pain processing is observed in individuals with PTSD (68–70). Some of the imaging studies, however have been equivocal and require cautious interpretation (68, 69, but see 70) as not all activated subregions of the amygdala could be evaluated and chronic stress may compromise neurovascular coupling to affect BOLD signals (71).

Similar to other brain regions with CRH expression, high levels of PACAP can be identified in the CeA, especially in the lateral capsular division (CeLC; 61). As in the BNST, PACAP staining was dense in fiber elements in this region and appeared distinct from CRH expression; however, unlike the BNST, PACAP expression was not increased following chronic variate stress (61). PACAP signaling has been shown to enhance excitatory CeLC neural activity from the BLA via VPAC1 receptors (72) and CeA PACAP infusions have been shown to increased passive (avoidant) behaviors in the shock-probe defensive burying test (73). Hence multiple lines of evidence have implicated PACAP in CeA function.

From immunocytochemical staining patterns, the CeLC PACAP was hypothesized to carry nociceptive signals via parabrachial nucleus (PBn) projection fibers along the spino-parabrachioamygdaloid tract. Peripheral nociceptive signals increase CeLC neuronal activity in a topographically organized manner; stimulation of PBn afferents to the CeLC evoke excitatory postsynaptic currents in CeLC neurons (74). The PBn highly expresses PACAP and calcitonin gene related peptide (CGRP); previous work had identified PBn-derived CGRP-positive terminals in the CeLC (75) and CeA CGRP infusions have been shown to decrease nociceptive thresholds (76). Hence, whether CeLC PACAP was a component of the same pathway was examined. From immunocytochemical PACAP and CGRP colocalization in anterograde tracing and excitotoxic lesion studies, CeLC PACAP was largely derived and coexpressed with CGRP from the lateral PBn (61). Bilateral PACAP infusions into the CeA dramatically reduced nociceptive thresholds on Hargreave’s thermal sensitivity tests which persisted for several hours; moreover, maxadilan infusions elicited similar effects implicating PAC1 receptors in the CeLC PACAP nociception response (61).

Interestingly, CeA PACAP infusion also produced anxiety-like behavior on the elevated-plus maze, which may be related to an augmentation of the emotional aspects of nociceptive input and/or activation of CeA afferents to the BNST (61). In aggregate, these data implicate CeA PACAP signaling in the spino-parabrachioamygdaloid, and the emotional processing of nociceptive stimuli (61).

PACAP and the BLA

A substantial body of work has implicated activity in the basolateral complex of the amygdala, including the basolateral (BLA) and lateral (LA) amygdala, as a critical locus for the changes in neuroplasticity that underlie Hebbian learning in standard fear conditioning paradigms (reviewed in 77). Since a hallmark feature of PTSD involves invasive fear memories, the BLA and LA have been extensively studies in preclinical research into the mechanisms underlying PTSD. While PACAP is not expressed as highly in this region as in the BNST and CeA, the BLA express PAC1 receptors, VPAC1 and VPAC2 receptors.

Ressler and colleagues reported that mRNA for the PAC1 receptor (*ADCYAP1R1*) increased 1.5 fold during the consolidation of fear following a standard fear conditioning protocol, and significantly correlated with fear expression (freezing behavior), and this effect can be attenuated with a NK3R antagonist (78). However, data from PAC1 receptor-null mice were less conclusive. These mice have been reported to exhibit reduced contextual fear conditioning (a hippocampal dependent task; 79–81), and this effect can be reduced by exposure to an enriched environment; however, PAC1 receptor-null mice exhibit intact cued fear conditioning, although fear extinction may be augmented in these mice (79–81). Notably, Ressler et al., (10) also found a similar increase in PAC1 receptor mRNA in the medial prefrontal cortex (mPFC). While these data are provocative, more investigation is required to determine whether PACAP signaling plays a key role in these aspects of emotional behavior.

