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Review

Endocannabinoid Signaling and Synaptic Function

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Endocannabinoids are key modulators of synaptic function. By activating cannabinoid receptors expressed in the central nervous system, these lipid messengers can regulate several neural functions and behaviors. As experimental tools advance, the repertoire of known endocannabinoid-mediated effects at the synapse, and their underlying mechanism, continues to expand. Retrograde signaling is the principal mode by which endocannabinoids mediate short- and long-term forms of plasticity at both excitatory and inhibitory synapses. However, growing evidence suggests that endocannabinoids can also signal in a nonretrograde manner. In addition to mediating synaptic plasticity, the endocannabinoid system is itself subject to plastic changes. Multiple points of interaction with other neuromodulatory and signaling systems have now been identified. In this Review, we focus on new advances in synaptic endocannabinoid signaling in the mammalian brain. The emerging picture not only reinforces endocannabinoids as potent regulators of synaptic function but also reveals that endocannabinoid signaling is mechanistically more complex and diverse than originally thought.

Introduction

Since the discovery of Δ^9-tetrahydrocannabinol (THC) as the main psychoactive ingredient in marijuana, and the cloning of cannabinoid receptors and the identification of their endogenous ligands (endocannabinoids [eCBs]), our understanding of the molecular basis and functions of the eCB signaling system has evolved considerably. Extensive research in the last 15 years has consolidated our view on eCBs as powerful regulators of synaptic function throughout the CNS. Their role as retrograde messengers suppressing transmitter release in a transient or long-lasting manner, at both excitatory and inhibitory synapses, is now well established (Alger, 2012; Chevaleyre et al., 2006; Freund et al., 2003; Kano et al., 2009; Katona and Freund, 2012). Apart from signaling in more mature systems, the eCB system has been implicated in synapse formation and neurogenesis (Harkany et al., 2008). It is also widely believed that by modulating synaptic strength, eCBs can regulate a wide range of neural functions, including cognition, motor control, feeding behaviors, and pain. Moreover, dysregulation of the eCB system is implicated in neuropsychiatric conditions such as depression and anxiety (Hillard et al., 2012; Mechoulam and Parker, 2012). As such, the eCB system provides an excellent opportunity for therapeutic interventions (Ligresti et al., 2009; Piomelli, 2005). Their prevalence throughout the brain suggests that eCBs are fundamental modulators of synaptic function. This Review focuses on recent advances in eCB signaling at central synapses.

The eCB signaling system comprises (1) at least two G protein-coupled receptors (GPCRs), known as the cannabinoid type 1 and type 2 receptors (CB_1R and CB_2R); (2) the endogenous ligands (eCBs), of which anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are the best characterized; and (3) synthetic and degradative enzymes and transporters that regulate eCB levels and action at receptors. An enormous amount of information on the general properties of the eCB system has accumulated over the last two decades (for general reviews on the eCB system, see Ahn et al., 2008; Di Marzo, 2009; Howlett et al., 2002; Pertwee et al., 2010; Piomelli, 2003). We discuss essential features of this system in the context of synaptic function.

The principal mechanism by which eCBs regulate synaptic function is through retrograde signaling (for a thorough review, see Kano et al., 2009). Here, postsynaptic activity leads to the production of an eCB that moves backward across the synapse, binds presynaptic CB_1Rs, and suppresses neurotransmitter release (Figure 1A). However, there is also evidence suggesting that eCBs signal in a nonretrograde or autocrine manner, in which they can modulate neural function and synaptic transmission by engaging transient receptor potential vanilloid receptor type 1 (TRPV1) and also CB_1Rs located on or within the postsynaptic cell (Figure 1B). Finally, recent studies indicate that eCBs can signal via astrocytes to indirectly modulate presynaptic or postsynaptic function (Figure 1C). This Review aims to highlight the emerging mechanistic diversity of synaptic eCB signaling.

Retrograde Endocannabinoid Signaling

The first demonstration of retrograde eCB signaling came from the discovery that eCBs mediate forms of short-term synaptic plasticity known as depolarization-induced suppression of inhibition (DSI) (Ohno-Shosaku et al., 2001; Wilson and Nicoll, 2001) and depolarization-induced suppression of excitation (DSE) (Kreitzer and Regehr, 2001). Shortly after it was shown that eCBs also mediate presynaptic forms of long-term depression (eCB-LTD) at both excitatory (Gerdeman et al., 2002; Robbe et al., 2002) and inhibitory (Chevaleyre and Castillo, 2003; Marsicano et al., 2002) synapses. eCBs have since emerged as the best characterized retrograde messengers (Regehr et al., 2009), with numerous examples of short- and long-term forms of synaptic plasticity reported throughout the brain (Heifets and Castillo, 2009; Kano et al., 2009).

CB_1/CB_2 receptors are $G_{i/o}$ protein-coupled receptors that mediate almost all effects of exogenous and endogenous

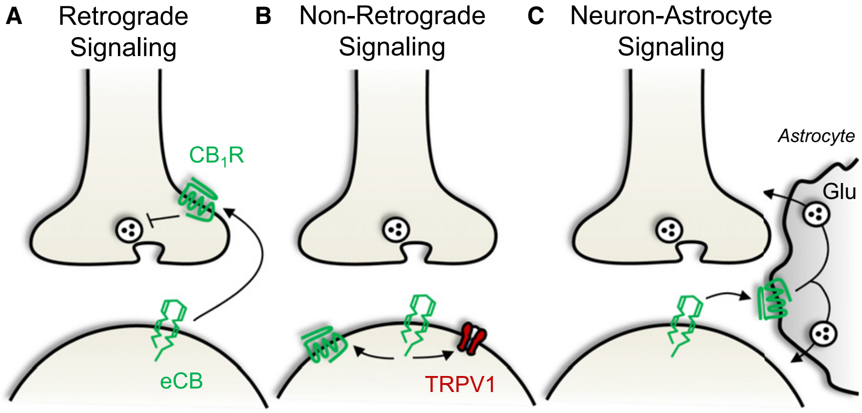

Figure 1. Endocannabinoid Signaling at the Synapse

(A) Retrograde endocannabinoid (eCB) signaling. eCBs are mobilized from postsynaptic neurons and target presynaptic cannabinoid type 1 receptors (CB_1Rs) to suppress neurotransmitter release.

