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MINI REVIEW

Emerging roles for endocannabinoids in long-term synaptic plasticity

[1]Gregory L. Gerdeman & *,[2]David M. Lovinger

[1]Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, AZ 85724, U.S.A. and [2]Laboratory for Integrative Neuroscience, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852, U.S.A.

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Abbreviations: AEA, anandamide; AMT, anandamide membrane transporter; 2-AG, 2-arachidonyl glycerol; BLA, basolateral amygdala; DAG, diacylglycerol; DSI/E, depolarization-induced suppression of inhibition/excitation; ERK, extracellular signal-regulated kinase; FAAH, fatty acid amide hydrolase; NAc, nucleus accumbens; PLC, phospholipase C; PPF, paired pulse facilitation; Δ^9-THC, Δ^9-tetrahydrocannabinol

Introduction

Preparations from the herb *Cannabis sativa* (such as marijuana, hashish and bhang) have been used across numerous cultures for thousands of years, and it is reasonable to say that this impressive history of use can be attributed in large part to profound effects of cannabis on mental state. There are commonly recognized euphoric or rewarding properties of cannabis (Maldonado & Rodriguez de Fonseca, 2002), but more negative consequences include impairments of attention, working memory (Hampson & Deadwyler, 1999) and executive function (Fried *et al*., 2002). These multiple behavioral effects are consistent with the findings that cannabinoids (the active constituents of cannabis, especially Δ^9-tetrahydrocannabinol or Δ^9-THC) have widespread actions upon neural function in the brain.

Recent years have seen a rapid series of discoveries about the targets and actions of cannabinoids, including the identification of cannabinoid receptors and their endogenous ligands, the endocannabinoids. This growing body of research has revealed numerous ways in which the endocannabinoid system functions to regulate fast synaptic transmission in multiple brain areas (Alger, 2002; Wilson & Nicoll, 2002). Important roles are emerging for endocannabinoid signaling in molecular pathways that underlie both transient and long-lasting alterations in synaptic strength (Alger, 2002). Thus, the critical involvement of endocannabinoids in some mechanisms of synaptic plasticity may refine current cellular models of learning and memory, and likewise these models may be pivotal in understanding both the rewarding and amnestic actions of cannabinoid drugs.

*Author for correspondence: Laboratory for Integrative Neuroscience, National Institute on Alcohol Abuse and Alcoholism, Room 158 H. Park 5 Building, 12420 Parklawn Drive, Rockville, MD 20852, U.S.A.; E-mail: lovindav@mail.nih.gov
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Synaptic plasticity

Synaptic plasticity–defined broadly as the dynamic adjustment of synaptic strength or efficacy–represents a general mechanism by which environmental or internal stimuli can alter brain neuronal responsiveness, such as for the storage of information gained through experience. The durability of such changes in synaptic strength is extremely variable, such that synaptic efficacy can fluctuate with time scales ranging from milliseconds to years. It is therefore not surprising that many different cellular and molecular processes have been implicated in the plasticity of synaptic function.

Long-term potentiation (LTP), a long-lasting increase in the strength of a synapse, and long-term depression (LTD), a long-lasting weakening of synaptic strength, are forms of synaptic plasticity that can persist for hours to weeks (Barnes & McNaughton, 1985). These phenomena have been studied extensively, and there is a large literature examining the roles of LTP and LTD in various forms of learning and memory that occur in different brain regions (see Martin *et al*., 2000; Kemp & Bashir, 2001; Silva, 2003; for review). It is now clear that LTP and LTD can be further subdivided based on the molecules involved in their induction and expression, as well as the synaptic locus of the primary change that underlies the alteration in efficacy. Some forms of plasticity are initiated and maintained by purely postsynaptic mechanisms, others by purely presynaptic mechanisms, and still others by mechanisms initiated in the postsynaptic neuron that are then communicated to the presynaptic neuron by the so-called retrograde messengers (Malinow *et al*., 2000; Kemp & Bashir, 2001; Tao & Poo, 2001). These retrograde messengers are molecules released from the postsynaptic neuron that participate in altering the presynaptic neurotransmitter release process. Recent studies have found that in multiple forms of synaptic plasticity, postsynaptically released endocannabinoids function as such a retrograde signal and are critical to the alteration of synaptic efficacy (Alger, 2002; Wilson & Nicoll, 2002). This review focuses primarily on certain subtypes of

LTD for which endocannabinoids are necessary, probably as retrograde messengers. In addition to these endocannabinoid-dependent LTD pathways, other forms of long-term synaptic plasticity have been shown to be disrupted by exogenous cannabinoid application and altered by endocannabinoids. Combining such observations, it is becoming clear that cannabinoid signaling functions as a widespread modulator of the molecular plasticity of brain synapses (Figure 1).

