Presynaptic Mechanisms of Endocannabinoid-Mediated Long-Term Depression in the Hippocampus

Carlos A. Lafourcade1,2

1Department of Physiology, Program in Neuroscience, University of Maryland School of Medicine, Baltimore, Maryland; and 2Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina

Lafourcade CA. Presynaptic mechanisms of endocannabinoid-mediated long-term depression in the hippocampus. J Neurophysiol 102: 2009–2012, 2009. First published June 24, 2009; doi:10.1152/jn.00441.2009. Endogenous cannabinoids (eCBs) are membrane-derived lipid molecules that target the same G-protein–coupled cannabinoid-1 receptor (CB1R) in the CNS as the main active ingredient (Δ²-THC) of the cannabis plant, Cannabis sativa. Many roles for eCBs have been discovered and, through their effects in the CNS, they influence stress and anxiety, feeding, neuroprotection, and learning and memory (for reviews see Alger 2002; Chevaleyre et al. 2006; Kano et al. 2009).

The mechanism by which eCBs could be exerting some of these actions on the CNS remained a mystery until 2001, when three independent research groups (Kreitzer and Regehr 2001; Ohno-Shosaku et al. 2001; Wilson and Nicoll 2001) discovered that eCBs are intercellular signaling molecules. Interestingly, eCBs are not released from presynaptic terminals acting on postsynaptic sites like typical neurotransmitters, but instead are produced and mobilized (it is not clear how they are synthesized or released) from the postsynaptic cell, and travel backward across the synapse to act on presynaptic terminals (Alger 2002). These “retrograde” messengers bind CB1Rs and transiently inhibit neurotransmitter release from the presynaptic terminal. This discovery brought together two large scientific fields of study: one group investigating eCBs and their receptors in the brain and another group of synaptic physiologists seeking to identify the retrograde messenger known to inhibit presynaptic neurotransmitter release. Since then, the mechanisms of eCB production, mobilization, receptor binding, and block of neurotransmitter release have been vigorously studied by many laboratories.

In the hippocampus, eCBs can be mobilized from the postsynaptic cell to suppress neurotransmitter release from presynaptic cells by repetitive action potential firing or strong depolarization, through a poorly understood mechanism that depends on elevated intracellular calcium ion concentration ([Ca²⁺]). This process has been named “depolarization-induced suppression of inhibition” (DSI), which refers specifically to the inhibition of γ-aminobutyric acid (GABA) release from presynaptic interneurons. Depolarization-induced suppression of excitation (DSE) refers to the eCB-mediated inhibition of glutamate release from excitatory nerve terminals. eCBs can also be mobilized through the activation of G-protein–coupled receptors, including group I metabotropic glutamate receptors (mGluRs), comprised of mGluR1 and mGluR5 (Nakanishi 1992), and M1/M3 muscarinic acetylcholine receptors (mAChRs). Membrane phospholipids are metabolized into inositol triphosphate and diacylglycerol, which are then converted into the eCB 2-arachidonyl glycerol (2-AG) by diacylglycerol lipase. G-protein–coupled eCB mobilization is less sensitive than DSI to increases in postsynaptic [Ca²⁺], and the molecular mechanisms seem to be different from those of DSI and DSE (Hashimotodani et al. 2005).

Whereas DSI and DSE result in short-term synaptic plasticity, typically inhibiting neurotransmitter release for only a few seconds, eCBs also mediate long-term synaptic changes. These synaptic changes always involve the depression of neurotransmitter release. When that neurotransmitter is GABA, this phenomenon is called eCB–iLTD (long-term depression of inhibitory inputs) and eCB–LTD when the inputs affected are excitatory. Although both short- and long-term eCB-mediated inhibition involve the retrograde action of eCBs on the same CB1Rs, important differences must exist to explain the very different temporal properties of the responses. eCB–iLTD requires prolonged mGluRI activation, but cannot be produced by prolonged mAChR activation, prolonged depolarization, or prolonged CB1R activation alone (Edwards et al. 2006; but see recent work of Heifets et al. 2008, discussed in the following text). Once established, eCB–iLTD maintenance does not require the continuous activation of CB1Rs or mGluRs. This suggests that postsynaptic mGluRI activation results not only in eCB mobilization, but also in long-term molecular changes in the presynaptic cell that inhibit vesicle release.

