Integration of Endocannabinoid Signaling into the Neural Network Regulating Stress-Induced Activation of the Hypothalamic–Pituitary–Adrenal Axis

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Abstract The evidence that has been gathered to date strongly argues for an inhibitory role of endocannabinoid (ECB) signaling in regulating HPA axis activity. Under basal conditions, ECB signaling appears to be a driving force in the maintenance of low HPA axis activity, as disruption of CB_1 receptor activity results in basal hyperactivity of the HPA axis. Under conditions of acute stress, ECB signaling likewise appears to constrain activation of the HPA axis, possibly via both distal regulation of incoming amygdalar inputs and local regulation of excitatory input to CRF neurosecretory cells in the PVN. ECB neurotransmission is, in turn, modulated by stress, possibly acting as either a "gatekeeper" of the HPA axis, or a recovery system aimed at limiting HPA axis activity. Consistently, pharmacological enhancement of ECB signaling attenuates stress-induced HPA axis activity while impairment of CB_1 receptor signaling results in an exaggerated cellular and neuroendocrine response to stress. Additionally, under conditions of repeated stress,

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D. Kendall and S. Alexander (eds.), *Behavioral Neurobiology of the Endocannabinoid System*, Current Topics in Behavioral Neurosciences 1,
DOI: 10.1007/978-3-540-88955-7_12,

a progressive increase in limbic 2AG/CB_1 receptor signaling contributes to the development and expression of neuroendocrine habituation.

Ultimately, these data demonstrate that the ECB system is likely to be an integral player in the neuronal response and plasticity to stress. The relevance of this relationship has not been fully explored with respect to both normal homeostasis and pathological states characterized by alterations in HPA axis function, but will be a focus of future research.

Keywords Cannabinoid • Anandamide • 2-AG • FAAH • Stress • PVN • HPA axis • Corticosterone • Amygdala • Adrenal

Abbreviations

2AG	2-Arachidonoylglycerol
ACTH	Adrenocorticotropic hormone
AEA	Anandamide
BLA	Basolateral nucleus of the amygdala
CeA	Central nucleus of the amygdala
CRF	Corticotrophin-releasing factor
ECB	Endocannabinoid
FAAH	Fatty acid amide hydrolase
GH	Growth hormone
HPA	Hypothalamic–pituitary–adrenal
MeA	Medial amygdala
MGL	Monoacylglycerol lipase
mPFC	Medial prefrontal cortex
PAG	Periaqueductal gray
PVN	Paraventricular nucleus of the hypothalamus

1 Stress and the Hypothalamic–Pituitary–Adrenal Axis

The hypothalamic–pituitary–adrenal (HPA) axis is a neuroendocrine system that responds in a coordinated manner to aversive stimuli. The integration center for the HPA axis is located within the paraventricular nucleus of the hypothalamus (PVN), where a dense population of neurons secreting corticotrophin-releasing factor (CRF) are located. The activity of these neurons is regulated by both excitatory and inhibitory signals arising from local hypothalamic nuclei such as the dorsomedial hypothalamic nucleus and the medial preoptic area, limbic structures such as the amygdala and prefrontal cortex, as well as monoaminergic cell bodies found in the midbrain and brainstem (Herman et al. 2002, 2003). Exposure of an organism to

aversive stimuli activates a characteristic neural network which encodes both the salience and the threat of the stimuli, and ultimately culminates in the activation of the CRF neurosecretory cells in the PVN. Upon activation, these neurons release CRF into the portal blood, where it is carried to the anterior pituitary. In the pituitary, CRF acts as a secretagogue and stimulates the release of adrenocorticotropic hormone (ACTH) into the general circulation, where it travels to the adrenal cortex to stimulate the secretion of glucocorticoids and other adrenal steroids. Blood-borne glucocorticoids, such as cortisol and corticosterone, perform a variety of functions. In particular, these hormones promote the reallocation of energy resources to deal appropriately with a potential threat, including the redirection of glucose from adipose to muscle tissue, heightened vigilance to contextual stimuli and a suppression of motivated and neurovegetative behaviors (Pecoraro et al. 2006). Under acute conditions, these effects of glucocorticoids can be beneficial to survival of the organism; however, long-term hypersecretion of glucocorticoids can lead to a plethora of psychological, metabolic, cardiovascular and immune dysfunctions (McEwen 2005; Pecoraro et al. 2006). Thus, regulation of the HPA axis is extremely important for maintaining optimal functioning of an organism.