Cannabinoid receptors

The cannabinoid receptors are G-protein-coupled, heptahelical receptors and number at least two–the CB_1 and CB_2 receptors–which have been extensively characterized (see Howlett *et al*., 2002; for review). The CB_1 cannabinoid receptor was originally identified as a binding site for Δ^9-THC and synthetic cannabimimetic compounds, and this receptor is abundantly expressed in the mammalian brain. The CB_2 receptor is expressed mostly in the periphery, where it has known roles in the immune system. There is also pharmacological evidence for at least two other metabotropic receptors that respond to cannabinoid compounds (Di Marzo *et al*., 2002; Freund *et al*., 2003), including a unique cannabinoid receptor that appears to modulate excitatory synaptic transmission in the hippocampus (Hajos & Freund, 2002). With the caveat that these putative receptors may share some pharmacological similarity to CB_1, the CB_1 receptor is believed to mediate most of the effects described in this review. The CB_1 receptor is coupled predominantly to G-proteins of the $G_{i/o}$ class. Thus, among its cellular actions are inhibition of adenylate cyclase (AC), inhibition of voltage-gated calcium channels, activation of GIRK-type potassium channels, and inhibition of synaptic transmission (Howlett *et al*., 2002; Freund *et al*., 2003). In addition, the CB_1 receptor activates several neurochemical pathways, including increasing phosphorylation of the MAP kinase extracellular signal-regulated kinase. Such downstream effectors of the CB_1 receptor, as well as some reported receptor-independent actions of anandamide (*n*-arachidonyl ethanolamide or AEA) (Chemin *et al*., 2001; Maingret *et al*., 2001), are quite likely to influence some forms of synaptic plasticity, but discussion of these possibilities goes beyond the scope of the present review.

What are endocannabinoids?

The endocannabinoids are lipid signaling molecules that bind to and activate cannabinoid receptors. These compounds are formed from phospholipid precursors within cells throughout the body, and are released from these cells in a nonvesicular manner (Wilson & Nicoll, 2002; Freund *et al*., 2003) to act in a juxtacrine or paracrine fashion. Two prominent endocannabinoids have been discovered to date (Mechoulam, 2002). AEA is believed to be made from phosphatidylethanolamine *via* a two-step synthesis involving an acyltransferase step followed by cleavage of the lipid by phospholipase D (Di Marzo *et al*., 1994; Freund *et al*., 2003). Notably, there is evidence for calcium dependence in both of these synthesis steps, which may underlie the requirement for postsynaptic Ca^{2+} in certain forms of synaptic plasticity (see below). AEA is metabolized to arachidonic acid and ethanolamine *via* the action of the fatty acid amide hydrolase (FAAH), and this activity plays a significant role in the rapid clearance of AEA from extracellular compartments (Deutsch *et al*., 2001; Glaser *et al*., 2003).

The second widely recognized endogenous CB_1 agonist is 2-arachidonyl glycerol (2-AG). This endocannabinoid can be formed in at least two molecular pathways, both of which involve the degradation of arachidonate-containing lipids by phospholipase C (PLC) activities (Freund *et al*., 2003). 2-AG, like AEA, is found in a variety of tissues throughout the body and brain, and appears to be released from cells in response to certain stimuli. 2-AG activates the CB_1 receptor with greater efficacy than does AEA, but in general less is known to date about the actions of 2-AG at the cell and tissue levels in comparison to what is known for AEA. A recent, intriguing study indicates that a previously characterized monoglyceride lipase is responsible for degradation of 2-AG (Dinh *et al*., 2002). It should be noted that other related lipids with endocannabinoid activity have been isolated from brain tissue (Mechoulam, 2002; Freund *et al*., 2003), but to date, there is

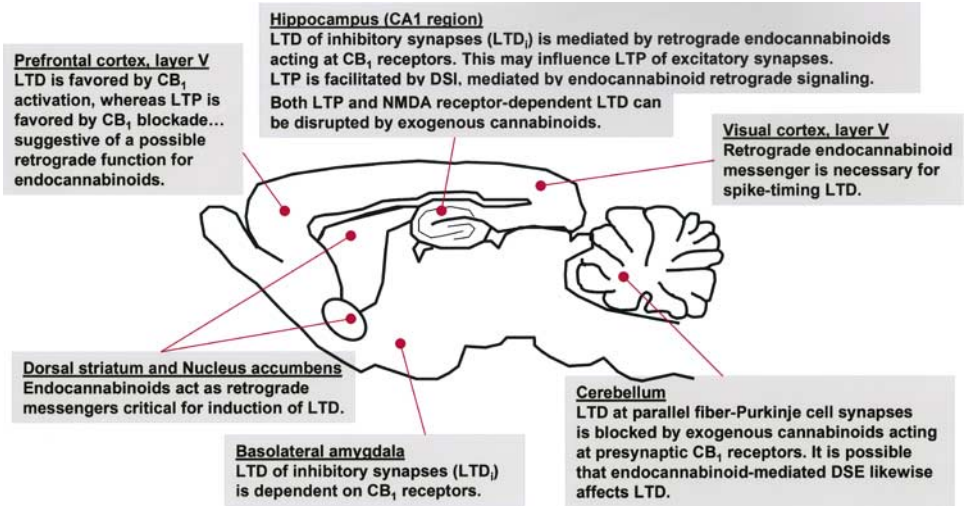

Figure 1 Schematic sagittal view of the rat brain, highlighting areas in which long-term synaptic plasticity is known to involve, or be influenced by, cannabinoid signaling.

little evidence about the physiological actions of these compounds. The most recently discovered endocannabinoid, virodhamine, appears to act as a partial CB_1 agonist that may behave as an antagonist to AEA signaling in the brain (Porter *et al*., 2002). Lastly, both AEA and 2-AG act as agonists at the vanniloid TRPV1 receptor, a nonspecific cation channel that is involved in pain perception (Di Marzo *et al*., 2002) and may also modulate synaptic transmission (Marinelli *et al*., 2003). Thus, the broad influence of the endocannabinoid system extends beyond the traditional cannabinoid receptors.