(B) Nonretrograde eCB signaling. eCBs produced in postsynaptic neurons activate postsynaptic CB_1Rs or transient receptor potential vanilloid type 1 (TRPV1) channels.

(C) Neuron-astrocyte eCB signaling. eCBs released from postsynaptic neurons stimulate astrocytic CB_1Rs, thereby triggering gliotransmission. Glu, glutamate.

cannabinoids. CB_1Rs are one of the most widely expressed GPCRs in the brain (Herkenham et al., 1990). Their localization to neuronal terminals (Katona et al., 1999, 2006) strongly suggests that CB_1Rs play important roles in regulating synaptic function. Indeed, CB_1R activation inhibits neurotransmitter release at synapses through two main mechanisms (Figure 2) (Chevaleyre et al., 2006; Freund et al., 2003; Kano et al., 2009). For short-term plasticity, in which CB_1Rs are activated for a few seconds, the mechanism involves direct G protein-dependent (likely via the $\beta\gamma$ subunits) inhibition of presynaptic Ca^{2+} influx through voltage-gated Ca^{2+} channels (VGCCs) (Brown et al., 2003; Kreitzer and Regehr, 2001; Wilson et al., 2001). For long-term plasticity, the predominant mechanism requires inhibition of adenylyl cyclase and downregulation of the cAMP/PKA pathway via the $\alpha_{i/o}$ limb (Chevaleyre et al., 2006; Heifets and Castillo, 2009). Moreover, CB_1Rs only need to be engaged during the induction, but not expression, phase of eCB-LTD. Induction also requires combined presynaptic firing with CB_1R activation, thereby providing a mechanism for input specificity; that is, only active synapses detecting eCBs express long-term plasticity (Heifets et al., 2008; Singla et al., 2007). The expression mechanism for eCB-LTD may involve presynaptic proteins Rab3B/RIM1α (Chevaleyre et al., 2007; Tsetsenis et al., 2011) or a reduction of P/Q-type VGCCs (Mato et al., 2008). While other effectors downstream of CB_1Rs have been described, mainly in cultured cells and expression systems (Howlett, 2005; Pertwee et al., 2010), their role in regulating synaptic function is presently less clear. In contrast to CB_1Rs, which are widely expressed in the brain, CB_2Rs are typically found in the immune system and are poorly expressed in the CNS. Although recent studies support a role for these receptors in the CNS (den Boon et al., 2012; Van Sickle et al., 2005; Xi et al., 2011), when compared with CB_1Rs, much less is known about the precise cellular mechanism(s) and contributions of CB_2Rs to brain function.

Although several eCBs have been identified, just two, AEA and 2-AG, emerged as the most relevant and prevalent regulators of synaptic function. While 2-AG seems to be the principal eCB required for activity-dependent retrograde signaling, the relative contribution of 2-AG and AEA to synaptic transmission is still debated. Functional crosstalk between 2-AG and AEA signaling was reported (Maccarrone et al., 2008), and recent findings suggest that 2-AG and AEA can be recruited differentially from the same postsynaptic neuron, depending on the type of presynaptic activity (Lerner and Kreitzer, 2012; Puente et al., 2011). A more complete signaling profile for 2-AG and AEA—including production, target identification, and degradation—is indispensable for better understanding their short- and long-term impact on synaptic function.

Synthesis and degradation of eCBs help shape their spatiotemporal signaling profile. For retrograde eCB signaling, postsynaptic neuronal depolarization elevates intracellular Ca^{2+} via VGCCs and elicits 2-AG production presumably by activating Ca^{2+}-sensitive enzymes. In addition, glutamate release onto postsynaptic group I metabotropic glutamate receptors (I mGluRs) (Maejima et al., 2001; Varma et al., 2001) can generate 2-AG by activating the enzyme phospholipase Cβ (PLCβ) (for a review, see Hashimotodani et al., 2007a). Most likely, Ca^{2+} influx through VGCCs and downstream signaling from I mGluR activation converge on the same metabolic pathway to mobilize 2-AG (Figure 2A). PLCβ is thought to act as a coincidence detector for postsynaptic Ca^{2+} and GPCR signaling (Hashimotodani et al., 2005; Maejima et al., 2005). This interaction might be important for integrating synaptic activity (Brenowitz and Regehr, 2005). On the other hand, it is worth noting that activation of I mGluRs is sufficient to mobilize eCBs to trigger short- and long-term forms of plasticity (Chevaleyre et al., 2006). For long-term plasticity, a few minutes of CB_1R stimulation is needed, which can result from a brief postsynaptic I mGluR activation triggering a relatively longer-lasting 2-AG mobilization (Chevaleyre and Castillo, 2003). Of general physiological relevance, many other $G_{q/11}$-GPCRs are known to promote eCB synthesis (Katona and Freund, 2012). Upon activation, PLCβ hydrolyzes phosphatidylinositol to generate diacylglycerol, which is converted to 2-AG by diacylglycerol lipase α (DGLα). DGLα is specifically localized to postsynaptic compartments (Katona et al., 2006; Lafourcade et al., 2007; Nomura et al., 2007; Yoshida et al., 2006). Whereas pharmacological studies inconsistently implicated DGLα in short-term synaptic plasticity, genetic deletion of DGLα indicates that this enzyme is required for Ca^{2+}-dependent 2-AG production and short- and long-term eCB-dependent synaptic plasticity (Gao et al., 2010; Tanimura et al., 2010; Yoshino et al., 2011). Once synthesized, 2-AG travels backward across the synapse; however, the precise mechanism by which this occurs is still unresolved.