PACAP and PTSD in humans

The investigations into PACAP function in animal models strongly implicated PACAP as a key signaling molecule at the interface between stress and emotion. In extending that hypothesis to PACAP dysregulation in humans and PTSD, blood PACAP levels from a highly traumatized population, matched on age, sex and trauma histories, recruited at Grady Hospital in Atlanta, were assessed by radioimmunoassay. Elevated blood PACAP38 levels were significantly correlated with PTSD symptoms (from all three symptom clusters necessary for PTSD diagnosis) in females, but not males, and these findings were replicated in a second cohort. Moreover, these effects were observed even after controlling for depression and substance abuse (10).

From these observations, a single nucleotide polymorphism (SNP) in the PAC1 receptor *ADCYAP1R1* gene was similarly associated with PTSD in females (10). An rs2267735 ‘CC’ genotype in the *ADCYAP1R1* gene was associated with higher levels of PTSD hyperarousal than ‘G’ carriers in women, but not men. Consistent with the apparent gender-dependent effects, rs2267735 resided within a predicted estrogen response element (ERE), suggesting a potential *ADCYAP1R1* regulatory interactions by sex hormones. Interestingly, *ADCYAP1R1*

polymorphism did not appear to be associated with depression, bipolar disorder or schizophrenia, although PACAP dysregulation has been associated with other psychopathologies. The potential changes in PACAP signaling were also associated with behavioral changes suggestive of enhanced BNST function. Hence, rs2267735 was associated with impaired fear discrimination in women (10), which has been shown to be BNST dependent (82), and enhanced dark-enhanced startle in women (10), a measure of anxiety in humans similar to the BNST-dependent light-enhanced startle paradigm in rodents. In addition to BNST function, rs2267735 has also been associated with increased amygdala and hippocampal activity in response to threatening faces in women (83). In sum, PACAP dysregulation in humans appears to alter activity in fear- and anxiety-pathways.

Associations between PACAP signaling and PTSD were not limited to females. Methylation at the first site within the *ADCYAP1R1* CpG island was found to be associated with PTSD in a sex-independent manner (10) suggesting that epigenetic mechanisms may also contribute to alter PAC1 receptor function in PTSD in females and males. Moreover associations between rs2267735 and dark-enhanced startle were observed in children of both sexes (84). Together these data implicate PACAP mechanisms in mediating an anxiety disorder associated with stress in humans. The sex specificity of these data suggested that PTSD mechanisms may differ between men and women, but additional studies are clearly needed.

Since the original report (10), associations between rs2267735 and PTSD have been assessed in several populations. Two independent samples have failed to replicate the original *ADCYAP1R1* polymorphism data but the populations were significantly different with respect to demographic characteristics, social context, and importantly, the nature and level of trauma (85). However, subsequent investigations were more consistent with the original findings (see Table 3). Associations between rs2267735 and startle behavior were observed in children (84); moreover, the *ADCYAP1R1* genotype was found to interact with childhood maltreatment, such that carriers of the risk allele exhibited enhance risk for PTSD if they had experienced childhood abuse (86). The risk allele was associated with the severity of emotional numbing in Chinese women who survived the 2008 Wenchuan earthquake (87) and the original association was replicated in a large sample of highly traumatized African-American women (88). Currently, it is not known how rs2267735 alters PAC1 receptor expression and function; further experiments will be needed to establish the consequences of PAC1 receptor polymorphism in stress-responses and behavior. In aggregate, these results support a relationship between rs2267735 and PTSD although more studies are required to define PACAP mechanisms in the disorder, and establish the determinants underlying the apparent differences in results, which may involve parameters such as the nature, level and duration of the trauma experienced, among other factors.

In addition to PTSD, PACAP signaling has been linked to other stress-related psychopathologies. Several SNPs in PACAP-related genes have been reportedly linked to schizophrenia (89) and major depressive disorder (MDD; (90), although neither rs2267735 nor blood levels of PACAP38 were linked to MDD in the Ressler et al., (2011) or Uddin et al., (2013) studies.