Endocannabinoids as a retrograde signaling system – how can we tell?

Perhaps, the first suggestion that endocannabinoids might act in a retrograde manner at synapses was based on a comparison between the subcellular localizations of CB_1 receptors (mostly presynaptic) and the FAAH enzyme (mostly postsynaptic) in the rat brain (Egertova *et al*., 1998). A key element of such a retrograde signaling model is that the activation of presynaptic cannabinoid receptors would serve to modulate neurotransmitter release. Indeed, presynaptic inhibition has now been shown to be a widespread function of CB_1 receptors in a number of brain regions (Schlicker & Kathmann, 2001; Doherty & Dingledine, 2003). By using techniques of single-cell, voltage-clamp electrophysiology, several measures can be used to demonstrate a presynaptic depression of transmission, either as an effect of agonist application or as a mechanism for expression of some forms of LTD. These measures include an increase in paired pulse facilitation (PPF), an increase in the coefficient of variation (cv) of transmission, and a decrease in the frequency of miniature synaptic responses with no change in their amplitude. Increases in PPF, a widely used measure, likely indicate decreased probability of neurotransmitter release associated with presynaptic depression. Also, all of the above-listed changes are classically associated with decreased quantal release at presynaptic sites, although it must be noted that alternative interpretations of each of these observations have been postulated (Clements, 1993; Nicoll & Malenka, 1999). For a more thorough review of the theoretical underpinnings of these measures, we refer the reader elsewhere (Clements, 1993). Suffice it here to say that when a presynaptic change in transmission occurs following a postsynaptic signaling event, this becomes evidence for a physiological retrograde messenger.

The first functional evidence – DSI and DSE

Among the most definitive examples of a change in transmission that requires a retrograde message is the phenomenon known as depolarization-induced suppression of inhibition (DSI) (Llano *et al*., 1991). This title refers to an experimental observation in which depolarization of a postsynaptic neuron produces a short-lasting suppression of GABAergic inhibitory synaptic transmission. This suppression, or disinhibition, is due to a presynaptic action that reduces GABA release (Pitler & Alger, 1994). Recent studies have strongly implicated endocannabinoids as the retrograde messenger, and activation of presynaptic CB_1 receptors as the mechanism of disinhibition (Alger, 2002; Wilson & Nicoll, 2002). Interestingly, DSI in hippocampal pyramidal neurons can also be triggered by the activation of metabotropic glutamate receptors (mGluRs) (Varma *et al*., 2001) or muscarinic acetylcholine receptors (Kim *et al*., 2002), presumably acting on the postsynaptic neuron to stimulate the formation and release of the endocannabinoid.

DSI has now been observed in several brain regions (Alger, 2002), and DSE, a similar depression of excitatory transmission, has also been observed (Kreitzer & Regehr, 2001; Maejima *et al*., 2001; Ohno-Shosaku *et al*., 2002). These short-term mechanisms of synaptic plasticity represent the first demonstrations of a retrograde signaling function for endocannabinoids, and may serve, among other probable roles, to influence longer-lasting modes of synaptic plasticity (Carlson *et al*., 2002). However, space does not permit further elaboration of the mechanisms of DSI and DSE, which have been the focus of excellent recent reviews (Alger, 2002; Wilson & Nicoll, 2002; Freund *et al*., 2003).

Evidence for endocannabinoid retrograde messengers in LTD – dorsal striatum

Based on the measures described briefly above, Choi & Lovinger (1997a, b) demonstrated that LTD at corticostriatal synapses is expressed as a presynaptic decrease in the probability of glutamate release. Initiation of this form of LTD depends on postsynaptic depolarization and increased postsynaptic intracellular Ca^{2+} (Calabresi *et al*., 1992; Choi & Lovinger, 1997a, b), and thus a retrograde messenger was postulated. Several aspects of the induction of corticostriatal LTD led to an investigation of endocannabinoids as a candidate for such a messenger. First and foremost was the critical role of postsynaptic intracellular Ca^{2+}, because there is strong evidence that AEA synthesis is stimulated by Ca^{2+} signaling (Di Marzo *et al*., 1994; Freund *et al*., 2003). Moreover, striatal medium spiny neurons grown in culture had been shown to synthesize and release AEA, in a Ca^{2+}-dependent manner, in response to depolarizing stimuli (Di Marzo *et al*., 1994). Furthermore, striatal LTD is dependent on activation of D2 (as well as D1) dopamine receptors. Accordingly, Giuffrida *et al*. (1999) found that both depolarization and D2 receptor activation led to an increased detection of AEA measured in the dorsal striatum of rats *in vivo*, and that these effects were additive. Finally, there was emerging evidence that presynaptic CB_1 receptors modulate transmission at corticostriatal synapses (Gerdeman & Lovinger, 2001; Huang *et al*., 2001), further suggesting that the commonalities between endocannabinoid synthesis and striatal LTD were related to the need for a retrograde messenger (Figure 2).

