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Corticotropin-Releasing Hormone in the Central Nucleus of the Amygdala: Link to Psychiatric Disorders

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Abstract
Corticotropin-releasing hormone (CRH) is a modulatory peptide that plays an essential role in the activation of the stress response. In the event of chronic stress, dysregulation of CRH expression occurs and is implicated in the pathology of psychiatric disorders. Individuals with depression and post-traumatic stress disorder (PTSD) show elevated levels of CRH protein in their cerebrospinal fluid. The source of this pathological increase in CRH expression is believed to be extrahypothalamic. One suspected extrahypothalamic source of CRH is the central nucleus of the amygdala (CeA). Normally, CRH in the CeA is believed to mediate autonomic and behavioral aspects of the stress response. However, dysregulation of this system has been linked to increased susceptibility to psychiatric disorders. The mechanism by which this increase in CRH levels in the CeA raises the risk for psychiatric disorders has not yet been elucidated. This paper will review current studies regarding the role of CeA CRH release in mediating the stress responses and how this relates to psychiatric disorders.

Keywords: *Corticotropin-releasing Hormone (CRH), Central nucleus of the amygdala (CeA), Lentivirus, Tetracycline-inducible System, Hypothalamic-pituitary-adrenal (HPA) axis*

Introduction

The Stress Response

Stress is a physiological response to a disruption in homeostasis caused by a physical or psychological element called a stressor[1]. The brain responds to stress by activating the sympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis. The former system functions to rapidly increase the release of epinephrine in order to mediate autonomic functions[2,3]. This results in increased arousal, vigilance and a decision to 'fight or flight'[2,3]. On the other hand, the HPA axis mediates the slow and sustained aspects of the stress response and is linked to corticotropin-releasing hormone (CRH)[A] mRNA increase in the amygdala[4].

The HPA axis is a system of interactions between the hypothalamus, pituitary gland and adrenal gland that regulate hormonal responses to internal or external stimuli. Stress activates the HPA axis, causing the secretion of CRH and vasopressin (AVP) from the paraventricular nucleus (PVN) of the hypothalamus to activate corticotroph cells in the anterior pituitary[1]. There, both hormones induce synthesis of adrenocorticotropin releasing-hormone (ACTH), which causes corticosterone (CORT) secretion from the adrenal cortex into the bloodstream[1] (Figure 1). CORT then binds to glucocorticoid receptors (GR), expressed in peripheral organs and limbic brain regions such as the amygdala, thereby facilitating responses aimed at adapting to the stressor[1]. Moreover, CORT binding to GR in the central nucleus of the amygdala (CeA)[5] has been demonstrated to increase CRH mRNA synthesis. This has been demonstrated in studies where CORT pellet implantation[4,6] or repeated CORT injections[7] elevated CRH mRNA in the CeA of rodent models[4,6-8]. Consequently, when GR is deleted in the CeA, it results in a subsequent decrease in the levels of CRH mRNA[9]. As a mechanism of control, after GR is occupied by CORT, the sustained increase in levels of plasma CORT cause it to negatively feedback at the level of the PVN and anterior pituitary to inhibit its own secretion, and thus return the system to homeostatic levels[1]. However, in the event of chronic stress, this hormonal response of the HPA axis is hyperactivated causing an aberrant rise in CORT that is resistant to negative feedback to inhibit its secretion[10,11]. Additionally, further increases in CRH mRNA in the amygdala leads to changes that heighten maladaptive emotional behavior[12]. This rise in amygdala CRH activates the HPA axis[13], possibly though CeA interaction with its targets described later in this review.

The CEA in Psychiatric Disorders

The aforementioned dysregulation of the HPA axis plays a vital role in the genesis of stress-related disorders such as anxiety and depression[11,14,15]. One characteristic these disorders share is an increased emotional response to neutral stimuli[16]. Given that the amygdala mediates emotional responses to stress[12,17], it is an important structure to study in order to further understand predispositions of certain individuals to psychiatric diseases.

The amygdala receives input from sensory modalities and integrates this information to activate behavioral and physiological responses[1]. Functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) studies[18] in individuals with depression and PTSD show enhanced amygdala activation[19]. Furthermore, ablation studies of the amygdala demonstrate decreased fear behavior and more docile demeanor in animals[20], implicating a role for this limbic structure in fear potentiation. Among the amygdala nuclei, the CeA stands out as the major nuclei of the amygdala for three main reasons.