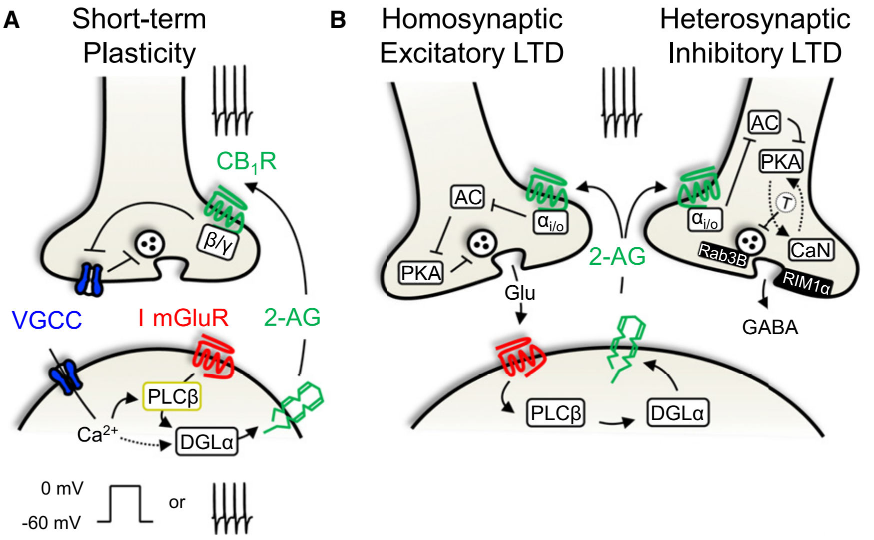

Figure 2. Molecular Mechanisms Underlying Endocannabinoid-Mediated Short- and Long-Term Synaptic Plasticity

(A) Short-term depression. Postsynaptic activity triggers Ca^{2+} influx via voltage-gated Ca^{2+} channels (VGCCs). Other Ca^{2+} sources, like NMDARs and internal stores, may contribute. Ca^{2+} promotes diacylglycerol lipase (DGLα)-mediated eCB production by an unknown mechanism. Presynaptic activity can also lead to eCB mobilization by activating postsynaptic group I metabotropic glutamate receptors (I mGluRs). Phospholipase-Cβ (PLCβ) can now act as a coincidence detector integrating pre- and postsynaptic activity. DGLα promotes 2-arachidonoylglycerol (2-AG) release, which retrogradely targets presynaptic CB_1Rs, and the βγ subunits probably couple to presynaptic VGCCs to reduce neurotransmitter release.
(B) eCB-mediated excitatory long-term depression (LTD) and inhibitory LTD (iLTD). Patterned presynaptic stimulation releases glutamate (Glu), which activates postsynaptic mGluRs coupled to PLCβ and DGLα. 2-AG homosynaptically targets CB_1Rs localized to excitatory terminals and heterosynaptically engages CB_1Rs at inhibitory terminals. A $G_{\alpha i/o}$-dependent reduction in adenylyl cyclase (AC) and protein kinase A (PKA) activity suppresses transmitter release. At inhibitory synapses, decreased PKA activity, in conjunction with activation of the Ca^{2+}-sensitive phosphatase calcineurin (CaN), shifts the phosphorylation status of an unidentified presynaptic target (T) required for iLTD. The active zone protein RIM1α and the vesicle-associated protein Rab3B are also necessary for iLTD. Induction of eCB-LTD may require presynaptic Ca^{2+} rise through VGCCs, NMDARs, or internal stores (not shown). Dashed lines indicate putative pathways.

The primary degradative enzyme for 2-AG is monoacylglycerol lipase (MGL) (Blankman et al., 2007). MGL is found presynaptically (Gulyas et al., 2004; Ludányi et al., 2011), but its expression seems to be heterogeneous across synapses (Tanimura et al., 2012; Uchigashima et al., 2011; Yoshida et al., 2011). The postsynaptically localized serine hydrolase ABHD6 also catabolizes a small fraction of 2-AG (Marrs et al., 2010), suggesting functional redundancy that could help fine-tune 2-AG signaling. Nevertheless, it seems clear that MGL controls the duration and magnitude of 2-AG-mediated synaptic plasticity (Hashimotodani et al., 2007b; Pan et al., 2011; Schlosburg et al., 2010; Szabo et al., 2006). While 2-AG probably signals within 20 μm of its site of origin (Chevaleyre and Castillo, 2004; Wilson and Nicoll, 2001), it would be useful to examine the relative contribution of MGL and ABHD6 to 2-AG diffusion.