Using both gene-targeted, CB_1 receptor-deficient mice, and the CB_1 receptor antagonist SR141716A, we showed that LTD was eliminated in the absence of CB_1 receptor activity (Gerdeman *et al*., 2002a). However, these findings alone do not constitute sufficient evidence for a retrograde signaling role of an endocannabinoid. Two other pieces of evidence reinforced this hypothesis. First, we observed that blockade of LTD by filling the postsynaptic neuron with EGTA (Choi & Lovinger, 1997a, b) could be reversed by extracellular application of the cannabinoid reuptake inhibitor AM404 (Gerdeman *et al*., 2002a). This finding suggests that an endocannabinoid retrograde messenger involved in LTD is normally formed and released from the postsynaptic neuron and if its formation is impaired then LTD cannot occur.

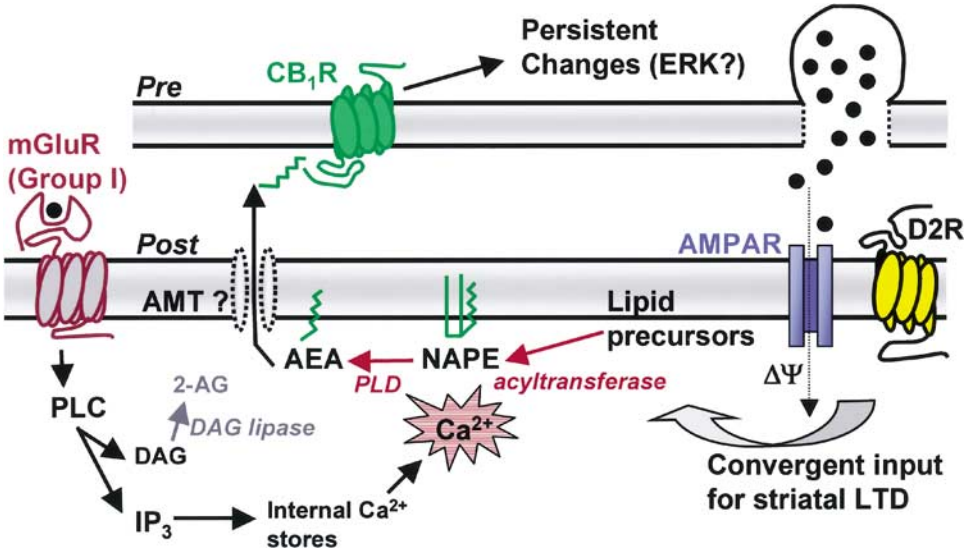

Figure 2 An abbreviated model for endocannabinoid synthesis and release during LTD in the striatum and NAc. In both areas, LTD is expressed presynaptically, due to the activity-dependent release of endocannabinoids as retrograde messengers. Activation of group I mGluRs is necessary for this plasticity, probably as a means for elevating postsynaptic Ca^{2+} (L-type voltage-gated Ca^{2+} channels are also involved in dorsal striatum). Ca^{2+}-dependent pathways of AEA synthesis are shown with red arrows. D2 dopamine receptors are also necessary for LTD in the dorsal striatum, where D2 receptors stimulate formation of AEA, especially in conjunction with depolarizing stimuli. Note that the activation of PLC by mGluRs may also lead to the formation of 2-AG through a Ca^{2+}-independent DAG lipase activity. The efflux of AEA or 2-AG from striatal neurons may involve an AMT, although this is controversial. Symbols and abbreviations: AMPAR, AMPA subtype glutamate receptor; DAG, diacylglycerol; IP_3, inositol trisphosphate; NAPE, *n*-acylphosphatidylethanolamine; PLD, NAPE-specific phospholipase D; $\Delta\Psi$, depolarization; ●, glutamate.

However, if reuptake of extracellular endocannabinoids is blocked, then presumably endocannabinoids that are released from neighboring cells can 'spill-over' to act on synapses onto the EGTA-filled cell. This observation reinforces the idea that local release of an endocannabinoid is a key step in LTD induction. We also demonstrated that filling the postsynaptic neuron with AEA produced synaptic depression that resembled LTD, indicating that this endocannabinoid can act as a retrograde messenger (Gerdeman *et al*., 2002a).