In the short term, glucocorticoids regulate their own secretion through several, distinct negative feedback processes (Dallman et al. 1994). Within adrenal, pituitary, hypothalamic and extrahypothalamic tissues, glucocorticoids act to inhibit their own release and/or synthesis. Within the hypothalamus, this negative feedback process can take the form of both fast feedback and delayed feedback. Glucocorticoid fast feedback occurs very rapidly within the PVN, where glucocorticoids appear to act through a nongenomic mechanism to inhibit glutamate release onto CRF neurosecretory cells (Dallman et al. 1994; Di et al. 2003). Delayed feedback elicited by glucocorticoids in the PVN occurs through a genomic mechanism in which glucocorticoids act to inhibit transcription of CRF (Dallman et al. 1994; Schulkin et al. 1998).

In the long term, adaptation to chronic stress is essential to prevent the detrimental effects of glucocorticoid hypersecretion. If an organism is exposed to the same stressor multiple times, habituation will occur even if the stressor is aversive (see Armario 2006). For example, physical restraint is an aversive, psychological stressor that will elicit a robust activation of the HPA axis during initial exposure in rats. However, following repeated restraint sessions, the magnitude of the HPA axis activation response is significantly reduced (Cole et al. 2000; Jaferi and Bhatnagar 2006; Viau and Sawchenko 2002). This plasticity of the HPA axis response is likely mediated by alterations in neuronal activity within extrahypothalamic limbic structures which regulate activation of CRF neurosecretory cells in the PVN, as lesions of specific neuroanatomical sites within the stress neural circuit can abrogate the development of habituation (Bhatnagar et al. 2002; Carter et al. 2004). In line with this hypothesis, activation of the neural stress circuit, as indicated via induction of immediate early genes in response to neuronal depolarization, declines in a similar fashion to adrenocortical secretion following repeated exposure to a common stressor (Melia et al. 1994; Patel et al. 2005; Viau and Sawchenko 2002; Watanabe et al. 1994). The flexibility of an organism to respond to disturbances of homeostasis in the short term, but to adapt to long-term exposure, is a process that is

critical to survival in a constantly changing environment. The importance of adaptation to stress in humans is emphasized by the association between maladaptive responses to stress and the development of neuropsychiatric disorders such as depression or post-traumatic stress disorder (Korte et al. 2005).

2 The Endocannabinoid System

Cannabis has been used recreationally for centuries by various cultures around the world, in part due to its relaxing and stress-alleviating properties. The psychoactive constituent of cannabis that is predominately responsible for eliciting most of these emotional alterations is Δ^9-tetrahydrocannabinol (THC; Isbell et al. 1967). The actions of THC are mediated by its ability to interact with specific cannabinoid receptors throughout the brain and periphery. Two receptors have been characterized to date. The CB_1 cannabinoid receptor is the predominant central cannabinoid receptor and exhibits widespread distribution in the brain (Herkenham et al. 1991; Moldrich and Wenger 2000; Tsou et al. 1998), and, at lower expression levels, in peripheral tissue, such as blood vessels, immune cells and reproductive tissues (Gorzalka and Hill 2006; Hillard 2000; Parolaro 1999). The CB_2 cannabinoid receptor is located mainly in cells of the circulating immune system, such as macrophages (Munro et al. 1993; Parolaro 1999), as well as resident immune cells, including microglia (Cabral and Marciano-Cabral 2005; Carrier et al. 2004). Recent evidence suggests that the CB_2 receptor may also be expressed by neurons in some species (Gong et al. 2006; Van Sickle et al. 2005). Both cannabinoid receptors are G-protein-coupled receptors; these receptors activate $G\alpha_{i/o}$ proteins resulting in inhibition of adenylyl cyclase activity and inhibition of calcium channel activation by depolarization (Felder and Glass 1998; Howlett and Mukhopadhyay 2000; Piomelli 2003). The CB_1 receptor is present at high densities on presynaptic axon terminals, where it functions to inhibit neurotransmitter release (Schlicker and Kathmann 2001; Vaughan and Christie 2005). The CB_1 receptor is expressed by sub-populations of glutamate, gamma-aminobutyric acid (GABA), acetylcholine, serotonin and noradrenergic neurons (Nakazi et al. 2000; Ohno-Shosaku et al. 2001; Schlicker and Kathmann 2001), indicating that cannabinoids possess the ability to suppress release of many neurotransmitters and neuromodulators. This distribution often complicates the interpretation of in vivo pharmacological data. In addition to these known targets for cannabinoid agents, there is also increasing, but not definitive, evidence that cannabinoids may also exhibit affinity for other receptor subtypes, such as vanilloid receptors (TRPV1; Ross 2003), peroxisome-proliferator-activated receptors (PPAR; Sun et al. 2006) and non-CB_1/CB_2 G-protein coupled receptors, such as GPR55 (Ryberg et al. 2007).