First, the CeA functions as the central integration point for most of the other amygdala nuclei and many other brain regions[21]. Specifically, the basolateral amygdala (BLA) receives fear memory information from the hippocampus and sends it to the CeA, causing output to regions involved in the behavioral expression of fear[22,23]. The CeA itself receives direct cortical innervations from the prefrontal cortex (PFC), sensory areas, brainstem and hypothalamus[21]. Information about the salience of danger and cognition associated with it is sent to the CeA directly from the mPFC or indirectly through the BLA[16]. This sensation of danger is heightened in individuals with anxiety disorders such that even neutral stimuli can evoke an emotional

[A] **Corticotropin Releasing Hormone (**CRH): a 41-amino acid peptide involved in modulation of neuroendocrine, autonomic, and behavioral responses to stress.

response[16]. Noradrenergic projections from the locus coeruleus to the CeA increases CeA activity and CRH mRNA in the CeA and affects autonomic activation[24]. The information sent into the CeA is thus integrated in a process that remains to be elucidated and is expressed in the form of autonomic and behavioral responses in CeA outputs.

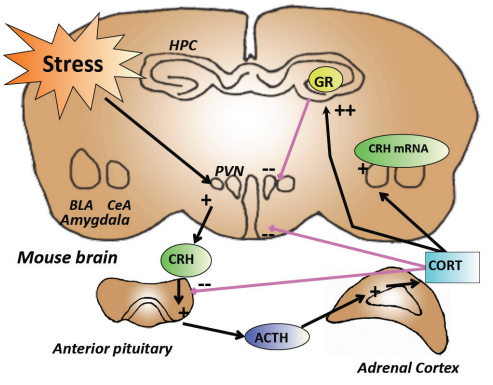

Figure 1: Hypothalamic-Pituitary-Adrenal (HPA) Axis Circuitry: Stress activation of the HPA axis as described in text leads to an increase in CRH in the central nucleus of the amygdala (CeA). GR, glucocorticoid receptor; HPC, hippocampus; BLA, basolateral amygdala; CRH, corticotropin releasing hormone; ACTH, adrenocorticotropin hormone, CORT, corticosterone.

+ activation, - inhibition

Second, the CeA is the major amygdala output structure[21] to regions involved in the stress response. Efferents of the CeA go to bed nucleus of the stria terminalis (BNST), hypothalamus, brainstem and midbrain nuclei, and modulate autonomic and behavioral functions[21]. The CeA mainly contains GABAergic output and as such, its projections to the BNST disinhibit BNST inhibition of the PVN[2,21]. Due to the limited direct CeA to PVN projections[25], this is believed to be the method by which the CeA activates the HPA axis[12], however there is no direct evidence for this. The CeA activates brainstem nuclei such as the periaqueductal gray to cause freezing and vocalization in the conditioned fear response to shock[21], and hence generates relevant fear responses to aversive stimuli[26]. In psychiatric diseases, stimuli can activate the sympathetic nervous system, possibly through CeA activation of the locus coeruleus[27], resulting in increased heart rate and blood pressure[28]. Electrical stimulation of the CeA also activates the sympathetic nervous system[21,28] and is believed to activate the HPA axis and lead to an increase in plasma CORT in rats[29].

Third, the CeA is a site containing large populations of CRH neurons[23,27] most of which colocalize with GAD65/67, implying their GABAergic inhibitory activity[27]. Moreover, electrophysiological data shows that CRH application increases GABA inhibitory post-synaptic currents (IPSCs) in the CeA[14,30]. Not only does CRH act at receptors in the CeA, but it also activates CeA targets through disinhibition of interneurons[14]. However, other studies using neuronal tract tracing methods demonstrate that CRH immunoreactive CeA neurons form excitatory synapses with locus coeruleus dendrites[31] to cause excitation of the sympathetic nervous system. This indicates that CeA CRH can function both through excitatory and inhibitory pathways to exert its function.

This circuitry of the CeA identifies a role for it in mediating pathways involved in behavioral, autonomic, and endocrine responses to stimuli[32], in part mediated by CRH. The role of CRH in affecting CeA targets may cause changes in behavior that increase risk for psychiatric disease. These roles of CeA CRH will be examined in the remainder of this review.

