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Sustained glucocorticoid exposure recruits cortico-limbic CRH signaling to modulate endocannabinoid function

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ABSTRACT

Sustained exposure to stress or corticosteroids is known to cause changes in brain endocannabinoid (eCB) signaling, such that tissue contents of the eCBs *N*-arachidonylethanolamine (AEA) are generally reduced while 2-arachidonoylglycerol (2-AG) levels increase. These changes in eCB signaling are important for many of the aspects of chronic stress, such as anxiety, reward sensitivity and stress adaptation, yet the mechanisms mediating these changes are not fully understood. We have recently found that the stress-related neuropeptide corticotropin-releasing hormone (CRH), acting through the CRH type 1 receptor (CRHR1), can reduce AEA content by increasing its hydrolysis by the enzyme fatty acid amide hydrolase (FAAH) as well as increase 2-AG contents. As extra-hypothalamic CRH is upregulated by chronic corticosteroid or stress exposure, we hypothesized that increased CRH signaling through CRHR1 contributes to the effects of chronic corticosteroid exposure on the eCB system within the amygdala and prefrontal cortex. Male rats were exposed to 7 days of systemic corticosterone capsules, with or without concurrent exposure to a CRHR1 antagonist, after which we examined eCB content. Consistent with previous studies in the amygdala, sustained corticosterone exposure increases CRH mRNA in the prefrontal cortex. As was shown previously, FAAH activity was increased and AEA contents were reduced within the amygdala and prefrontal cortex following chronic corticosterone exposure. Chronic corticosterone exposure also elevated 2-AG content in the prefrontal cortex but not the amygdala. These corticosteroid-driven changes were all blocked by systemic CRHR1 antagonism. Consistent with these data indicating sustained increases in CRH signaling can mediate the effects of chronic elevations in corticosteroids, CRH overexpressing mice also exhibited increased FAAH-mediated AEA hydrolysis in the amygdala and prefrontal cortex compared to wild type. CRH overexpression increased 2-AG content in the amygdala, but not the prefrontal cortex. These data indicate that chronic elevations in CRH signaling, as is seen following exposure to chronic elevations in corticosterone or stress, drive persistent changes in eCB function. As reductions in AEA signaling mediate the effects of CRH and chronic stress on anxiety, these data provide a mechanism linking these processes.

Abbreviations: ACTH, adrenocorticotropic hormone; AEA, *N*-arachidonylethanolamine; CB1, cannabinoid type 1 receptor; CORT, corticosterone; CRH, corticotropin-releasing hormone; CRHR1, CRH receptor type 1; CRH-OE, CRH overexpressing mice; FAAH, fatty acid amide hydrolase; GABA, gamma-aminobutyric acid; PFC, prefrontal cortex; WT, wild-type; 2-AG, 2-arachidonoylglycerol.

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1. Introduction

Over the last several years, endocannabinoids (eCBs) have become widely appreciated as a neuromodulatory signaling system that is capable of dampening endocrine stress responses, decreasing activation of stress-sensitive brain circuits, and reducing the detrimental impacts of stress on mood and anxiety (see Hill and Tasker, 2012; Morena et al., 2016; Gray et al., 2014 for reviews). To better understand how eCBs exert these effects, recent studies have characterized eCB changes in response to acute and chronic stress in animal and human models (Morena et al., 2016). Although the scope of human studies is currently limited (see Hillard et al., 2012 for review), the accumulating wealth of animal studies indicates that eCB responses show similarities across a variety of stress models, suggesting the neural basis coordinating these changes is likely similar (Morena et al., 2016). A notable commonality across acute psychological stress and chronic stress paradigms is that the eCBs *N*-arachidonylethanolamine (AEA) and 2-arachidonoylglycerol (2-AG) typically change in opposite directions. While decreases in AEA content are consistently observed in the prefrontal cortex (PFC), amygdala and hippocampus, and to a lesser extent in the hypothalamus (Patel et al., 2005; Rademacher et al., 2008; Hill et al., 2010b; McLaughlin et al., 2012; Hill et al., 2009; Dubreucq et al., 2012; Gray et al., 2015b; Jennings et al., 2016), these same regions show increases in 2-AG concentrations, which are generally most prominent during repeated and chronic stress conditions (Patel et al., 2005; Hill et al., 2010b; Dubreucq et al., 2012; Wang et al., 2012; Evanson et al., 2010; Hill et al., 2011; see Gray et al., 2014 and Morena et al., 2016 for review).