The endogenous ligands of the cannabinoid receptors have been called ECBs. The two primary molecules that have been functionally identified as ECBs are the arachidonate-derived lipophilic molecules *N*-arachidonoylethanolamine (anandamide; AEA; Devane et al. 1992) and 2-arachidonoylglycerol (2AG; Sugiura et al.

1995). Several other lipid molecules, including virodhamine (Porter et al. 2002), noladin ether (Hanus et al. 2001) and *N*-arachidonoyldopamine (Bisogno et al. 2000) have been identified as putative ECB ligands; however, far less is known about these compounds than is known about AEA and 2AG. The ECBs are not typical neurotransmitters but are synthesized post-synaptically by activity-dependent cleavage of phospholipid head-groups by activation of specific enzymes. The ECB molecules are not released vesicularly; instead, these molecules are thought to be synthesized "on demand" in response to increased neuronal excitation and/or increased intracellular calcium. The ECBs are released into the synapse, where they act in a retrograde manner to activate the presynaptically located CB_1 receptor and inhibit neurotransmitter release (Bisogno et al. 2005; Schlicker and Kathmann 2001; Wilson and Nicoll 2002). Regulation of ECB content is also maintained by degradative enzymes such as fatty acid amide hydrolase (FAAH), which is the primary enzyme class capable of AEA hydrolysis, and monoacylglyceride lipase (MGL), which is the primary, but not exclusive, catabolic enzyme for 2AG (Deutsch et al. 2002; Dinh et al. 2002; Ueda 2002). A more detailed examination of the biochemical and pharmacological properties of the ECB system can be found in earlier chapters in this book.

3 Endocannabinoid-Mediated Regulation of the HPA Axis

3.1 Endocannabinoid Signaling within the HPA Axis

As cannabis is known typically to evoke stress-reducing and relaxing effects, it is not surprising that increasing evidence has indicated that the ECB system may contribute to regulation of the HPA axis. In terms of functional expression, all the major players of the ECB system are widely distributed throughout the central stress circuit as well as within peripheral endocrine tissue. Within the brain, the CB_1 receptor and both ECB ligands are found throughout all of the extrahypothalamic sites that regulate PVN neuronal activation, such as the hippocampus, prefrontal cortex, amygdala, bed nucleus of the stria terminalis and midbrain monoaminergic nuclei such as the locus coeruleus and dorsal raphe (Bisogno et al. 1999; Cadas et al. 1997; Herkenham et al. 1991; Tsou et al. 1998). Given that the ECB system modulates synaptic transmission via effects on neurotransmitter release (Freund et al. 2003), it is well-suited to regulate neuronal activation in stress-sensitive anatomical circuits.

The ECB system is strategically located both externally at sites regulating the HPA axis as well as internally throughout the HPA axis in a fashion conducive to an integral regulator. Particularly, the CB_1 receptor has been characterized on glutamatergic afferents within the PVN, and activation of this receptor attenuates excitatory activation of CRF neurosecretory cells (as well as oxytocinergic and

vasopressinergic; Di et al. 2003). Microdialysis studies have revealed that antagonism of the CB_1 receptor in the PVN increases excitatory amino acid release and subsequent activation of neuropeptidergic cells (Succu et al. 2006). Thus, as well as regulating activity within extrahypothalamic structures that regulate the PVN, ECB signaling within the PVN proper can also potentially modulate activation of the HPA axis.

Within the periphery, both the CB_1 receptor and ECB ligands have been identified within the pituitary gland (Gonzalez et al. 1999; Pagotto et al. 2001; Wenger et al. 1999). Co-expression analysis in human pituitaries has identified that CB_1 receptor mRNA is only seen in the anterior lobe of the pituitary, and particularly within cells synthesizing ACTH, GH or prolactin (Pagotto et al. 2001). Finally, at the termination point of the HPA axis, CB_1 receptor expression has been documented within the adrenal gland (Galiegue et al. 1995). Collectively, these data indicate that the ECB system is present in all the major structures integrated into the HPA axis, making it an ideal candidate for regulating stress responsivity.