Nucleus accumbens

Studies from other brain areas have found remarkably similar roles for endocannabinoids in the induction of LTD. Manzoni and co-workers, examining glutamatergic synapses made by afferents from the prelimbic cortex in the nucleus accumbens (NAc) demonstrated that LTD produced by prolonged, moderate-frequency stimulation (10 min at 13 Hz) was blocked by CB_1 antagonists and eliminated in the CB_1 knockout mouse (Robbe *et al*., 2002). The demonstration that this form of LTD was blocked by postsynaptic Ca^{2+} chelation and involved a presynaptic expression mechanism was consistent with the idea that an endocannabinoid retrograde signal was involved in this form of plasticity. Importantly, LTD was blocked by interfering with postsynaptic signaling mediated by group I mGluRs, and conversely, the mGluR agonist DHPG was reported to cause an LTD-like synaptic depression that was prevented by SR141716A. Thus, postsynaptic endocannabinoid release in the NAc appears to be downstream from an mGluR-induced elevation in Ca^{2+} (see Figure 2 and below). These authors also reported that preincubation of slices with either WIN 55,212-2, a CB_1 agonist, or with AM404 caused a synaptic depression that occluded subsequent induction of LTD by a 13 Hz train. This finding suggests overlapping mechanisms between CB_1 agonist effects and LTD, such that CB_1 activation may be sufficient on its own to induce LTD in the NAc. An alternative interpretation, however, is that LTD was prevented by low glutamatergic drive due to presynaptic inhibition (as in cerebellum and hippocampus; see below).

Recently, Hoffman *et al*. (2003) have repeated the observation that endocannabinoid-dependent LTD occurs in the NAc, using a stimulus paradigm (5 min at 10 Hz) fairly similar to that used by Robbe *et al*. (2002). In addition, these authors have found that LTD is disrupted in rats following chronic treatment with Δ^9-THC. Also in these rats, glutamatergic transmission in the NAc was less sensitive to exogenous cannabinoids, indicating a functional desensitization of cannabinoid receptors (Breivogel *et al*., 1999), which is a probable mechanism for the loss of stimulus-induced LTD.

Visual cortex, spike-timing plasticity

LTD at another excitatory, glutamatergic synapse also appears to involve an endocannabinoid retrograde signal. The term spike-timing plasticity refers to long-lasting changes in the efficacy of transmission that are brought about in thick-tufted layer-V neurons of the visual cortex by paired action potential firing in pre- and postsynaptic neurons. The direction of plasticity, LTP vs LTD, depends on the relative timing, such that LTD is elicited when postsynaptic firing precedes presynaptic firing (Sjostrom & Nelson, 2002). This form of LTD, which depends on postsynaptic activation (including Ca^{2+} signaling) and appears to be expressed presynaptically, is blocked by CB_1 antagonists (Sjostrom *et al*., 2003). Moreover, CB_1 agonists produce presynaptic inhibition at these synapses and allow LTD to occur without postsynaptic spiking

(Sjostrom *et al*., 2003). When either endocannabinoid uptake or the FAAH enzyme was blocked, the LTD-permissive time window between postsynaptic and presynaptic spiking was significantly increased (Sjostrom *et al*., 2003). Thus, it appears that spike-timing LTD also involves an endocannabinoid retrograde signal, which sets the critical time window determining the direction of synaptic plasticity at this cortical synapse.

Endocannabinoid-mediated LTD of inhibitory inputs – basolateral amygdala

Endocannabinoids have also been implicated in LTD within the basolateral nucleus of the amygdala (BLA), only in this case the lasting decrease in efficacy takes place at a GABAergic inhibitory synapse onto principal neurons of the BLA (Marsicano *et al*., 2002). This LTD of inhibitory inputs (LTD_i) is induced by low-frequency (1 Hz) stimulation, and PPF evidence indicates that expression of this form of plasticity involves a presynaptic decrease in neurotransmitter release. LTD_i may be functionally related to the extinction of aversive memories, since Marsicano *et al*. (2002) found that this process is dependent on CB_1 receptor activation, and that re-exposure to an aversive conditioned-stimulus (a tone previously learned to predict a foot shock) results in a specific increase in the endocannabinoid content of the BLA. However, many molecular details of LTD_i in the BLA remain to be elucidated, such as possible postsynaptic induction mechanisms or the involvement of other neurotransmitter receptors that may influence endocannabinoid release. Thus, it remains inconclusive that the role of endocannabinoids in LTD_i is as a retrograde messenger, but this is a tempting possibility given the presynaptic expression and function of CB_1 receptors in the BLA (Katona *et al*., 2001; Azad *et al*., 2003).

