REVIEW

NEURONAL POPULATIONS MEDIATING THE EFFECTS OF ENDOCANNABINOIDS ON STRESS AND EMOTIONALITY

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Abstract—An adequate emotional response to stress is essential for survival and requires the fine-tuned regulation of several distinct neuronal circuits. Therefore, a precise control of these circuits is necessary to prevent behavioral imbalances. During the last decade, numerous investigations have evidenced that the endocannabinoid (eCB) system is able to crucially control stress coping. Its central component, the cannabinoid type 1 receptor (CB1 receptor), is located at the presynapse, where it is able to attenuate neurotransmitter release after its activation by postsynaptically produced and released eCBs. To date, the eCB system has been found to control the neurotransmitter release from several neuron populations (e.g. GABA, glutamate, catecholamines and monoamines), suggesting a general mechanism for tuning neuronal activity, and thereby regulating emotion and stress responses. In this review, we aim at summarizing the anatomical and functional relation of the eCB system to an adequate response to stressful situations. Of special interest will be neuronal connections to the hypothalamic-pituitary-adrenal axis, but also circuits between cortical structures, such as prefrontal cortex, amygdala and hippocampus, and subcortical regions, such as raphe nuclei and locus coeruleus. We further like to step toward allocating eCB system functions to distinct cellular subpopulations in the brain. It has emerged that the eCB system is spatially well defined, and its detailed knowledge is a prerequisite for understanding the eCB system in the context of controlling behavior. Thus, advanced approaches combining different genetic and pharmacological tools to dissect specific eCB system functions are of particular interest.

This article is part of a Special Issue entitled: Stress, Emotional Behavior and the Endocannabinoid System. © 2011 IBRO. Published by Elsevier Ltd. All rights reserved.

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Key words: cannabinoid receptor, endocannabinoid, stress, mouse genetics, adeno-associated virus, Cre/loxP system.

Contents

ENDOCANNABINOIDS, EMOTION AND STRESS

Emotionality describes a highly complex behavior in response to various environmental stimuli. An appropriate emotional outcome requires fine-tuned neurotransmitter release processes and functional neuronal circuits. Therefore, prevention of an imbalanced signaling is highly important, especially in stressful situations. One of the endogenous control mechanisms is constituted by the endocannabinoid (eCB) system, which is named according to its sensitivity to Δ^9-tetrahydrocannabinol (THC), the main psychoactive compound of *Cannabis sativa*. Since the cannabinoid type 1 receptor (CB1 receptor) has been discovered (Matsuda et al., 1990), the members of the eCB system have steadily increased, comprising different ligands, synthesizing and degrading enzymes as well as other cannabinoid receptors (Petrosino and Di Marzo, 2010). There are two major endogenous ligands (named endocannabinoids; eCBs), *N*-arachidonoyl ethanolamine (anandamide, AEA) (Devane et al., 1992) and 2-arachidonoyl glycerol (2-AG) (Sugiura et al., 1995). Unlike "classical" neurotransmitters, the eCBs are not stored in vesicles at the presynapse, but are synthesized from lipid membrane precursor molecules on demand by Ca^{2+} dependent and independent mechanisms in the postsynapse (Kano et al., 2009). The ligands travel by a still unknown

mechanism retrogradely across the synaptic cleft to the presynaptically located CB1 receptor. Activation of the CB1 receptor induces the major feature of the eCB system, namely the inhibition of neurotransmitter release by modulation of several ion channels and kinases (Kano et al., 2009; Turu and Hunyady, 2010). Cannabinergic signaling is limited by a still poorly defined uptake process and rather well-characterized intracellular hydrolysis by fatty acid amide hydrolase (FAAH) for AEA, monoacylglycerol lipase (MAGL), and serine hydrolase α,β-hydrolase domain 6 (ABHD6) for 2-AG (Kano et al., 2009; Marrs et al., 2010). Experimental evidence has suggested that the uptake process is mediated by a transporter mechanism (Hillard et al., 1997). In fact, a truncated FAAH protein lacking the amidase activity has recently been identified as an AEA transporter (Fu et al., 2011). Interestingly, the degrading enzymes for the two major eCBs display distinct subcellular and synaptic localization, suggesting different signaling properties for AEA and 2-AG (Cristino et al., 2008; Kano et al., 2009). Although FAAH is mostly found in the postsynapse, MAGL is primarily colocalized with the CB1 receptor in the presynaptic structure (Egertova et al., 2003; Gulyas et al., 2004; Kano et al., 2009; Keimpema et al., 2010). Moreover, differential functions of AEA and 2-AG in the modulation of neuronal transmission processes have recently been described in the bed nucleus of the stria terminalis (BNST) (Puente et al., 2011). To date, there is clear evidence for other receptors in the CNS that are modulated by eCBs, such as the transient receptor potential cation channel vanilloid type 1 (TRPV1) (Chávez et al., 2010; De Petrocellis and Di Marzo, 2010), G protein-coupled receptor 55 (GPR55) (Baker et al., 2006; Nevalainen and Irving, 2010), peroxisome proliferator-activated receptor (PPAR) family of nuclear receptors (O'Sullivan and Kendall, 2010), and GABA$_A$ receptor (Sigel et al., 2011), making the understanding of distinct eCB system functions even more difficult. The existence of the cannabinoid type 2 receptor (CB2 receptor) in brain tissue has been under discussion for the last years. Accumulating evidence has shown the presence and physiological importance of the CB2 receptor in the CNS (Onaivi et al., 2006; García-Gutiérrez et al., 2010). In the present review, we will focus on CB1 receptor-related functions. Nevertheless, the role of the CB2 receptor as well as the degrading enzymes FAAH and MAGL will also be discussed.