PACAP, estradiol, and stress

PTSD is greater than two times more prevalent in females than males (91–94) and given the associations between altered PACAP signaling and PTSD in women, estrogen has been implicated in the regulation of PACAP or PAC1 receptor expression and function. The BNST is a sexually dimorphic nucleus and accordingly, chronic estradiol (E2) treatment substantially upregulated BNST PACAP and PAC1 receptor transcripts in ovariectomized female rats (10). These observations demonstrated E2 - PACAPergic pathway interactions in a central stress- and anxiety-associated brain region, and were consistent with literature implicating PACAP signaling in the regulation of sex hormone secretion (10). In assessing BNST PACAP-mediated stress-related behavioral effects in ovariectomized female rats with or without E2 replacement, acute BNST PACAP infusions increased circulating corticosterone levels and dramatically reduced food intake and caused weight loss over 24 h, comparable to changes observed in male rats (57, 58). Surprisingly, PACAP interactions with E2 treatment were not observed. The PACAP treatment regimens may have obscured the E2 effects or additional hormonal regulators may be needed. Hence, while suggestive, how E2 and reproductive cycling might impact PACAP signaling to influence behavior is still relatively unclear awaiting future investigations.

Conclusion and future directions

The studies described above illustrate how data from animal models can inform potential mechanisms underlying human behavioral disorders. Hence, PACAP is a relatively novel mediator poised at the junction of stress and anxiety, and the dysregulation of the PACAPergic system may represent one mechanism capable of altering the physiological balance leading to behavioral disorders. However, PACAP signaling clearly has multiple functions in multiple organs and physiological systems, and mechanisms for specifically targeting PACAP pathways to ameliorate psychopathologies will be critically important if therapeutic approaches are to be considered. The development of molecular tools to study and directly target PACAP or PAC1 receptor-expressing neurons are rapidly becoming available and the mechanistic insights from these investigations may provide approaches for PACAP-related tools, used either alone or with other reagents, in clinical applications.

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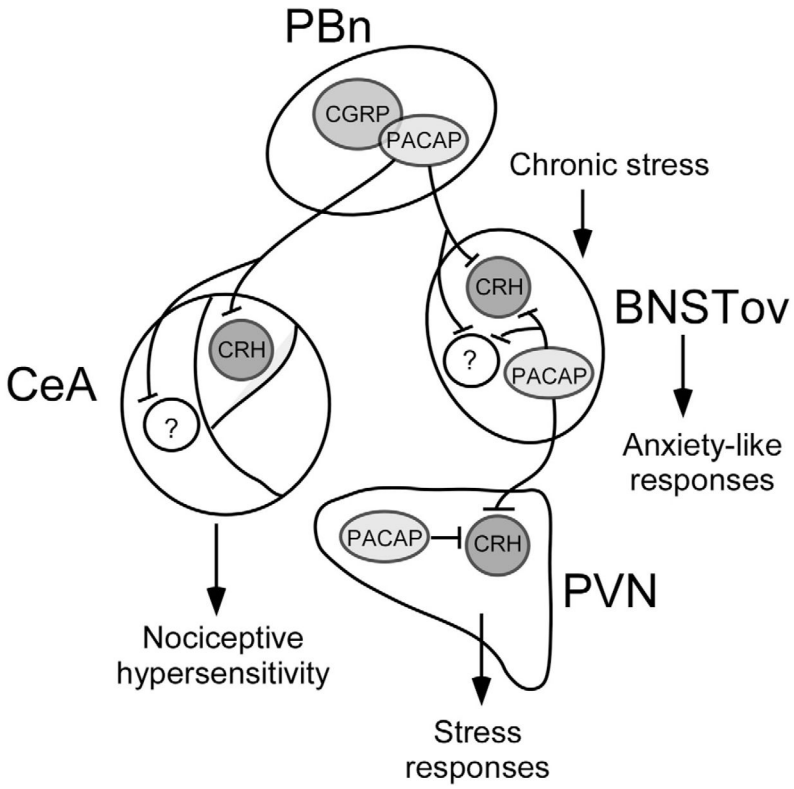

Figure 1.

Schematic of proposed PACAP - CRH interactions. Chronic stress increases endogenous PACAP expression and levels in the oval nucleus of the BNST (BNSTov) which may impact local CRH function to facilitate anxiety-related behaviors, and produce long distance effects in the hypothalamic paraventricular nucleus (PVN) to mediate stress responses (54, 58). Endogenous PVN PACAP signaling may also participate in chronic stress processes. Recently, PACAP and CGRP have been shown to be colocalized in a large population of the lateral parabrachial nucleus (PBn) neurons which project not only to the BNSTov but the central nucleus of the amygdala which may have significant roles in nociception hypersensitivity (61). In addition to CRH, the various PACAP-producing cells may also project to other yet unidentified neurons to affect behavior and hormonal stress responses.