Heterosynaptic LTD_i of the hippocampus

A second form of endocannabinoid-dependent LTD_i has also been recently demonstrated, with elegant mechanistic detail that links excitatory neurotransmission and postsynaptic mGluRs with a heterosynaptic depression of GABA release from cannabinoid-sensitive interneurons in the *stratum radiatum* of the hippocampus (Chevaleyre & Castillo, 2003; note that 'LTD_i' is being used here for consistency, rather than 'I-LTD' as originally reported). Chevaleyre & Castillo (2003) showed that two brief 100 Hz trains, activating glutamatergic afferents, induced LTD_i that was blocked by a CB_1 receptor antagonist, and was mutually occlusive of presynaptic inhibition caused by the CB_1 agonist WIN 55,212-2. Heterosynaptic LTD_i was dependent on group I mGluRs, was mimicked and occluded by application of DHPG, and both forms of depression were dependent on endocannabinoid signaling (Chevaleyre & Castillo, 2003). However, LTD_i was not blocked by postsynaptic BAPTA, indicating that the requisite endocannabinoid synthesis was not dependent on Ca^{2+}, but LTD_i was prevented by inhibitors of DAG lipase, applied intracellularly through the patch pipette. Thus, synaptic activation of group I mGluRs on hippocampal pyramidal neurons can lead to the Ca^{2+}-independent formation of 2-AG, which then acts as a retrograde messenger binding to CB_1 receptors on GABAergic axon terminals.

Commonalities in induction of endocannabinoid-dependent LTD: importance of mGluRs and Ca^{2+}

These forms of LTD have more in common than just postsynaptic initiation and presynaptic expression. Increased postsynaptic Ca^{2+} is a common mechanism in the types of LTD described here for excitatory synapses. As has been determined from studies of DSI and DSE, postsynaptic Ca^{2+} appears to be a primary trigger for endocannabinoid synthesis, and perhaps release (Alger, 2002; Freund *et al*., 2003). However, Ca^{2+}-independent mechanisms of endocannabinoid synaptic release have also been reported (Maejima *et al*., 2001; Kim *et al*., 2002). As mentioned, this appears to be the case in hippocampal LTD_i, even though the induction of endocannabinoid-mediated DSI in the same neurons was strongly dependent on Ca^{2+} signaling (Chevaleyre & Castillo, 2003). Experiments testing the role of Ca^{2+} in LTD_i of the BLA have not been reported.

Activation of certain metabotropic receptors, in particular the group I mGluRs, also appears to play a prominent role in these endocannabinoid-mediated forms of synaptic plasticity (Doherty & Dingledine, 2003). It has been known since 1992 that corticostriatal LTD requires activation of mGluRs, and recent evidence suggests involvement of the group I subclass, with mGluR1 being an especially attractive candidate (Calabresi *et al*., 1992; Gubellini *et al*., 2001; Sung *et al*., 2001). Manzoni and co-workers have also implicated group I mGluRs, in particular mGluR5, in endocannabinoid-dependent NAc LTD (Robbe *et al*., 2002). It is known that group I mGluRs can stimulate rises in postsynaptic Ca^{2+} *via* activation of PLC, and accordingly, Robbe *et al*. (2002) prevented LTD in the NAc by blocking the activation of Ca^{2+}-releasing ryanodine receptors that would be downstream from PLC. However, it is also possible that group I mGluR stimulation of DAG lipase or other phospholipase activities may participate in LTD induction in a Ca^{2+}-independent manner, as demonstrated for LTD_i in the hippocampus (Chevaleyre & Castillo, 2003). Nonetheless, while mGluRs, the CB_1 receptor and LTD appear to be linked in many circumstances, mGluR antagonists were reportedly without effect on spike-timing LTD of the visual cortex (Sjostrom *et al*., 2003).

There are other subtle, but important, differences in the mechanisms of induction of the different endocannabinoid-dependent forms of LTD observed in different brain regions. For example, corticostriatal LTD is dependent on dopamine and activation of D2 dopamine receptors (Calabresi *et al*., 1992; Tang *et al*., 2001), while endocannabinoid-mediated LTD in the NAc does not involve dopamine (Robbe *et al*., 2002). Presynaptic NMDA receptors have been implicated in spike-timing LTD in visual cortex (Sjostrom *et al*., 2003), but appear not to be involved in the endocannabinoid-mediated forms of LTD observed in NAc, striatum or hippocampus (Calabresi *et al*., 1992; Choi & Lovinger, 1997b; Robbe *et al*., 2002; Chevaleyre & Castillo, 2003). Finally, the patterns of synaptic activation that have been used to evoke these forms of LTD differ in different brain regions, with stimulus frequencies varying from 0.1 Hz (Sjostrom *et al*., 2003) to 100 Hz (Gerdeman *et al*., 2002a; Chevaleyre & Castillo, 2003). The reasons for these differences are not clear at this time, but may reflect the need for different patterns of release of glutamate and other neurotransmitters that are necessary to stimulate

endocannabinoid formation and release at the different synapses (Freund *et al*., 2003).