The mechanisms by which stress modulates eCB signaling are not entirely understood. We have recently demonstrated the importance of the stress-related neuropeptide, corticotropin-releasing hormone (CRH), acting through the CRH type 1 receptor (CRHR1), in mediating the rapid decline in amygdalar AEA tissue contents by increasing AEA hydrolysis by the enzyme fatty acid amide hydrolase (FAAH; Gray et al., 2015b). While these acute AEA/FAAH amygdala effects appear to be independent of corticosterone (CORT) changes, acute stress-induced CORT increases are required for elevations in 2-AG content in the PFC (Hill et al., 2011), hippocampus (Wang et al., 2012) and the hypothalamus (Evanson et al., 2010). Following chronic stress, however, reductions in tissue levels of AEA and elevations of 2-AG in limbic structures, including the amygdala, become amplified and both of these effects are recapitulated by sustained elevations in corticosteroids (Patel et al., 2005; Dubreucq et al., 2012; Hill et al., 2005; Bowles et al., 2012). The inability of acute CORT exposure to induce the same AEA changes found following chronic CORT exposure (Hill et al., 2010a), or chronic stress, suggest that the effects of chronic CORT on AEA are likely indirect, and could involve secondary signaling mechanisms downstream of glucocorticoid receptor activation.

In this regard, a hallmark feature of clinical studies examining stress-related disorders, such as major depression, is a significant increase in central CRH levels (Nemeroff et al., 1984). Rodent models of chronic stress also show an increased capacity for central and extra-hypothalamic CRH signaling to modulate neuronal function. These studies have consistently described CORT-dependent CRH mRNA increases in the amygdala and bed nucleus of the stria terminals (BNST) following sustained exposure to glucocorticoid elevations (Swanson and Simmons, 1989; Makino et al., 1994a,b) and the facilitation of acute stress-induced CRH release in the amygdala and PFC using microdialysis approaches (Merali et al., 2008). Similarly, repeated restraint stress also increases CRH mRNA levels in the amygdala, which is thought to be due to CORT-dependent upregulation (Makino et al., 1999; Gray et al., 2010). Given the role of CRH in the acute regulation of FAAH activity and AEA content by stress, these data suggest that the progressive recruitment of CRH signaling by sustained CORT elevations could also mediate the effects of chronic stress on the eCB system. The aim of the current study was to determine the necessity of CRH signaling in the effects of chronic CORT exposure on the eCB system, and determine if CRH overexpression alone is sufficient to precipitate the eCB changes associated with chronic stress and prolonged exposure to glucocorticoid elevations.

2. Methods and materials

2.1. Animals

Adult male Sprague Dawley rats (200–225 g) from Charles River Laboratories (Kingston, NY) were used. Rats were pair housed under standard conditions of light (lights on at 0900 h and off at 2100 h) and temperature ($22 \pm 2\,^{\circ}C$) and given one week of acclimatization to the animal facility upon arrival. Rats were provided Purina Rodent Chow (Labdiet 5012, Wilkes-Barre, PA) and tap water *ad libitum*.

Adult male C57BL/6J mice bred at the Max Planck Institute of Psychiatry were used to study the effects of centrally restricted CRH overproduction. Breeding details for the generation of homozygous mice that conditionally overexpress CRH in the brain (CRH-COE-Nes) have been previously described (Lu et al., 2008). In brief, the CRH-COE-Nes mice were generated by first inserting a single copy of the murine CRH cDNA, preceded by a *loxP*-flanked transcriptional terminator into the ubiquitiously expressed ROSA26 (R26) locus, to produce a subset of homozygous $R26^{flopCrh/flopCrh}$ mice (Dedic et al., 2012). These animals were crossed with mice expressing Cre under the nestin promoter ((Nes)-Cre mice) to ensure CRH overproduction is limited to the central nervous system. This approach permits CRH expression to be conditionally activated throughout the brain as early as embryonic day 10.5, when nestin expression is initiated. This mouse model, unlike other CRH overexpressing mouse lines which are associated with peripheral corticosteroid elevations, does not display elevations of basal adrenocorticotropic hormone (ACTH) or CORT which allows the effects of CRH overproduction to be assessed independent of changes in circulating corticosteroids under resting conditions (Lu et al., 2008).

Male wild-type (WT) and CRH overexpressing mice (CRH-OE) were group housed 2–4 per cage under standard conditions of light (lights on at 0700 h and off at 1900 h), temperature ($22 \pm 2\,^{\circ}C$) and provided with food and tap water *ad libitum*. All mice were 2–3 months old at the time of tissue collection.

2.2. Experimental methods

2.2.1. CRH mRNA in situ hybridization

Adjacent series of tissue from each rat were used for *in situ* hybridization and morphological analysis. *In situ* hybridization was performed using a ^{35}S-labeled (Amersham Biosciences Inc., Arlington, IL, USA) antisense CRH cRNA probe. Techniques for riboprobe synthesis are described in greater detail elsewhere (Makino et al., 1994b). A thorough description of tissue preparation and the *in situ* hybridization protocol can also be found elsewhere (Kinlein et al., 2015). Based on the strength of autoradiographic signal on test slides exposed to X-ray film (Kodak BioMax MR film, Sigma, St. Louis, MO, USA), hybridized slides containing the PFC were exposed to film for 2 days to optimize the detection of possible treatment differences. Films were then digitized with a scanner and semi-quantitative densitometric analysis of relative levels of CRH mRNA was performed. Optical densities were determined bilaterally and averaged across 3 adjacent coronal sections for each rat. Every measurement of optical density was corrected by background subtraction. Film images were analyzed using MCID-M4 software