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Review

Brain endocannabinoid signaling exhibits remarkable complexity

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ABSTRACT

The endocannabinoid (eCB) signaling system is one of the most extensive of the mammalian brain. Despite the involvement of only few specific ligands and receptors, the system encompasses a vast diversity of triggered mechanisms and driven effects. It mediates a wide range of phenomena, including the regulation of transmitter release, neural excitability, synaptic plasticity, impulse spread, long-term neuronal potentiation, neurogenesis, cell death, lineage segregation, cell migration, inflammation, oxidative stress, nociception and the sleep cycle. It is also known to be involved in the processes of learning and memory formation. This extensive scope of action is attained by combining numerous variables. In a properly functioning brain, the correlations of these variables are kept in a strictly controlled balance; however, this balance is disrupted in many pathological conditions. However, while this balance is known to be disrupted by drugs in the case of addicts, the stimuli and mechanisms influencing the neurodegenerating brain remain elusive. This review examines the multiple factors and phenomena affecting the eCB signaling system in the brain. It evaluates techniques of controlling the eCB system to identify the obstacles in their applications and highlights the crucial interdependent variables that may influence biomedical research outcomes.

1. Introduction

The human endocannabinoid system comprises two principal ligands and two key receptors. The major endogenous ligands for CB receptors are two fatty acid derivatives: the eicosanoids N-arachidonoyl-ethanolamide (anandamide) and 2-arachidonoyl glycerol (2-AG). Two endocannabinoid receptors are known: CB1R, which is widely expressed in the brain, with lower levels observed in the peripheral circulation, and CB2R, which is mostly expressed in the peripheral circulation, predominantly in immune-related organs and cells. Both receptors contain seven transmembrane domains and are members of the class A G-protein coupled receptor (GPCR) superfamily.

CB1R has a phylogenetically conserved function, sharing 97–99% amino acid sequence identity with various mammalian species. Other species also demonstrate similar patterns of CB1R distribution in the brain (Matsuda et al., 1990a). Compared to the density of GABA, glutamate or striatal dopamine receptors, CB1 receptors appear to be as abundant in the brain as pivotal neurotransmitter receptors (Herkenham et al., 1990). In the brain, CB1Rs are widespread in the hippocampus, cerebellum, cerebral cortex, basal ganglia, amyglada and sensory motor sectors of the striatum; however, they are sparsely distributed in the brainstem, diencephalon and spinal cord (Herkenham et al., 1991; Glass et al., 1997; Van Waes et al., 2012; Chevaleyre et al., 2006). The activity, quantities and distribution pattern of the CB1 receptors seem to be specific to cell type, synapse type and even microdomain. CB1Rs are mostly distributed presynaptically, with much higher density in GABA-ergic than in glutamatergic neurons (Katona et al., 2006a; Kawamura et al., 2006; Stempel et al., 2016), and are most abundant in the hippocampal GABA-ergic interneurons, on presynaptic axon terminals (Katona et al., 1999). Even though the highest CB1R content is found on the GABA-ergic synapses of hippocampus CCK-positive interneurons, their density varies between distinct types of interneuron, resulting in differential efficacy of synaptic activity control (Katona et al., 2000; Lee et al., 2010a).

In addition, the enzymes involved in endocannabinoid metabolism, such as the AEA-degrading enzyme FAAH (fatty acid amide hydrolase), several AEA synthesizing enzymes, the 2-AG synthesizing enzymes DGL-α and DGL-β (diacylglycerol lipase α/β) and the 2-AG degrading enzyme MAGL (monoacylglycerol lipase), also demonstrate considerable subcellular segregation and varied distribution patterns in the brain (Nyilas et al., 2009; Ludanyi et al., 2011; Gao et al., 2010; Uchigashima et al., 2007; Yoshida et al., 2006; Matyas et al., 2008; Yoshida et al., 2011; Katona et al., 2006a; Gulyas et al., 2004; Uchigashima et al., 2011; Tanimura et al., 2010). In consequence, the localization, quantity and thus the activity of 2-AG, AEA and CBRs are highly controlled and site specific, resulting in a highly-complex arrangement of endocannabinoid signaling outputs. For example, the psychotropic effects of Δ^9-tetrahydrocannabinol (Δ^9-THC), a major