Taken together, the eCB system with its property of modulating neurotransmission is an interesting candidate to control behavior (Kano et al., 2009). In fact, various pharmacological and genetic studies show a variety of behavioral responses, in particular changes in mood and emotionality (Lutz, 2009; Hill and McEwen, 2010; Moreira and Wotjak, 2010). Interestingly, in animal models, cannabinergic drugs have been shown to possess biphasic effects depending on the dose (Kathuria et al., 2003; Gobbi et al., 2005; Hill and Gorzalka, 2005; Viveros et al., 2005; Bambico et al., 2007). This is in accordance with effects in humans, where opposite (depressive or euphoric) experiences after cannabis use were reported (Fusar-Poli et al., 2009).

However, ubiquitous pharmacological and genetic approaches might not be sufficient to precisely dissect the mechanisms underlying this biphasic phenomenon. It is our belief that the CB1 receptor, but also the other components of eCB system, on distinct neuronal populations is responsible for these opposing effects. Hence, a general activation or inhibition of the eCB system might shade specific effects. In addition, some neuronal populations might be differently affected by lower or higher availability of cannabinoids, which is based on an unequal sensitivity and/or availability of the eCB receptors and/or their respective signaling cascades.

A major drawback of a pharmacological approach is the lack of cellular specificity of the applied drug. Similarly, insufficient for the detailed functional analysis of the eCB system is a ubiquitous genetic deletion of a respective eCB system component, as distinct effects might be shaded. Thus, a more local and cell type-specific understanding of the eCB system is necessary to pinpoint particular eCB related effects. To target this problem, more complex approaches combining different state-of-the-art genetic and pharmacological tools are required. In fact, recent publications on mice lacking the CB1 receptor only in GABAergic or glutamatergic neurons do show opposite responses to stressful situations, which was only partially seen in mice with ubiquitous deletion (Lafenêtre et al., 2009; Jacob et al., 2009; Häring et al., 2011). Also viral gene delivery systems combined together with transgenic animals seem to be a promising strategy (Guggenhuber et al., 2010).

NEURONAL CIRCUITS INVOLVED IN STRESS AND EMOTION

The hypothalamic-pituitary-adrenal (HPA) axis is the major circuit involved in the response to a stressful situation (Ulrich-Lai and Herman, 2009; Hill and McEwen, 2010). Upon exposure to stressful stimuli, neurons of the hypothalamic paraventricular nucleus (PVN) secrete corticotropin-releasing hormone (CRH) into the portal vessels of the median eminence. In the pituitary, CRH initiates the secretion of adrenocorticotropic hormone (ACTH), which in turn induces the synthesis and release of glucocorticoid hormones (corticosterone in mice and rats and cortisol in humans) in the inner adrenal cortex into the bloodstream. Besides a fast mobilization of stored energy, released glucocorticoids inhibit HPA axis activity by feedback mechanisms (Steiner et al., 2008a; Ulrich-Lai and Herman, 2009; Hill et al., 2010a).

Despite of its central role, the HPA axis is only appropriately operational in connection with additional networks spanning from brainstem nuclei to specific limbic system structures (Fig. 1). How specific regulatory networks control glucocorticoid release in response to stress is influenced by a number of factors, such as different stressor types (reactive vs. anticipatory stressor, physical vs. psychological stressor) (Dedovic et al., 2009). These brain regions execute their regulatory functions on HPA axis activity by targeting the PVN of the hypothalamus (Herman et al., 2003; Jankord and Herman, 2008). Brainstem struc-