In contrast to synaptic 2-AG signaling, AEA synthesis and degradation seems more complex. Postsynaptic depolarization and intracellular Ca^{2+} influx support AEA production, but how this occurs is not fully understood (Di Marzo, 2011). AEA is in part synthesized by N-acyl-phosphatidylethanolamine-hydrolyzing phospholipase-D (NAPE-PLD). However, alternative synthetic pathways exist (Okamoto et al., 2007). NAPE-PLD can be expressed postsynaptically (Cristino et al., 2008) but was also observed on axonal membranes, in particular at CA3 mossy fiber terminals (Egertová et al., 2008; Nyilas et al., 2008), where AEA could locally modulate presynaptic function. AEA transport across membranes might be facilitated by a lipophilic carrier protein (Beltramo et al., 1997; Fu et al., 2012; Hillard et al., 1997). This protein presumably supports AEA delivery to intracellular compartments where fatty acid amide hydrolase (FAAH), the enzyme primarily responsible for AEA degradation, is localized (Gulyas et al., 2004). While 2-AG and AEA are hydrolyzed by MGL and FAAH, respectively, oxidizing enzymes like cyclooxygenase and lipoxygenase can also utilize these substrates (Vandevoorde and Lambert, 2007). Of interest, some of these eCB metabolites are biologically active (Nomura et al., 2011) and probably modulate synaptic function, a possibility that needs to be further investigated. Continued exploration of the mechanisms underlying eCB synthesis and degradation will advance our understanding of how lipids shape synaptic function.

Nonretrograde Endocannabinoid Signaling

Besides the classical cannabinoid receptors (CB_1R/CB_2R), there is growing evidence that TRPV1 channels also participate in eCB signaling (De Petrocellis and Di Marzo, 2010; Pertwee et al., 2010). TRPV1, originally VR1 for vanilloid receptor type 1 (Caterina et al., 1997), is a polymodal transient receptor potential (TRP) ion channel largely expressed in afferent peripheral sensory neurons, where its activation regulates synaptic transmission associated with pain sensation (Caterina and Julius, 2001). Interestingly, TRPV1 can bind lipophilic substances, such as AEA (Di Marzo et al., 2002). Of note, AEA is a partial agonist at the CB_1R but a full agonist at TRPV1 channels (Smart et al., 2000; Zygmunt et al., 1999). In addition to their expression in the periphery, TRPV1 channels have been found in the CNS (Cristino et al., 2006, 2008; Mezey et al., 2000; Puente et al., 2011; Roberts et al., 2004; Tóth et al., 2005) (but see Cavanaugh et al., 2011), where they appear to regulate synaptic function.

Recent studies revealed that AEA acting on TRPV1 mediates a postsynaptic form of LTD (Figure 3A). This TRPV1-LTD has been observed in dopamine receptor-2 (D_2)-positive medium spiny neurons of the nucleus accumbens (Grueter et al., 2010), in dentate granule cells (Chávez et al., 2010), and in the bed nucleus of the stria terminalis (Puente et al., 2011). In each case, activation of mGluR5, presumably via PLC (Liu et al., 2008) and Ca^{2+} release from intracellular stores, promotes the synthesis of AEA that activates TRPV1 channels. In addition,

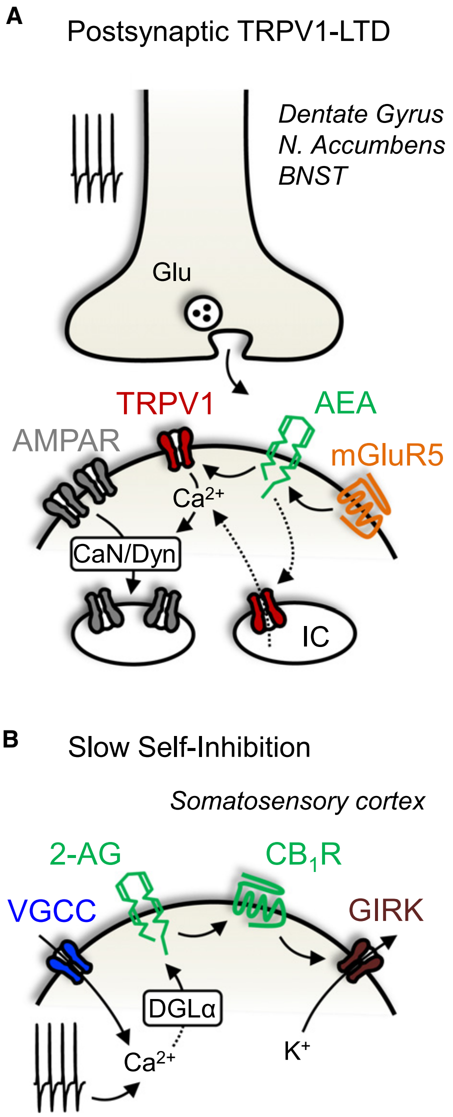

Figure 3. Nonretrograde eCB Signaling

(A) Mechanism underlying postsynaptic TRPV1-LTD. Presynaptic activity releases glutamate that stimulates mGluR5. Postsynaptic depolarization may also be required. mGluR5 couples to anandamide (AEA) production, which activates TRPV1, leading to enhanced Ca^{2+} signaling. Ca^{2+} engages calcineurin/dynamin (CaN/Dyn), causing AMPA receptor (AMPAR) endocytosis and LTD. IC, intracellular compartment; N. Accumbens, nucleus accumbens; BNST, bed nucleus of the stria terminalis.

(B) Mechanism responsible for slow self-inhibition (autocrine signaling). Postsynaptic activity-induced Ca^{2+} elevation facilitates 2-AG production. 2-AG activates postsynaptic CB_1Rs that signal to a G protein-coupled inwardly rectifying K^+ (GIRK) channel to hyperpolarize the membrane potential and inhibit neuronal firing. Dashed lines indicate putative pathways.

TRPV1-LTD relies on AMPA receptor (AMPAR) endocytosis. These findings are consistent with the notion that AEA can act as an intracellular messenger (van der Stelt and Di Marzo, 2005) but differs from a presynaptic, TRPV1-dependent LTD at glutamatergic synapses onto CA1 hippocampal interneurons (Gibson et al., 2008). While CB_1Rs mediate excitatory and inhibitory synaptic plasticity, whether brain TRPV1 channels mediate inhibitory synaptic plasticity is unknown. There is also evidence that TRPV1 localizes to neuronal intracellular compartments like the endoplasmic reticulum, *trans*-Golgi network, and perhaps even vesicles (Dong et al., 2010). The functional significance of such receptors warrants further investigation.