3.2 Endocannabinoid Signaling Inhibits HPA Axis Activity

Genetic and pharmacological studies have revealed a critical role of ECB signaling as a negative regulator of the HPA axis. Under non-stress conditions, deletion of the CB_1 receptor results in an increase in adrenocortical secretion, particularly during the daily peak of the circadian rhythm (Barna et al. 2004; Cota et al. 2007; Haller et al. 2004; Steiner et al. 2008). Similarly, those transgenic animals lacking the CB_1 receptor also exhibit an increase in the expression of CRF within the PVN under basal conditions (Cota et al. 2003, 2007). It should be noted, however, that there are also reports of either no change or even a reduction in basal corticosterone (Aso et al. 2008; Fride et al. 2005; Uriguen et al. 2004; Wade et al. 2006). These findings do appear to be strain-specific, and are likely a compensatory response that occurs in some strains, as pharmacological studies in mice and rats have supported the hypothesis that ECB activity suppresses basal HPA axis activity. Specifically, both acute and chronic administration of CB_1 receptor antagonists have reliably been found to elevate basal levels of ACTH and corticosterone (Doyon et al. 2006; Hill et al. 2007; Lamota et al. 2008; Manzaneres et al. 1999; Patel et al. 2004; Steiner et al. 2008; Wade et al. 2006). These data would suggest that ECB signaling dampens HPA axis activity under non-stress conditions.

While somewhat equivocal outcomes occur under basal conditions, under stress conditions the ECB system appears consistently to constrain activation of the HPA axis. Deletion of the CB_1 receptor results in a robust increase in corticosterone and/or ACTH secretion in response to restraint stress (Uriguen et al. 2004), tail suspension stress (Aso et al. 2008), forced swim stress (Steiner et al. 2008) and novelty stress (Barna et al. 2004; Haller et al. 2004), but not audiogenic stress (Fride

et al. 2005). Similarly, pharmacological antagonism of the CB_1 receptor potentiates stress-induced glucocorticoid secretion and neuronal activation within the PVN (Evanson et al. 2007; Ginsberg et al. 2006; Patel et al. 2004; Steiner et al. 2008). Similarly, antagonism of the CB_1 receptor is capable of reversing the ability of chronic antidepressant treatment to dampen stress-induced neuronal activation within the PVN and corticosterone secretion (Hill et al. 2006). Consistent with the hypothesis that ECB signaling constrains stress-induced activation of the HPA axis, pharmacological inhibition of ECB uptake or FAAH activity attenuates corticosterone secretion in response to restraint stress (Patel et al. 2004). It should be noted that administration of the ECB AEA has been found to activate the HPA axis (Hao et al. 2000; Weidenfeld et al. 1994; Wenger et al. 1997, 2003); however, this effect is not mediated by the CB_1 receptor (Wenger et al. 1997, 2003), but is likely mediated by the rapid metabolism of AEA by FAAH and subsequent synthesis of prostaglandins, which themselves are potent activators of the HPA axis (Malcher-Lopes et al. 2008), and accordingly cannot be assumed to represent an ECB effect.

Despite some discrepancies, most of which appear to be a function of methodological issues, the bulk of current evidence argues that ECB signaling negatively regulates HPA axis activity, both under basal conditions and following exposure to acute stress.

3.3 At What Sites of Action Does Endocannabinoid Signaling Modulate HPA Axis Activity?

Through a collection of lesion and immediate early gene studies, several critical brain structures have been highlighted as exerting regulation of the HPA axis (Herman et al. 2003, 2005). Thus, it is possible that the inhibitory effects of ECB signaling are due not to local actions within the HPA axis, but to modulation of neuronal circuits which subserve activation of the HPA axis. Given the inhibitory nature of ECB neurotransmission, these effects would likely be localized to structures which promote HPA axis activation, and not to those which inhibit it.

The prefrontal cortex is an important site for coordinating incoming stimuli and behavioral/visceral responses. Lesion studies have highlighted that dorsal regions of the prefrontal cortex (such as the anterior cingulate cortex and prelimbic cortex) function to inhibit HPA axis activation, while ventral regions of the prefrontal cortex (such as the infralimbic cortex) appear to facilitate HPA axis activity (Diorio et al. 1993; Radley et al. 2006). We have recently examined the effects of a local infusion of a CB_1 receptor agonist or antagonist into the infralimbic cortex on stress-induced adrenocortical secretion (Hill et al. 2009a). Neither of these manipulations modulated basal glucocorticoid levels or the increase in plasma corticosterone following restraint stress (Hill et al. 2009a), suggesting that ECB signaling in the prefrontal cortex is not relevant for regulation of the HPA axis.