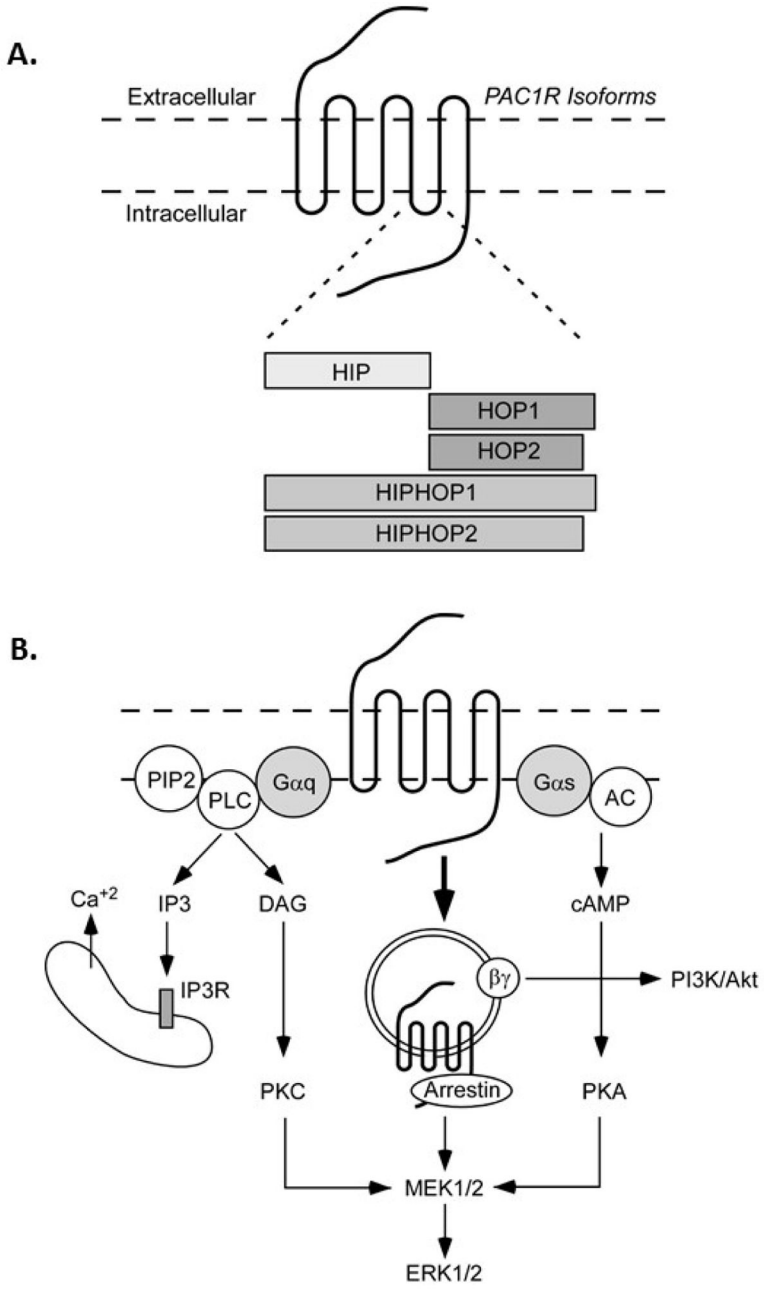

Figure 2.