Nonretrograde eCB signaling has been observed in other contexts. Repetitive activation of a subtype of neocortical GABAergic interneuron triggers a CB_1R-dependent postsynaptic hyperpolarization, which reduced its excitability (Figure 3B) (Bacci et al., 2004). This slow self-inhibition resulted from activity-dependent rises in intracellular Ca^{2+}, mobilization of 2-AG, and activation of CB_1Rs that couple to a G protein-coupled inwardly rectifying K^+ channel (Bacci et al., 2004; Marinelli et al., 2008). This form of autocrine signaling was also observed in a fraction of layer 2/3 neocortical pyramidal neurons (Marinelli et al., 2009). Unexpectedly, CB_2Rs were recently shown to mediate an activity-induced self-inhibition in medial prefrontal cortical pyramidal neurons (den Boon et al., 2012). CB_2Rs were localized to intracellular compartments and coupled to calcium-activated chloride channels to decrease neuronal firing. The generalizability of autocrine eCB signaling to other brain regions should be examined.

Endocannabinoid-Mediated Communication between Neurons and Glia

Growing evidence indicates that glia participate in eCB signaling (Stella, 2010). The synthetic machinery for eCB production was observed in oligodendrocytes (Gomez et al., 2010), astrocytes, and microglial cells (Hegyi et al., 2012). Likewise, cultured astrocytes and microglial cells can produce 2-AG or AEA (Stella, 2009). It is not yet clear whether eCBs produced by glial cells modulate synaptic transmission. On the other hand, several recent findings support a role for eCBs signaling to astrocytes and their ability to indirectly mediate synaptic function.

At Schaffer collateral excitatory synapses onto hippocampal CA1 pyramidal neurons, postsynaptic activity-dependent release of eCBs was shown to target not only presynaptic CB_1Rs but also astrocytic CB_1Rs (Figure 4A). Astrocytic CB_1Rs unexpectedly coupled to PLC via $G_{q/11}$, which increased intracellular Ca^{2+} and triggered glutamate release (Navarrete and Araque, 2008). In support of these functional observations, CB_1Rs in hippocampal astrocytes have recently been observed using immunoelectron microscopy (Han et al., 2012). Glutamate activated NMDA receptors (NMDARs) on CA1 pyramidal neurons and, at some synapses, triggered short-term facilitation of transmitter release, presumably by stimulating presynaptic mGluR1s (Navarrete and Araque, 2008, 2010). Interestingly, this short-term facilitation was not spatially restricted, being observed over 70 μm away from the active pyramidal cell. Thus, eCBs could concomitantly suppress synaptic transmitter release by triggering DSE and indirectly potentiate synaptic transmission through astrocytes, both in a CB_1R-dependent manner. While the functional significance of such plasticity is not yet clear, astrocytes may have long-distance neuromodulatory effects that are mediated by eCB signaling.

eCB-mediated neuron-astrocyte communication has also been implicated in long-term plasticity. Spike timing-dependent LTD (tLTD) between neocortical pyramidal neurons is known to

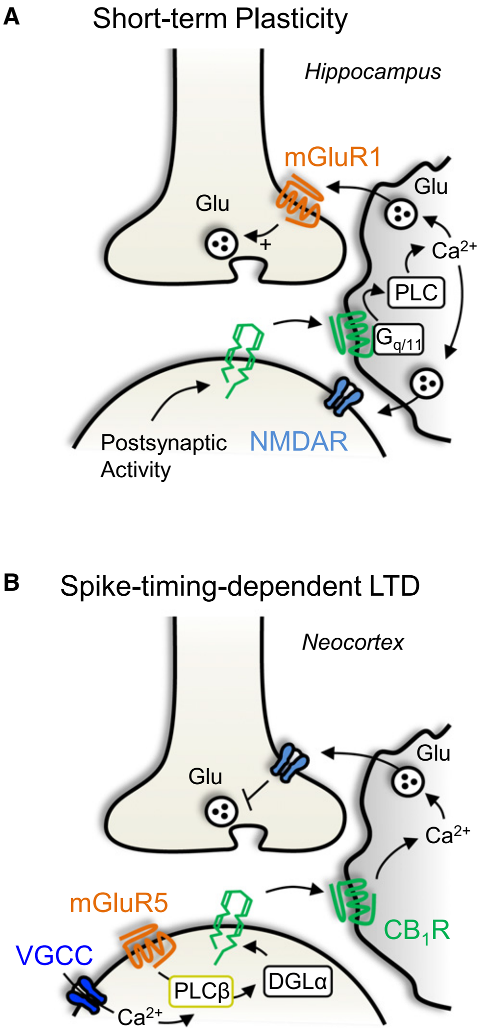

Figure 4. Astrocytic CB$_1$R Modulation of Synaptic Transmission

(A) Short-term plasticity. Postsynaptic neuronal activity leads to eCB release. eCBs target G$_{q/11}$-coupled CB$_1$Rs localized to astrocytes. As a result, PLC activity facilitates astrocytic Ca^{2+} signaling. Glu released from the astrocyte activates presynaptic mGluR1s to potentiate release and postsynaptic NMDARs to trigger a slow inward current.