The amygdala is one of the main limbic structures which promotes HPA axis activity (Herman et al. 2003, 2005). Incoming sensory stimuli activate neurons within the basolateral nucleus of the amygdala (BLA), which in turn activates the central nucleus (CeA) or medial amygdala (MeA), which projects both directly and indirectly to the PVN to modulate HPA axis activation (Herman et al. 2003, 2005). We have recently found that infusion of a CB_1 receptor agonist into the BLA prior to restraint stress significantly attenuated the subsequent increase in plasma corticosterone (Hill et al. 2009a). In line with this, infusion of a CB_1 receptor antagonist into the BLA increased HPA axis output (Hill et al. 2009a). These effects were relatively specific to the BLA, as local administration of CB_1 receptor ligands into either the CeA or the MeA did not exert these effects (Hill et al. 2009a). Thus, it would appear that ECB activity within the amygdala, and particularly the BLA, may act to curb HPA axis activation.

In addition to these effects in the amygdala, there also appears to be a role for local ECB signaling within the PVN to modulate HPA axis activity. As mentioned, CB_1 receptors can attenuate glutamatergic activation of CRF neurosecretory cells in the PVN, likely via an inhibition of glutamate release (Di et al. 2003). This hypothesis is consistent with both the basal increase in CRF in the PVN seen in CB_1 receptor knockout mice (Cota et al. 2003, 2007) and the finding that systemic administration of a CB_1 receptor antagonist increases excitatory amino acid release within the PVN (Succu et al. 2006). As such, it appears quite plausible that ECB signaling directly inhibits HPA axis activation by suppressing incoming excitatory input onto CRF neurons in the PVN.

The presence of ECB activity in the pituitary suggests that this endocrine tissue may also be a locus of action; however, it should be noted that intracerebroventricular administration of a CB_1 receptor antagonist increases HPA axis activity, suggesting that this effect is mediated by a central site of action (Manzaneres et al. 1999). An initial study demonstrated that pituitary function of CB_1 receptor knockout mice is unaltered, in that both basal and CRF-stimulated ACTH secretion were not different between mutant and wild-type mice (Barna et al. 2004). However, a more recent study has revealed that while basal ACTH secretion is unchanged in pituitary cells derived from transgenic mice lacking the CB_1 receptor, both forskolin- and CRF-stimulated ACTH secretion is increased significantly (Cota et al. 2007). It is not clear why these differences exist between these studies, but it does appear that CB_1 signaling within the pituitary may contribute to the inhibitory effects of ECBs on the HPA axis. The same argument cannot be made for the adrenal gland, as no hypertrophy or gross morphological changes have been found within the adrenal glands of mice lacking the CB_1 receptor (Barna et al. 2004; Cota et al. 2007).

Ultimately, the current data would suggest that the ability of ECB signaling to negatively regulate both basal and stress-induced HPA axis activity is mediated through a coordinated suppression of incoming amygdalar input and local excitatory tone within the PVN following activation of the CB_1 receptor in the BLA and PVN, respectively. However, ongoing research is seeking to determine if other neuroanatomical sites are relevant for these effects.

4 Acute Stress-Induced Modulation of Endocannabinoid Signaling: Contributions to Glucocorticoid Negative Feedback

While ECB signaling may attenuate the neuroendocrine responses to stress, the question remains as to whether ECB activity itself is modulated by stress. The first evidence suggesting that the ECB system could be modulated by stress emerged from a series of in vitro studies employing glucocorticoid treatment to hypothalamic tissue. Specifically, bath application of glucocorticoids (such as corticosterone and dexamethasone) to slices of hypothalamic tissue, largely composed of the PVN, resulted in a rapid and significant elevation in both AEA and 2AG tissue contents (Di et al. 2005; Malcher-Lopes et al. 2006). These effects were mediated by the activation of a putative membrane-bound glucocorticoid G-protein coupled receptor that enhanced intracellular cAMP-protein kinase A signaling and resulted in rapid synthesis of ECB ligands (Malcher-Lopes et al. 2006).