Cannabinoid effects on plasticity of excitatory synapses in the hippocampus, cerebellum and prefrontal cortex

A number of studies have found that cannabinoids disrupt or otherwise influence synaptic plasticity, with or without special relevance to endocannabinoid signaling. For example, it has been demonstrated by multiple groups that application of CB agonists can prevent induction of LTP at Schaffer collateral/commissural synapses onto CA1 pyramidal neurons in hippocampal slices (see Alger 2002; for review). This result suggests that ingestion of exogenous cannabinoid drugs may alter learning and memory through disruption of this form of plasticity. Cannabinoid inhibition of LTP appears to be due predominantly to a decrease in neurotransmitter release taking place during LTP-inducing high-frequency stimulation, presumably *via* activation of CB receptors on these glutamatergic afferent presynaptic terminals (Misner & Sullivan, 1999). It stands to reason that any modulatory neurotransmitter capable of inhibiting synaptic transmission in this way would disrupt LTP in a manner similar to cannabinoid agonist treatment. Thus, this LTP inhibiting action is likely not a unique action of cannabinoid drugs, but given the widespread presynaptic expression of CB_1 receptors throughout the brain, it may be a common mechanism by which cannabinoids regulate multiple forms of synaptic plasticity. For example, similar mechanisms appear to explain why cannabinoids also inhibit LTD at parallel fiber–Purkinje neuron synapses in the cerebellum (Levenes *et al*., 1998), as well as NMDA receptor-dependent LTD in the hippocampus (Misner & Sullivan, 1999).

While these studies demonstrate that exogenous cannabinoid agonists applied continuously to brain slices can inhibit these forms of synaptic plasticity, the manner in which endocannabinoids may serve to regulate the induction of LTP and LTD in these areas *in vivo* remains largely speculative. However, a recent intriguing study by Alger and co-workers suggested that the transient release of endocannabinoids in response to depolarization, causing DSI and thus briefly disinhibiting the pyramidal neuron, would serve to facilitate LTP of excitatory inputs (Carlson *et al*., 2002). Specifically, these authors showed that LTP can be induced by stimulation that is normally insufficient to do so, provided that stimulation is preceded by DSI induction. The authors proceeded to demonstrate that this LTP-enhancing effect depended on CB_1 receptor activation and disinhibition. This study demonstrates that the role of endocannabinoids may be to enhance selectively plasticity at particular Schaffer collateral–CA1 synapses. It is worth noting that Collingridge and co-workers reported that activation of $GABA_B$ receptors on presynaptic interneuron terminals promotes LTP through a similar disinhibitory action (Davies *et al*., 1991). While the notion has been challenged that DSI actually occurs at these synapses in response to physiologically relevant stimuli (Hampson *et al*., 2003), a very similar (but more persistent) function may exist for endocannabinoid-mediated LTD_i. In support of this idea, Chevaleyre & Castillo (2003) demonstrated that increased neuronal excitability (measured as 'E–S coupling') following a high-frequency stimulus and normally associated with the induction of LTP in pyramidal neurons, is blocked by antagonists to either the CB_1 receptor or the mGluRs1/5 that appear to promote endocannabinoid release. Therefore, multiple mechanisms exist whereby prolonged exposure to exogenous cannabinoid receptor agonists, during cannabis use for example, would tend to inhibit plasticity of excitatory pathways in the hippocampus, an effect that might underlie effects of Δ^9-THC on short-term memory.

Lastly, two studies of LTD and LTP in slices of rodent prefrontal cortex (PFC) indicate that bath application of cannabinoids facilitates LTD, at the expense of LTP (Auclair *et al*., 2000; Barbara *et al*., 2003). Conversely, blockade of CB_1 receptors using the antagonist SR141716A led to an increased likelihood of observing LTP, although LTD was not entirely absent. Thus, the endocannabinoid system may serve to promote LTD in layer-V pyramidal neurons of the PFC, without being absolutely necessary for the phenomenon. It stands to reason that a natural balance between LTD and LTP in the PFC, regulated by endocannabinoids, could be significantly altered by exogenous cannabinoid compounds, either agonists or antagonists.

Discussion

Recent studies described in this review demonstrate that the endocannabinoid system influences processes of long-lasting synaptic plasticity in multiple brain areas, either as a potential regulator of these pathways or as a mechanism for transducing a retrograde synaptic message necessary in certain forms of LTD. Starting with the discovery that endocannabinoids mediate hippocampal DSI, these molecules have rapidly become the foremost example of retrograde signaling in the mammalian brain. It should be stated, however, that a retrograde signaling mechanism is difficult to prove conclusively in LTD and LTP, where many neurotransmitters are active and where methods of induction are more complicated than, for example, inducing DSI by injecting current into a single cell. These studies are very compelling however, especially given the commonalities among them and consistency with what is known about endocannabinoid synthesis and presynaptic function of CB_1 receptors.

It is however, not the intention of this review to detract from potentially important postsynaptic effects of cannabinoids, such as regulation of cAMP levels and activation of MAP kinase pathways. Also, questions remain as to what cellular effectors, downstream of CB_1 activation, are responsible for inducing LTD in certain systems. Some intriguing observations have been made regarding the CB_1 receptor, including a capacity to sequester G proteins from other neurotransmitter receptors (Vasquez & Lewis, 1999). Conversely, coactivation of D2 and CB_1 receptors in isolated striatal neurons was reported to induce a shift in the G-protein transduction of the CB_1 receptor, causing it to stimulate AC activity *via* G_s (Glass & Felder, 1997). Further study of these distinctive signaling pathways could provide hints as to why activation of the CB_1 receptor appears to be so prominently associated with long-lasting alterations in presynaptic function.