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terpenophenolic compound of marijuana, are mediated by CB1Rs, and its prolonged use leads to CB1R-dependent long-term memory deficits (Ledent et al., 1999; Puighermanal et al., 2009). In addition, CB1R may well play a protective role against age-dependent cognitive decline as the absence of a CB1R receptor induced degeneration of pyramidal neurons and enhanced neuroinflammation in *CNR1* knock out mice: this being the gene encoding CB1R (Albayram et al., 2011).

Cannabinoid signaling involves non-cannabinoid factors that increase CB system complexity. eCBs can non-specifically activate many other receptors. For example, AEA, but not 2-AG, activates type-1 transient receptor potential vanilloid (TRPV1) ion channels that naturally serve to respond to low pH and noxious heat (Tominaga et al., 1998; Di Marzo and De Petrocellis, 2010). However, while only high concentrations of AEA preferentially activate TRPV1, low AEA concentrations activate CB1R (Moreira et al., 2012). Some other non-cannabinoid receptors that are vulnerable to eCB activation include peroxisome proliferator-activated receptors (PPARs), non-selective cation channels such as TRPA1 or TRPM8, ligand-gated ion channels including 5-HT3, metabotropic receptors including GPR55, glycine and nicotinic acetylcholine receptors, voltage-gated ion channels including T-type calcium channels and the TASK potassium channels (Witkamp, 2016; Pertwee et al., 2010). Also some non-cannabinoid ligands can activate CB receptors and these include FAAs, glycerol esters, prostamides or PG esters (Witkamp, 2016).

The present review discusses the known factors forming parts of the vast network of structural and functional dependence in the cannabinoid system of the brain. It involves the activation or inhibition of diverse signaling pathways by cannabinoid and non-cannabinoid ligands and receptors. It highlights the significance of conformational changes within receptors which allow incoming signals to be distinguished, thus inducing various interactions with distinct G-proteins and other endogenous non-G proteins. It outlines known phenomena which influence downstream signaling such as receptor oligomerisation. When emphasizing the immense complexity of the cannabinoid system in the brain, it is important to note two key points: numerous variables and their combinations are specific for a cell type and its condition, and that no two cells are the same, according to the cogent concept of somatic mosaicism (Katona and Freund, 2012; McConnelll et al., 2017; Lodato et al., 2015; Hazen et al., 2016).

2. Astrocytes boost eCB signaling complexity

The human brain comprises approximately 86 billion neurons that generate a dense web with around 100 trillion synapses (Azevedo and Ludmila, 2009). Each synaptic connection is literally a tripartite synapse composed of two neuronal terminals, surrounded by one astrocyte; hence astrocytes are associated with all synapses and participate in information exchange between pre- and postsynaptic neurons. Surprisingly, a single rat hippocampus astrocyte has been found to link with about 140,000 synapses (Oberheim et al., 2012). This phenomenon shows a potential transmission of each neuronal impulse and may explain astrocytic involvement in learning and memory formation.