These in vitro data lead to the hypothesis that stress, via elevation of glucocorticoids, could result in a rapid induction of ECB signaling. However, the first in vivo study published on this topic actually demonstrated the converse. Specifically, Patel and colleagues (2004) exposed male mice to 30 min of restraint stress, after which they were immediately sacrificed and ECB contents were measured in the hypothalamus. 2AG content was found to be significantly reduced, while AEA content was not modified by stress (Patel et al. 2004). This group reported subsequently that 2AG levels did not change in the forebrain, amygdala, cerebellum (Patel et al. 2005; Rademacher et al. 2008), hippocampus (S. Patel and C.J. Hillard, unpublished findings), ventral striatum or medial prefrontal cortex (mPFC; Rademacher et al. 2008) of mice exposed to 30 min restraint stress. Intriguingly, preliminary data from our laboratory has found that, unlike mice, in rats 30 min of restraint stress resulted in a significant increase in 2AG content in both the mPFC and the hypothalamus, but not the amygdala (Hill et al. 2009a). While this finding does support the hypothesis that stress-induced glucocorticoid secretion may induce ECB synthesis, these increases in 2AG did not correlate with the magnitude of the adrenocortical response to stress (Hill et al. 2009a). Similarly, a second study employing rats has demonstrated an immediate and transient increase in 2AG content in the periaqueductal gray (PAG) of rats as early as 2 min following exposure of the rats to foot shock stress (Hohmann et al. 2005). Collectively, these data do illustrate the potential for dramatic species differences in the effects of stress and/or glucocorticoids on 2AG synthesis (increase in rats, decrease in mice), especially since all of the in vitro studies examining glucocorticoid-induced ECB synthesis have been performed in tissue harvested from rats and not mice (Di et al. 2003, 2005; Malcher-Lopes et al. 2006). This species difference is not unprecedented. Restraint stress has previously been shown to have opposite effects on neurogenesis in mice and rats (Bain et al. 2004) and other stressors have been shown to have opposite behavioral effects in mice and rats (De Catanzaro and Gorzalka 1979). With regards to relevance to humans, it should be noted that we

have recently determined that circulating 2AG levels rapidly elevate in response to stress (Hill et al. 2009b), similar to what is seen in central tissue in rats.

On the other hand, exposure of mice to restraint stress resulted in significant reductions in amygdalar (Patel et al. 2005; Rademacher et al. 2008) and hippocampal (S. Patel and C.J. Hillard, unpublished findings) AEA contents, while AEA content in the forebrain, cerebellum, ventral striatum and mPFC did not change (Patel et al. 2005; Rademacher et al. 2008). Unlike the discrepancy that was seen with 2AG, we have recently found a significant reduction in amygdalar, but not prefrontal cortical or hypothalamic, AEA content following 30 min restraint stress in rats (Hill et al. 2009a). Similarly, hippocampal AEA content is reduced following acute administration of corticosterone to male rats (Hill et al. 2008). Thus, while stress and/or glucocorticoids appear to have differential effects on 2AG synthesis in mice and rats, in both species a reliable reduction in AEA content, specifically in the amygdala and hippocampus, is seen following these treatments. The effects of acute stress and/or glucocorticoid treatment on ECB content can be seen in Table 1.

As discussed previously, ECB signaling constrains activation of the HPA axis, and has been hypothesized to function as a stress-recovery system (Di Marzo et al.

Table 1 The effects of acute stress (or glucocorticoid treatment) on the tissue content of the endocannabinoid ligands anandamide (AEA) and 2-arachidonylglycerol (2AG) in discrete brain regions

Species/strain	Stressor	Brain region	AEA	2AG	Reference
ICR mice	30 min restraint stress	Hypothalamus	NC	−	Patel et al. (2004)
ICR mice	30 min restrain stress	Forebrain	NC	NC	Patel et al. (2005)
		Amygdala	−	NC	
		Cerebellum	NC	NC	
ICR mice	30 min restraint stress	Hippocampus	−	NC	Patel and Hillard, unpublished data
ICR mice	30 min restraint stress	Prefrontal cortex	NC	NC	Rademacher et al. (2008)
		Amygdala	−	NC	
		Ventral striatum	NC	NC	
Sprague–Dawley rats	30 min restraint stress	Prefrontal cortex	NC	+	Hill et al. (2009a)
		Amygdala	−	NC	
		Hypothalamus	NC	+	
Sprague–Dawley rats	3 min foot shock	Periaqueductal gray (PAG)	+	+	Hohmann et al. (2005)
		Occipital cortex	NC	NC	
Long Evans rats	Single injection corticosterone (20 mg kg^{-1})	Hippocampus	−	NC	Hill et al. (2008)