Furthermore, many questions remain to be elucidated regarding the cellular regulation of endocannabinoid synthesis, release and reuptake. It appears that numerous receptor systems can stimulate the formation of AEA or 2-AG, including mGluRs, muscarinic and nicotinic acetylcholine

receptors, and dopamine D2 receptors (see Freund *et al*., 2003; for review). This list is likely to grow, since any pathway that activates PLC or raises intracellular Ca^{2+} could hypothetically stimulate endocannabinoid synthesis.

Cannabinoid uptake and release

The mechanisms by which endocannabinoids travel across cellular membranes and synapses are of profound interest for further study. Such mechanisms represent sites of regulating numerous endocannabinoid-mediated processes, either on the physiological level or through pharmacological intervention for the treatment of disease. As mentioned above for AEA, the enzymatic inactivation of endocannabinoids by FAAH appears to play a substantial role in governing the rate of cellular uptake of these compounds (Deutsch *et al*., 2001; Glaser *et al*., 2003). In addition, there is evidence supporting the existence of an AEA membrane transporter (AMT) that moves both AEA and 2-AG across membranes (Piomelli *et al*., 1999; Freund *et al*., 2003), perhaps in a bidirectional fashion (Hillard & Jarrahian, 2000). In the brain, the AMT appears to be important in the removal of endocannabinoids from the synapse, raising the converse possibility that an AMT-like activity is responsible for endocannabinoid efflux in response to relevant cellular activation. We have obtained preliminary evidence for this model, in that AMT blockers such as AM404, which are pharmacologically characterized as competitive substrates for transport (Piomelli *et al*., 1999), appear to prevent the induction of corticostriatal LTD when these agents are applied intracellularly *via* a whole-cell patch electrode (Gerdeman *et al*., 2002b; and unpublished observations). However, there is still little information about the molecular characteristics of a putative AMT, and a recent biochemical study of AEA hydrolysis argues that the existence of an AMT is not necessary to explain the transport of lipophilic endocannabinoids across membranes (Glaser *et al*., 2003). If indeed the AMT is real, it is not clear if this is an energy-independent protein transporter (Hillard & Jarrahian, 2000) or some lipid domain specialized for transmembrane transport. Elaboration of this controversy through future studies will be necessary to refine our understanding of endocannabinoid biology as it relates to synaptic function.

Physiological relevance for learning, memory and development

Much research over the last 20 years has focused on the relevance of long-lasting synaptic plasticity as a model for learning and memory (Martin *et al*., 2000). This general hypothesis has appeared to strengthen with the emergence of sophisticated genetic techniques that have allowed investigators to manipulate single genes and observe the contribution of these genes to both memory and synaptic plasticity (Silva, 2003). The cannabinoid system provides a relatively new focus for this avenue of research. In accordance with the described mechanisms by which cannabinoids mediate or disrupt LTD and LTP, it is known that CB_1 receptor agonists impair certain memory functions, especially involving the hippocampus (Hampson & Deadwyler, 1999; Freund *et al*., 2003). Moreover, genetic deletion of the CB_1 receptor has been reported to improve performance of some learning tasks in rodents (Reibaud *et al*., 1999), as well as enhancing hippocampal LTP (Bohme *et al*., 2000).

It is also important to note that addiction can be viewed as a complex process of learning and memory. Growing evidence supports the congruent notion that mechanisms of long-term synaptic plasticity are involved in the molecular and cellular development of addictive behaviors (Berke & Hyman, 2000), and processes of LTD in the striatum may be of particular relevance (Gerdeman *et al*., 2003). This is intriguing in the light of recent behavioral studies, which have employed multiple rodent models to show that CB_1 receptor signaling is involved in the chronic intake of ethanol (Hungund *et al*., 2002; Wang *et al*., 2003). Such an endocannabinoid involvement in addiction-related neural plasticity (Gerdeman *et al*., 2003) may indicate a therapeutic role for cannabinoid-based medicines in the treatment of certain addictions, as suggested by the ability of cannabinoid agonists and uptake blockers to mitigate symptoms of opiate withdrawal in rodents (Vela *et al*., 1995; Yamaguchi *et al*., 2001; Del Arco *et al*., 2002).

Various expressions of synaptic plasticity, including corticostriatal LTD (Tang *et al*., 2001), are thought to play roles in the cellular and synaptic organization that occurs during development. In addition to endocannabinoid-dependent mechanisms of LTD, some investigators have reported that the CB_1 receptor plays important roles in synapse formation (Kim & Thayer, 2001) and growth cone guidance (Williams *et al*., 2003) *in vitro*. Such observations may involve processes similar to those of LTD and LTP reviewed here, and they should be included in a general picture of how the endocannabinoid system might influence synaptic organization *in vivo*. Thus, while the cannabinoid system represents a target for numerous potential therapeutic approaches, the widespread mechanisms of synaptic plasticity that utilize these endogenous pathways are likely to mediate a diversity of functions that are important to mental state and behavior. This should be kept in mind when considering the clinical use of cannabinoid antagonists, especially in children.

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