Role of CRH in CEA Function

The CRH peptide is distributed throughout the brain but found more concentrated in the PVN, CeA, and BnST[23,33]. CRH has two receptors, CRH-R1 and CRH-R2, which have primarily non-overlapping expressions in the brain[34,35]. CRH-R1 is more potently activated by CRH than the CRH-R2 receptors[36] and is known to be the major CRH receptor that activates the HPA axis and the stress response[35]. Restraint stress in rodents significantly increases the levels of CRH and CRH-R1 mRNA in the PVN[37]. CRH-R2 function is not clearly understood, but it is implicated in reducing stress sensitivity[38] as its deletion leads to a rise in anxiety behavior[8]. In the amygdala, CRH-R1 receptors are the predominant type expressed[35]. Functionally, inhibition of CRH-R1 receptors prevent the CRH induced increase in GABAergic IPSCs in the CeA previously described[14] and may also affect autonomic and behavioral functions of the CeA.

Studies in patients with depression and PTSD show distinct elevations in CRH levels in the cerebrospinal fluid[8,39-43]. Treatment of these depressed patients with CRH-R1 antagonist ameliorates the symptoms of anxiety and depression[44] and decreases HPA axis activity[45]. Many anxiety and despair symptoms can be recapitulated in rodent models upon intracerebroventricular CRH administration, through a pathway that is thought to be HPA axis–independent[46]. This implies that extrahypothalamic sources of CRH are the main source of CSF CRH[46]. Since CeA CRH is anxiogenic[26] and CeA stimulation exacerbates the effects of intracerebroventricular CRH[17], the CeA is a probable extrahypothalamic source of CSF CRH. This hypothesis is supported in rodent models using both in-vivo microdialysis studies that demonstrate elevated CeA CRH following restraint stress[47], as well as chronic CORT administrations. Furthermore, elevated CeA CRH in animal models also demonstrate elevated anxiety[7] suggesting that changes in CeA CRH levels are a potential model of CeA dysfunction in psychiatric disease.

Evidence for the Role of CeA CRH in Stress-Related Behavior

Numerous brain functions are sensitive to stress and can be evaluated in behavioral paradigms that have been validated over the years. Two such brain functions are learning and memory, some aspects of which are mediated by the CeA[9]. CeA lesions in rodents impair memory retention in the inhibitory avoidance task[48], the defensive burying task[49], and conditioned fear test[23]. Although there are other peptides found in the CeA, such as neuropeptide Y, neurotensin, and enkephalin[21], the role of CRH as the major HPA axis activator in the stress response, as well as activator of sympathetic nervous system[1] is the reason for its focus in this review.

When CeA CRH is reduced with the use of antisense oligonucleotide[23] or by GR deletion in the CeA (CeAGRKO)[9], memory retention in conditioned fear test is impaired. Furthermore, the CeAGRKO mice show rescue of conditioned fear behavior if intracerebroventricular CRH is administered before conditioned fear training[9]. It appears therefore that there is a homeostatic level of CRH required in the CeA for normal function and any deviation above or below this level can result in behavioral and neuroendocrine problems. CRH receptor antagonist applied to the CeA reduces elevated plus maze[50] and CRH-mediated anxiety behavior[51]. CRH-R1 antagonists diminish anxiety and HPA axis response, and increases exploratory behavior in

primates[52]. Although the anxiety attenuating effects of CRH R1 antagonist was not observed in a study looking at novelty-suppressed feeding in rat[53], most studies to date do suggest a role for CeA CRH in anxiogenic behaviors[54], fear memory consolidation[9], as well as autonomic responses[2].

Behavioral data also shows that the CeA is involved in the psychological aspect of stress. Studies comparing the effects of the physical stress of treadmill running to psychological restraint stress show that the increase in CeA CRH throughout the restraint stress was not seen to the same extent in treadmill running rats[55]. This implies that the CeA is more a mediator of the psychological stress response and this mediation may occur through modulation by CRH[26].

Evidence from Development Studies of CRH-induced Behavioral Changes

Early life stress increases the risk of psychiatric disorders later in life. Therefore, the developmental time period that stress occurs also plays a role in the neuroendocrine and behavioral outcomes[56]. Adults who experienced some form of childhood stress show elevated basal cortisol, increases in ACTH responsiveness, and heightened emotional responses to stressful stimuli than control patients[56]. In the CSF of these patients, increased CRH was predicted by perceived stress during pre-school years but not during preteen years[56]. These models of early life stress have been recapitulated by studies of maternal deprivation in both primate and rodent models. The maternal deprivation model consists of taking the young away from the mothers a few hours a day for 2 weeks[57] and studying the behavior of the young when they reach adulthood. These studies show increased anxiety and depression behavior in rodents and primates that were maternally deprived[56,58]. In adulthood, maternally deprived primates show an increase in CSF CRH and rodents show increases in anxiety behavior[56]. Furthermore, CRH mRNA in the amygdala and hypothalamus, and CRH immunoreactivity in the median eminence are increased due to maternal deprivation[57]. This indicates that the time period during which one is exposed to a stress can affect the CRH system's response later in life. There is a need for animal models to explore temporal effects of CRH over-expression in the CeA.