(B) Spike-timing-dependent LTD. Repetitive pairings of post-before-pre synaptic activity mobilizes eCBs through the neuronal PLCβ-coincidence detection mechanism. Released eCB stimulates astrocytic CB$_1$Rs, leading to Ca^{2+} signaling. Astrocyte-mediated Glu release activates presynaptic NMDARs to depress release.

require activation of presynaptic NMDARs and CB$_1$Rs (Bender et al., 2006; Nevian and Sakmann, 2006; Sjöström et al., 2003). Surprisingly, a recent study found that astrocytic CB$_1$Rs were necessary and sufficient to mediate tLTD (Min and Nevian, 2012). eCBs originating from layer 2/3 pyramidal neurons activated astrocytic CB$_1$Rs, which increased intracellular Ca^{2+}, thereby releasing glutamate and stimulating presynaptic NMDARs (Figure 4B). Given the anatomical and functional evidence for presynaptic CB$_1$Rs in neocortex (Domenici et al., 2006; Hill et al., 2007; Lafourcade et al., 2007), future studies could use astrocyte- and neuron-specific CB$_1$R knockout mice to identify the exact conditions required to activate neuronal and/or astrocytic CB$_1$Rs.

Attesting to the possible physiological relevance of astrocytic CB$_1$Rs, a recent in vivo study showed that intraperitoneal injection of THC induced long-lasting suppression of excitatory synaptic transmission in hippocampal area CA1, an effect that required astrocytic CB$_1$Rs (Han et al., 2012). Previous work in acute hippocampal slices from global CB$_1$R knockout mice suggested that agonist-mediated suppression of excitatory transmission in CA1 depends solely on CB$_1$Rs expressed at Schaffer collateral terminals (Katona et al., 2006; Kawamura et al., 2006; Takahashi and Castillo, 2006). Unexpectedly, however, THC-mediated suppression of synaptic transmission in vivo was intact in glutamatergic- and GABAergic-specific CB$_1$R knockout mice, whereas it was abolished in glia-specific CB$_1$R knockout mice (Han et al., 2012). Mechanistically, glutamate, presumably released from astrocytes, activated postsynaptic NMDARs, triggering AMPAR endocytosis and subsequent synaptic depression. These results contrast with those observed in vitro in which eCBs indirectly facilitated synaptic transmission via astrocytic CB$_1$Rs (Navarrete and Araque, 2008, 2010). A thorough examination of the conditions necessary for activating synaptic and astrocytic CB$_1$Rs is clearly needed.

Tonic Endocannabinoid Signaling

In addition to the classical, activity-dependent phasic mode of eCB mobilization, tonic eCB signaling has been reported. Tonic signaling can be observed as an increase in basal synaptic transmission after pharmacological blockade of CB$_1$Rs (Auclair et al., 2000; Hentges et al., 2005; Losonczy et al., 2004; Neu et al., 2007; Oliet et al., 2007; Slanina and Schweitzer, 2005; Zhu and Lovinger, 2010). However, CB$_1$R blockade in this manner does not always reveal an eCB tone (Chevaleyre and Castillo, 2003; Pan et al., 2011; van Beugen et al., 2006; Wilson and Nicoll, 2001; Zhong et al., 2011). Buildup of an eCB tone can occur when inhibiting eCB uptake (Wilson and Nicoll, 2001) or genetic deletion of MGL (Pan et al., 2011; Zhong et al., 2011). The fact that most 2-AG is hydrolyzed by MGL (Blankman et al., 2007; Chanda et al., 2010; Nomura et al., 2011) suggests that 2-AG mediates tonic eCB signaling, which is consistent with a constitutive release of 2-AG in cultured neurons (Hashimotodani et al., 2007b). On the other hand, AEA can also contribute to tonic eCB signaling. Chronic inactivity in hippocampal slice cultures reduced an AEA tone presumably by augmenting AEA uptake and degradation (Kim and Alger, 2010). Together, these studies suggest that tonic eCB signaling can control, under some conditions, basal synaptic neurotransmitter release. It is currently unclear whether regional differences in the expression pattern of enzymes responsible for eCB metabolism can fully account for synapse specificity. Moreover, most of these studies were performed in vitro, and therefore the impact of an eCB tone on synaptic function in vivo should be further examined.

Interaction between Endocannabinoids and Other Signaling Systems

The eCB system allows for multiple points of interaction with other signaling and neuromodulatory systems. In addition to regulating release of classical neurotransmitters like glutamate and GABA, CB_1Rs can also control the release of several neuromodulators including serotonin, acetylcholine, dopamine, opioids, norepinephrine, and cholecystokinin (Alger, 2002; Kano et al., 2009; Schlicker and Kathmann, 2001). On the other hand, many of these neuromodulators actually couple to eCB synthesis by activating their respective $G_{q/11}$ protein-coupled receptors (for a comprehensive list, see Katona and Freund, 2012). Additionally, regulators of G protein signaling were recently shown to control $G_{q/11}$-coupled receptors and eCB mobilization (Lerner and Kreitzer, 2012), indicating how GPCRs themselves can fine-tune eCB release. Together, these studies not only support a general theme by which $G_{q/11}$-coupled GPCRs mobilize eCBs but demonstrate the existence of multiple routes for eliciting and regulating eCB release.