NC = no change; − = significant reduction; + = significant increase

1998; Gorzalka et al. 2008). With regards to data obtained from rats, this hypothesis seems plausible given that hypothalamic tissue from rats exhibits an increase in 2AG synthesis following glucocorticoid exposure (Di et al. 2005; Malcher-Lopes et al. 2006), and in vivo rats exposed to 30 min restraint exhibit increased hypothalamic levels of 2AG (Hill et al. 2009a). Accordingly, it can be predicted that stress-induced glucocorticoid secretion may induce 2AG synthesis within the PVN, in which 2AG binding to presynaptic CB_1 receptors will reduce the excitatory drive on CRF neurosecretory cells and aid in shutting down the HPA axis. However, in mice the biochemical studies discussed above indicate that hypothalamic ECBs are reduced following stress, not increased (Patel et al. 2004). Given that pharmacological modulation of the ECB system exhibits comparable regulation of the HPA axis in both mice and rats, to reconcile this apparent discrepancy in hypothalamic 2AG responses to stress, a "gatekeeper" hypothesis has been proposed (Patel et al. 2004). According to this hypothesis, ECB content within the PVN of the hypothalamus is high during the basal (non-stressed) state, resulting in a tonic inhibition of excitatory inputs to the HPA axis. Upon exposure to stress, 2AG levels rapidly decline, through an undetermined mechanism, resulting in a disinhibition of glutamatergic projections to the PVN and allowing activation of the HPA axis (Patel et al. 2004). If ECB levels are maintained at a high level (through administration of an ECB uptake inhibitor, for example [Patel et al. 2004]) then this inhibition is maintained and HPA axis activation is attenuated. If this inhibition is disrupted by the administration of a CB_1 receptor antagonist or genetic deletion of the CB_1 receptor, then an exaggerated activation of the HPA axis occurs. The fact that the CB_1 receptor appears to constrain HPA axis activation under non-stress conditions in mice (Cota et al. 2007; Patel et al. 2004) attests to the "gatekeeper" theory of ECB regulation of the HPA axis. The same argument can theoretically be proposed for ECB signaling within the amygdala. As discussed, activation of amygdalar CB_1 receptors can inhibit activation of the HPA axis and disruption of amygdalar CB_1 receptor activity can promote HPA axis function (Hill et al. 2009a). Across both rats and mice, stress evoked a reduction in amygdalar AEA content; in rats, this reduction has been found to negatively correlate with the magnitude of the adrenocortical response, such that larger reductions in amygdalar AEA equated to larger increases in plasma corticosterone (Hill et al. 2009a). Thus, AEA/CB_1 receptor coupling in the amygdala, particularly the BLA, may provide a similar "gatekeeper" function as described for 2AG within the mouse hypothalamus (Patel et al. 2004). Taken together, the available data indicate that the ECB/CB_1 receptor signaling within the PVN of the hypothalamus, and possibly the amygdala, is likely a critical player in the regulation of the HPA axis under conditions of acute stress. While the mechanism by which ECBs fine tune HPA axis activity may slightly differ between rats and mice, the end result appears to be the same.

In addition to these hypotheses, it has also been suggested that ECB signaling may modify HPA axis activity through a modulation of glucocorticoid-mediated negative feedback. The in vitro studies examining the effects of glucocorticoid activity on ECBs revealed that the induction of ECB synthesis mediated glucocorticoid-suppression of PVN neuronal activation, or "glucocorticoid fast-feedback"

(Di et al. 2003). Accordingly, ECBs provided the missing link through which glucocorticoids rapidly suppressed CRF neural activity to inhibit subsequent activation of the HPA axis (Di et al. 2003). This phenomenon appears to be specific to the PVN, however, as deletion of the CB_1 receptor does not impair glucocorticoid-mediated suppression of ACTH secretion from pituitary cells (Barna et al. 2004), nor does it affect CRF expression in other brain regions such as the amygdala and prefrontal cortex (Cota et al. 2007). In line with a hypothetical role of ECBs in glucocorticoid feedback, in vivo glucocorticoid administration prior to stress induction can attenuate ACTH secretion, a phenomenon which has been found to be sensitive to genetic or pharmacological blockade of the CB_1 receptor (Cota et al. 2007; Evanson et al. 2007). Thus, the hyperactivity of the HPA axis that occurs in response to disruption of CB_1 receptor signaling may occur via a deficiency in glucocorticoid feedback that promotes a state of glucocorticoid hypersecretion.

5 Chronic Stress-Induced Regulation of Endocannabinoid Signaling: A Driving Force for Stress Habituation

The picture to emerge thus far indicates that stress modulates ECB signaling in limbic structures, which in turn appears to contribute to the activation, and subsequent suppression, of the HPA axis. Habituation to stress is a progressive reduction in neuronal and neuroendocrine responses to exposure to an aversive stimulus, such as restraint stress (Bhatnagar et al. 2002; Cole et al. 2000; Patel et al. 2005; Viau and Sawchenko 2002). Given the neuroanatomical distribution of the ECB system, as well as its robust ability to modulate HPA axis activation under conditions of acute stress, it is reasonable to hypothesize that the ECB system may be important for regulation of the HPA axis under conditions of chronic stress.