Although both neurons and astrocytes express CB1Rs on cell surfaces, their CB1R ligands trigger different mechanisms. The electrical impulse stimulating the synapse causes neurotransmitter release from presynaptic neurons and depolarization of the postsynaptic neuron membrane, leading to the release of endocannabinoids to the synaptic cleft, where they stimulate the receptors of presynaptic neurons and astrocytes. In a presynaptic neuron, CB1R agonists trigger mechanisms which cause the inhibition of neurotransmitter release, a process called retrograde signaling (Stempel et al., 2016). There are two critical eCB mediated variants of this phenomenon. The first is depolarisation-induced suppression of inhibition (DSI), which is a retrograde signaling from a strongly depolarised post-synaptic cell which results in a reduction of the release of inhibitory neurotransmitter γ-aminobutyric acid (GABA). The other is depolarisation-induced suppression of excitation (DSE), which involves suppressing excitatory neurotransmitter glutamate (GLU) release from GLU-releasing cells. However, DSE requires longer depolarisation for induction than DSI and is about 30-fold less prominent in the hippocampus, presumably because the CB1Rs on the pyramidal cells have lower expression and are less sensitive. Both DSI and DSE in the hippocampus can be blocked by CB1R antagonists (Ohno-Shosaku et al., 2002). While endocannabinoids inhibit the release of GABA neurotransmitters in GABAergic terminals, they inhibit the release of glutamate in glutamergic neurons (Kano et al., 2009). However, it has been shown that anandamide specifically inhibits glutamate transmission, whereas 2-AG inhibits GABA release in the striatum (Maccarrone et al., 2008). This suggests three possibilities: either the ligands affect the same receptor in different ways, inducing diverse conformational changes resulting in the activation of distinct signaling in the cell, or that CB1R differs structurally in glutamatergic and GABA-ergic synapses, or that CB1R complexes with different receptors in these synapses, thus affecting its functionality (Kano et al., 2009).

The same CB1R agonist released from postsynaptic neurons causes an elevation of intracellular Ca^{2+} in astrocytes surrounding the activated synapse, which stimulates astrocytic glutamate release. The glutamate released from astrocytes activates mGluR1 receptors in presynaptic neurons, which can cause persistent synaptic potentiation of neurotransmitter release (Covelo and Araque, 2016; Navarrete and Araque, 2010). It has been demonstrated that the coincidence of NO release from postsynaptic neurons together with the glutamate released by astrocytes leads to activation of both mGluR1 and PKC in presynaptic neurons, and such coordinated activity induces long-term potentiation (LTP). However, this effect is observed only in synapses located at a distance from the source of eCB release (Covelo and Araque, 2016). eCBs are thought to play a role in the modulation of neuronal signaling by silencing the presynaptic neuron of the synapse following stimulation by the impulse, and by triggering the mechanism used to stimulate lateral quiet synapses by astrocytes. This remote stimulation is facilitated by astrocytic calcium signaling that can spread over large distances, through adjacent astrocytes, leading to a regulation of many lateral synapses (Covelo and Araque, 2016; Navarrete and Araque, 2010). It has been shown that blocking Ca^{2+} elevation in astrocytes prevented endocannabinoid-mediated synaptic potentiation (e-SP) of transmitter release in 100% of cases when BAPTA (Ca^{2+} chelator) was used and in 94% of cases when GDPβS (G protein-mediated intracellular signaling inhibition) was used, compared to 73% in control samples (Navarrete and Araque, 2010). These results indicate that e-SP requires G protein-mediated Ca^{2+} elevation in astrocytes. In addition, neuronal DSE was observed in 40% of cases where BAPTA was used to block Ca^{2+} elevation, and in 37% of cases with GDPβS, versus 27% in control samples. Hence, astrocytic Ca^{2+} elevation appears to have only a slight influence on DSE in neurons or none at all (Navarrete and Araque, 2010).

3. Heterogeneous activity of eCB signaling

Numerous varied systems and mechanisms regulating organism functions are affected by the activity of eCB signaling. Some events induce changes in eCB synthesis. For example, the levels of eCBs, especially 2-AG, are elevated after brain injury, convulsions or stroke (Panikashvili et al., 2001; Wettschureck et al., 2006), suggesting that the system played a remedial function. Inversely, any disruption in the eCB system can result in specific functional disorders. For example, the level of CB1 receptors is decreased in the brains of Huntington Disease patients, due to suppression of *CNR1* transcription, the CB1R gene, by the mutant huntingtin (Van Laere et al., 2010; Blázquez et al., 2011). CB1R protein level and receptor binding efficacy is also decreased in Alzheimer's disease; however, the expression and abundance of CB1R mRNA remain unaffected (Kalifa et al., 2011; Westlake et al., 1994; Lee et al., 2010b). Overexpression of CB1R in the hippocampus protects