On the other side of the synapse, functional interactions between CB_1Rs and other receptors have been identified. For example, at inhibitory terminals in the prefrontal cortex, D_2-like receptors colocalize with CB_1Rs where they appear to facilitate CB_1R-mediated suppression of transmitter release (Chiu et al., 2010). This is probably due to a cooperative lowering of PKA activity, consistent with similar observations in the ventral tegmental area (Pan et al., 2008). In addition, work in visual cortical slices from young mice suggests that BDNF interferes with CB_1R downstream signaling, thereby disrupting eCB-mediated suppression of neurotransmitter release (Huang et al., 2008). This might result from, at least in part, BDNF inhibiting CB_1R function through a mechanism requiring cholesterol metabolism and altered membrane lipid raft function (De Chiara et al., 2010). At Schaffer collaterals, adenosine A_1 receptors (A_1Rs) colocalize with CB_1Rs. Tonic activation of A_1Rs can reduce the efficacy of CB_1R-mediated inhibition of glutamate release (Hoffman et al., 2010). Also in the hippocampus, stimulating GluK1-containing kainate receptors at inhibitory terminals appears to actually facilitate CB_1R signaling (Lourenço et al., 2010). The mechanism by which this occurs is not yet clear. Adding to the complexity of eCB signaling, evidence suggests that CB_1Rs can associate with other GPCRs to form heteromeric complexes. Such interactions have been detected for CB_1-D_2, CB_1-opioid, CB_1-A_{2A}, and CB_1-orexin-1 receptor pairs (Hudson et al., 2010; Mackie, 2005; Pertwee et al., 2010). Strikingly, higher-order heteromeric complexes consisting of CB_1, D_2, and $A_{2A}Rs$ have also been observed (Carriba et al., 2008). Intriguingly, these macromolecular interactions can significantly change the downstream G proteins recruited during receptor activation. Much more work is needed to determine the physiological impact of these heteromeric complexes in the brain and, in particular, at the synapse.

Plasticity of the Endocannabinoid System

In addition to triggering various forms of synaptic plasticity like DSI/DSE, eCB-LTD, and TRPV1-LTD, the eCB system itself undergoes plastic changes. Mechanistically, plasticity of the eCB system can arise by modifications to any of its components, for example, CB_1R number and function or eCB production and degradation. These changes have been observed both in vivo and in vitro and can be triggered by several natural and experimental conditions including neural activity and agonist-induced CB_1R activation. Of clinical relevance, changes in eCB signaling are also associated with several brain disorders. Here, we illustrate how plasticity of the eCB system can profoundly affect synaptic physiology and, ultimately, brain function.

An interesting example of agonist-induced plasticity of eCB signaling comes from the observation that a single in vivo exposure to THC abolished for a few days eCB-mediated retrograde signaling in the hippocampus and nucleus accumbens of mice (Mato et al., 2004). This effect was associated with a reduction in CB_1R maximal efficacy without modifications in total binding or coupling. Prolonged exposure to agonists in humans and animal models results in behavioral tolerance, which is classically attributed to receptor desensitization and internalization (Coutts et al., 2001; Jin et al., 1999; Wu et al., 2008). However, a reduction in CB_1R lateral mobility may also contribute (Mikasova et al., 2008). Understanding the impact of synaptic CB_1R signaling and trafficking in vivo should further reveal how eCBs control physiological responses to drugs of abuse.

The eCB system also undergoes developmental changes (Harkany et al., 2008). In the hippocampus, both the magnitude of eCB-mediated iLTD and the ability of a CB_1R agonist to suppress inhibitory transmission were greater in juvenile than in adult rats (Kang-Park et al., 2007; see also Zhu and Lovinger, 2010). In addition, a form of eCB-mediated heterosynaptic LTD at excitatory synapses was observed in young animals, attenuated across development, and disappeared in the mature brain (Yasuda et al., 2008). Lower expression levels of CB_1Rs at excitatory synapses in the adult brain may underlie these changes (Kawamura et al., 2006). Along these lines, developmentally expressed CB_1Rs at mossy fiber terminals in the CA3 region of the hippocampus mediate eCB-LTD at immature but not mature synapses (Caiati et al., 2012). Postsynaptic eCB production is also modulated over time. A developmental shift from long-term potentiation (LTP) to eCB-LTD was reported in the striatum (Ade and Lovinger, 2007). Whereas CB_1R sensitivity to its agonist was not changed, the shift in plasticity was associated with developmental increases in AEA levels, suggesting that AEA determines the direction of synaptic plasticity. Similarly, it was shown that the magnitude of hippocampal DSI is developmentally regulated, such that DSI is modest in early postnatal days and becomes more robust at 2 weeks postnatal (Zhu and Lovinger, 2010). The mechanism is not entirely clear but it might rely on a postsynaptic change in eCB release. In addition, tonic eCB release suppresses GABAergic transmission in the mature but not the neonatal hippocampus (Kang-Park et al., 2007; Zhu and Lovinger, 2010). While these studies argue that synaptic eCB signaling is developmentally regulated, the exact mechanisms underlying these changes remain unclear.

In mature animals, eCB signaling can be modified in an activity-dependent manner. High-frequency (Chen et al., 2007) or low-frequency (Zhu and Lovinger, 2007) stimulation of Schaffer collaterals, as well as brief pharmacological activation of I mGluRs (Edwards et al., 2008), triggered a long-lasting potentiation in the magnitude of DSI. Remarkably, the transient postsynaptic Ca^{2+} rise that occurs during a single episode of DSI facilitated subsequent I mGluR-dependent mobilization of eCBs and the induction of iLTD (Edwards et al., 2008). The molecular components that undergo priming are unknown. A similar DSI potentiation was observed after a single episode of experimentally induced febrile seizures (Chen et al., 2003). This potentiation was due to an increase in the number of CB_1Rs associated with perisomatic inhibitory inputs. In contrast, the epileptic human hippocampus showed a reduction in the expression of CB_1Rs at glutamatergic terminals (Ludányi et al., 2008). Nevertheless, both upregulation of CB_1Rs at GABAergic terminals and downregulation of CB_1Rs at excitatory terminals are potentially epileptogenic, suggesting that dysregulation of the eCB system could play a role in epilepsy. Identifying the molecular basis for these activity-dependent changes in CB_1R expression levels is important because it may uncover novel therapeutic targets.