The effects of repeated, homotypic stress exposure on the ECB system has been examined in male mice exposed to 30 min of restraint sessions for 5–10 consecutive days. Repeated exposure to homotypic stress does not change CB_1 receptor density (Rademacher et al. 2008). Repeated restraint stress has no effect on hypothalamic AEA content, but 2AG content is significantly increased compared to a control condition and compared to a single restraint exposure (Patel et al. 2004). 2AG content is also significantly elevated in both the amygdala and limbic forebrain as a whole, and the mPFC in particular, following consecutive stress exposures (Patel et al. 2005; Rademacher et al. 2008). Similar to what was seen under acute stress conditions, AEA content in the amygdala is significantly reduced following 5, 7 or 10 days of repeated stress, as is AEA content in the mPFC following 7 or 10 days of repeated restraint (Patel et al. 2005; Rademacher et al. 2008). However, AEA content is not changed if the tissue piece includes the entire limbic forebrain, demonstrating the AEA changes within forebrain structures may be limited to specific structures such as the mPFC (Patel et al. 2005). Thus, repeated homotypic stress is associated with an increase in limbic 2AG/CB_1 receptor signaling and a concomitant reduction in AEA/CB_1 receptor coupling.

Differences in the synaptic functions of AEA and 2AG have been suggested; one hypothesis being that 2AG/CB_1 receptor activity represents a rapid, burst-like signal while AEA/CB_1 receptor coupling may provide more of a tonic level of CB_1 receptor activation (Gorzalka et al. 2008). In line with this hypothesis, it can be predicted that repeated restraint stress is associated with a reduction in the tone of limbic ECB signaling, but an increase in the rapid, intense signal. This increase in 2AG may be relevant for the suppression of neuronal activation within the stress circuit during the development of habituation; there are several lines of evidence to support this hypothesis. First, acute treatment with a CB_1 receptor antagonist to mice repeatedly exposed to restraint stress reverses the habituation of stress-induced neuronal activation within limbic structures (as assessed by immediate early gene induction; Patel et al. 2005). Similarly, administration of a CB_1 receptor antagonist, either on the final day of stress or daily throughout the stress exposure, to rats repeatedly exposed to restraint stress reversed neuroendocrine habituation to stress (Hill et al. 2007). Second, 2AG signaling is known to suppress excitatory neurotransmission (Uchigashima et al. 2007; Yoshida et al. 2006); thus a repeated stress-induced increase in 2AG release may function to attenuate excitatory activation of the neuronal stress circuit, thus contributing to the manifestation of stress habituation. Third, we have found that potentiation of AEA/CB_1 signaling (via pharmacological inhibition of FAAH) does not modulate habituation to stress (Hill et al. 2007), indicating that 2AG is likely the signaling molecule at the CB_1 receptor driving the habituation response. Fourth, ECB signaling promotes other forms of habituation to aversive stimuli, such as behavioral inhibition in response to shock (Kamprath et al. 2006) and audiogenic stress (Fride et al. 2005). Taken together, these data collectively argue that ECB signaling is recruited under conditions of repeated stress to suppress neuronal activation in stress circuits, thereby facilitating cellular, neuroendocrine and behavioral adaptations to stress.

6 Conclusion

The aim of the current chapter was to review the current literature examining the interactions between ECBs and the HPA axis. The evidence that has been gathered to date strongly argues for an inhibitory role of ECB signaling in regulating HPA axis activity. Under basal conditions, ECB signaling appears to be a driving force in the maintenance of low HPA axis activity, as disruption of CB_1 receptor activity results in basal hyperactivity of the HPA axis. Under conditions of acute stress, ECB signaling likewise appears to constrain activation of the HPA axis, possibly both via distal regulation of incoming amygdalar inputs and local regulation of excitatory input to CRF neurosecretory cells in the PVN. ECB neurotransmission is, in turn, modulated by stress, possibly acting as either a “gatekeeper” of the HPA axis, or a recovery system aimed at limiting HPA axis activity. Consistently, pharmacological enhancement of ECB signaling attenuates stress-induced HPA axis activity while impairment in CB_1 receptor signaling results in an exaggerated

cellular and neuroendocrine response to stress. Additionally, under conditions of repeated stress, a progressive increase in limbic 2AG/CB_1 receptor signaling contributes to the development and expression of neuroendocrine habituation.

Ultimately, these data demonstrate that the ECB system is likely an integral player in the neuronal response and plasticity to stress. The relevance of this relationship has not been fully explored with respect to both normal homeostasis and pathological states characterized by alterations in HPA axis function, but will be a focus of future research.

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