Figure 2A. G protein-coupled PAC1 receptor isoforms within the third cytoplasmic loop. PAC1 receptors can be expressed in various isoforms depending on the absence or presence of two 84 - bp Hip and/or Hop cassettes encoding segments within the third cytoplasmic loop of the 7-transmembrane receptor (14–16, 19–21). The PAC1 receptors can be contain neither Hip nor Hop (null isoform), Hip, Hop1, Hop2 (shortened form of Hop1), HipHop1 and HipHop2; the principal forms in the CNS are the null and the Hop1 receptor isoforms. Figure 2B. PAC1 receptor signaling cascades. The various PAC1 receptor isoforms can be differentially coupled to Gαs and Gαq to initiate adenylate cyclase (AC) and phospholipase

C (PLC) signaling cascades, respectively (16, 19–21, 23). In addition, as in other Class B GPCRs, the PAC1 receptor contains consensus Ser sequences in the intracellular cytoplasmic tail for high affinity arrestin binding for endosome signaling following PAC1 receptor internalization. Arrestin molecules can serve as scaffolds for adaptor proteins and enzymes for ERK pathway activation; in addition to downstream PKA/PKC - dependent signaling events, both PLC/PKC and AC/PKA pathways may also intersect with ERK signaling (19, 95–97). The internalization of G protein βγ subunits may result in other pathway activation including PI3K/Akt (97).

Table 1

CNS PACAP effects in animal models

Locomotor activity	
PVN and CeA PACAP infusions decrease locomotor activity (rats) ICV PACAP decrease activity (fish)	(61, 98, 99)
VMN PACAP increase activity (rats)	(100)
Anxiety-like responses	
ICV PACAP increase stress-like responses (rats)	(30)
BNST, CeA and ICV PACAP infusions increase anxiety-like behavior in open field/EPM (rats)	(34, 54, 61)
BNST PACAP increases startle responses (rats)	(53)
Feeding/weight change	
Hypothalamic, BNST and ICV PACAP infusions decrease feeding/weight gain (anorexigenic) (rats, mice, chick, fish)	(34, 54, 57, 99 – 105)
Memory/cognition	
ICV PACAP infusion improves learning memory in passive avoidance task (rats)	(106 – 108)
Systemic PACAP administration increases learning (invertebrates)	(109)
Sensory responsiveness	
CeA PACAP administration increases nociceptive sensitivity (rats)	(61)
HPA responsiveness	
ICV and BNST PACAP infusion increase corticosterone levels (rats, chick)	(30, 34, 54, 58, 110)

Table 2

CNS PACAP functions from PACAP or PAC1 receptor transgenic animal models

Locomotor activity	
Increase locomotor activity (PACAP KO)	(89, 111 – 116)
Increased locomotor activity (PAC1 KO)	(79, 80)
Anxiety-like responses	
Decrease anxiety responses in open field/EPM (PACAP KO)	(89, 111 – 115)
Decreased startle behavior (PACAP KO)	(115)
Decreased anxiety-like responses EPM (PAC1 KO)	(79, 80)
PAC1 participates in stress/anxiety responses from zebrafish knockdown studies	(117)
Feeding/weight change	
Decreased carbohydrate food intake (PACAP KO)	(118)
No changes in food intake (PACAP KO)	(119, 120)
PACAP regulates feeding after fasting (PACAP-Ires-Cre)	(121)
Memory/cognition	
Mild memory impairment (PACAP KO)	(115, 122)
Mild memory impairment (PAC1 KO)	(79 – 81, 123)
Sensory responsiveness	
Mechanical hyperalgesia absent (PACAP KO)	(124)
Induced PACAP expression in zebrafish increases sensory responsiveness	(125)
Diminished chronic pain responses (PAC1 KO)	(126)
HPA responsiveness	
Impaired corticosterone responses to emotional and chronic stressors (PACAP KO)	(31, 127, 128)
Normal corticosterone levels (young PAC1 KO before premature death)	(129)

Table 3

PACAP associations with PTSD

Altered blood PACAP levels associated with PTSD in women	(10)
PAC1 receptor gene SNP associated with PTSD in women	(10)
PAC1 receptor gene methylation associated with PTSD (both sexes)	(10)
PAC1 receptor gene SNP association in highly traumatized African-American women	(88)**
PAC1 receptor SNP associated with startle behavior in children (both sexes)	(84)
Interaction between PAC1 receptor SNP and childhood maltreatment increases PTSD risk	(86)
PAC1 receptor gene SNP associated with emotional numbing in Chinese women earthquake survivors	(87)
Associations of PAC1 genetics and stress with childhood asthma	(130)

** The same group failed to identify PAC1 receptor gene associations with PTSD in previous samples, Chang, 2012