Altered eCB signaling has been reported in experimental models for disorders like fragile X syndrome. Upregulation of eCB signaling was found in fragile X mental retardation protein knockout mice as indicated by facilitation of I mGluR agonist-induced iLTD. Facilitated iLTD might result from aberrant coupling between I mGluR activation and eCB mobilization (Zhang and Alger, 2010). Aberrant coupling might be due to changes in Homer 1a protein, which reportedly interacts with mGluRs to regulate eCB release in cultured hippocampal neurons (Roloff et al., 2010). Another possible mediator of aberrant coupling includes the excitatory synapse-specific scaffolding protein SAPAP3, which can modulate postsynaptic mGluRs and eCB-mediated synaptic plasticity in the striatum (Chen et al., 2011). Continued exploration of the mechanisms underlying mGluR-coupled eCB production should provide clues as to how to treat patients with fragile X syndrome.

Several studies indicate that physiological responses to stress modulate the expression levels of key components of the eCB system (Riebe and Wotjak, 2011). In general, how stress modulates eCB signaling is largely dependent on brain regions, stress paradigm, and duration of stress exposure. In the striatum and nucleus accumbens, chronic stress inhibited CB_1R-mediated suppression of synaptic transmission (Rossi et al., 2008; Wang et al., 2010). Downregulation of CB_1R function might underlie this eCB signaling deficiency since stress-induced downregulation of CB_1R function was observed in the hypothalamus (Wamsteeker et al., 2010). There is also evidence that stress can enhance eCB signaling. Repeated restraint stress increased 2-AG levels and enhanced DSI in the basolateral amygdala (Patel et al., 2009). Similarly, restraint stress increased 2-AG levels and enhanced DSI in hippocampal CA1 pyramidal neurons (Wang et al., 2012).

Food intake is another physiological process that modulates the eCB system (Banni and Di Marzo, 2010; DiPatrizio and Piomelli, 2012). For example, CB_1R agonists increase food intake, whereas antagonists reduce food consumption. Providing mechanistic insight as to how this modulation may occur, a recent study showed that diet-induced obesity in mice increased hippocampal DGLα protein, 2-AG and AEA production, as well as CB_1R expression (Massa et al., 2010). Levels of DGLβ, MGL, and FAAH were unchanged. Consistently, DSI and eCB-mediated iLTD were augmented in these mice (Massa et al., 2010). Diet restrictions likewise cause significant changes in the eCB system. In hypothalamic feeding circuits, food deprivation downregulated CB_1R signaling, converting eCB-mediated LTD-expressing synapses into ones that show nitric-oxide-dependent LTP (Crosby et al., 2011). In addition, polyunsaturated fatty acid diet-deficient mice showed impaired eCB-mediated LTD in both prefrontal cortex and nucleus accumbens (Lafourcade et al., 2011). Lack of eCB-LTD was attributed to reduced coupling of the CB_1R to its downstream $G_{i/o}$ protein. Intriguingly, these mice exhibited defects in mood and emotional behavior, implicating synaptic eCB signaling in affective behaviors. Taken together, these studies highlight how behavioral manipulations profoundly regulate eCB signaling and synaptic function.

Conclusions and Future Directions

In this Review, we have highlighted essential properties of eCB signaling at the synapse. Research in the last decade has bolstered eCBs as powerful regulators of synaptic function throughout the CNS. Exciting developments have uncovered new mechanisms underlying eCB-mediated regulation of synaptic transmission. Moreover, the dynamics of synaptic eCB signaling display an intricate, and sometimes reciprocal, set of interactions with other neuromodulatory systems. These emerging levels of complexity clearly indicate that much more work lies ahead in our pursuit to fully understand eCB signaling at the synapse.

While an overwhelming body of evidence strongly suggests that eCBs are retrograde synaptic messengers, a major outstanding issue with the model is how a lipid traverses an aqueous synaptic cleft. Furthermore, once produced, how far do eCBs diffuse? While AEA seems to be transported by a lipid carrier protein, whether 2-AG is also transported by a lipid chaperone is unknown. Alternatively, specialized protein/lipid bridges, akin to synaptic intercellular adhesion molecules, could adopt a structural conformation that exposes lipophilic patches to reduce the retrograde energy barrier. Regardless of the exact mechanism, it is clear that eCB signaling powerfully regulates synaptic function. Developing new technologies to image lipid signaling, in real time, should dramatically propel the field of eCB research forward.

Apart from their more traditional role in retrograde signaling, eCBs also appear to act in a nonretrograde manner to modulate postsynaptic function as well as trigger gliotransmission. However, the general physiological relevance of nonretrograde signaling mediated by TRPV1 in the CNS is not yet clear. While experimental evidence for eCBs targeting postsynaptic receptors is growing, whether presynaptically produced eCBs activate presynaptic CB_1Rs or TRPV1 channels to modulate synaptic function remains unknown. In addition, the role of CB_1Rs in regulating gliotransmission and, indirectly, synaptic plasticity warrants further investigation. Given the myriad of evidence supporting synaptic CB_1Rs in modulating synaptic transmission, the precise conditions necessary for activating neuronal versus astrocytic CB_1Rs must be defined.

Several other fundamental mechanistic questions remain unanswered. What are the rules governing CB_1R trafficking into and out of membranes? What are the conditions required for CB_1R heteromerization with other neuromodulatory receptors, and what is their impact on synaptic function? As for the two main eCBs, 2-AG and AEA, are there specific patterns of activity that predominantly mobilize one lipid versus the other? Perhaps these eCBs subserve specific functions at the synapse. If so, which ones? What is the precise role of tonic eCB release in the brain? In vitro approaches are unquestionably useful for addressing fundamental mechanisms underlying synaptic eCB signaling, but much more work in vivo is required to determine their contribution to physiological and pathological conditions. While a great deal of progress has been made in our understanding of eCB signaling and synaptic function, the greatest challenges lie ahead.

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