These studies have laid the foundation for identifying behavioral and neuroendocrine effects of CRH in the CeA. However more research is needed using more physiologically relevant models to better understand the role CRH in the CeA plays in stress and psychiatric disorders.

Current Studies that Analyze the Effects of CRH Overexpression on Anxiety and Despair Behaviors in Rodents: Use of Lentiviral Vectors and Tetracycline-inducible Systems

There are a few rodent studies that have demonstrated the effects of CRH overexpression in anxiety and despair. Transgenic mice that overexpress CRH show reduced locomotor activity in a novel environment, which is exacerbated by social defeat stress[46]. In the elevated plus maze test, these mice spent less time in the open arm of the maze compared to the closed arm, indicating increased anxiety[46]. This anxiety response was abolished when α-helical CRH 9-41, a CRH antagonist, was injected into the intracerebroventricular region of the brain[46]. Transgenic mice that have an inducible tetracycline system[B] have also been used to demonstrate that forebrain CRH overexpression in the first three weeks of life produces anxiety-like behavior in the open field and light-dark preference test later in adulthood[59]. These mice show despair behavior in tail suspension and forced swim tests as well as increased CRH-R1 mRNA that are reversed upon treatment with antidepressant[59]. Recently, however, transgenic models have been developed that can overexpress CRH in specific brain regions to determine site-specific CRH function.

Regev *et al*. used lentiviral vectors[C] to specifically overexpress CRH in the CeA and the BNST of male mice and tested the effects of chronic expression of CRH in these regions using behavioral test for anxiety and depression[60]. The data showed that under non-stressed conditions, chronic CRH overexpression in the CeA had no effect on anxiety in the open field and light/dark preference tests[60]. However when the mice underwent 30 minutes of restraint stress before the behavioral tests, anxiety was attenuated, implying that chronic overexpression can cause habituation to a stressor[60]. In contrast, Keen-Rhinehart *et al*., also using lentiviral vectors to overexpress CRH in the CeA of female rats, showed increased anxiety, increased despair, and impaired negative feedback of HPA axis, all changes associated with stress pathology[12]. The discrepancy in these two studies may be a result of the length of over-expression time, and gender and/or species differences.

In order to combine the use of lentiviral vectors with the tetracycline inducible system, our lab has developed a transgenic mouse model[59] that will allow for spatial and temporal CRH overexpression (unpublished data). These mice have the CRH gene under the tetracycline responsive promoter[59]. This model allows for the use of stereotaxic injections of lentiviral reverse tetracycline transactivator into the brain region of interest to overexpress CRH as well as control the period of overexpression. Targeting the CeA will allow the study of specific changes that occur in this brain region upon HPA-axis dysregulation and how this affects diseases such as depression and PTSD.

Conclusion

Stress plays an important role in precipitating psychiatric disorders. One key mediator of an organism's response to stress is the HPA axis, which is associated with increased CRH mRNA levels in the CeA. The CeA receives sensory information from many brain regions and sends output to regions mediating autonomic, neuroendocrine, and behavioral responses. CeA CRH produces anxiogenic behavior in rodents and affects HPA axis and autonomic functions vital to the stress response. CRH overexpression during development as well as in adulthood can increase stress-related behavior and lead to increased risk for psychiatric diseases.

The use of tetracycline-inducible transgenic mice models to overexpress CRH during specific periods of development, in combination with stereotaxic injections of viral vectors, will allow for the determination of region-specific effects of CRH. By understanding region-specific functions of CRH in the stress response, the function of the CRH system can be elucidated to determine when a response will either cause a return to homeostasis or a drive towards psychiatric disease. Ultimately, this will enable the identification of more effective treatment of psychiatric diseases, thereby increasing human quality of life.

[B] **Tetracycline-inducible system**: Transgenic mice are generated with the gene of interest under the tetracycline responsive promoter. In the presence or absence of doxycycline, transcription can be turned on or off in these mice.

[C] **Lentiviral Vectors**: Vectors used to efficiently introduce genes into *in vivo* systems.

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