The physiological significance of eCB–iLTD, a process that occurs widely throughout the brain (e.g., hippocampus, amygdala, striatum, neocortex, and cerebellum), is beginning to emerge. It has been proposed that eCBs could help strengthen synaptic changes relevant for the consolidation of certain types of memories. Long-term potentiation (LTP) is a long-lasting (hours to days) increase in signaling strength of glutamatergic synapses and is believed to be the cellular event that occurs during learning and memory formation in the hippocampus. eCB-mediated depression of GABA release may increase the excitability of postsynaptic cells and facilitate or modulate the induction of LTP. A role for eCB–iLTD on specific behaviors has also been proposed. In the amygdala, eCB–iLTD may be relevant for the extinction of aversive memories (Marsicano et al. 2002). In these experiments, wild-type animals first learn to associate a tone (conditioned stimulus) with a foot shock and
continue to freeze (the fear response) for an extended time even when no foot shock follows the tone. Eventually, however, the fear response is reduced with time (extinguished) when not coupled with the conditioned stimulus. In CB1R knockout animals (or in those injected with a CB1R antagonist) the time for memory extinguishing was prolonged and the animals continued to freeze whenever the tone was played.

Although behavioral studies have implicated eCB–iLTD in important phenomena, little was known about the physiological mechanisms underlying this process. Several recent studies have deepened our understanding of the molecular processes necessary for the induction of eCB–iLTD. Chevaleyre and Castillo (2003) first reported the occurrence of eCB–iLTD in the hippocampus. Since eCB–iLTD expression does not require continued eCB production, it was natural to look for changes in the presynaptic terminal that were responsible for maintenance of the long-term decrease in the release of GABA. CB1R activation seems to trigger two different biochemical cascades that inhibit GABA release. With DSI, CB1Rs inhibit adenylyl cyclase, thereby preventing the activation of protein kinase A (PKA; Chevaleyre et al. 2007). PKA is a cyclic AMP (cAMP)–dependent protein kinase that mediates a broad array of biological processes (e.g., cell development, synaptic plasticity, and cellular metabolism) by the reversible phosphorylation of diverse proteins. By blocking the effects of presynaptic PKA, eCB–iLTD is prevented, although DSI is not. The machinery for producing eCBs and activating CB1Rs remained intact, but the downstream pathways that mediate DSI were not able to induce eCB–iLTD. The involvement of PKA in eCB–iLTD made researchers look for candidate molecules that could be phosphorylated by this kinase. Because eCB–iLTD is expressed as a decrease in neurotransmitter release, the next logical step was to look for molecules regulated by PKA that were involved in the release machinery.

Downstream of PKA are many active-zone proteins that regulate vesicle release. One protein called RIM1α (Rab3-interacting-molecule-α) is required for a presynaptic form of LTP at hippocampal mossy fiber synapses and is phosphorylated by PKA (for review see Schoch and Gundelfinger 2006). Chevaleyre et al. (2007) found that eCB–iLTD is absent in RIM1α knockout mice (RIM1α−/−), although DSI is normal. These results imply that, during eCB–iLTD consolidation, CB1R activation produces a long-lasting modification of RIM1α protein through the PKA pathway. Follow-up studies, however, yielded some unexpected results.

The previous data suggest that RIM1α is an essential component of iLTD. A prediction of this model is that mutating this protein so that it cannot be phosphorylated by PKA should

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**FIG. 1.** Model of the presynaptic pathways involved in consolidation of endocannabinoid–long-term depression of inhibitory inputs (eCB–iLTD). A rise in Ca2+ or activation of group I metabotropic glutamate receptors (mGlur1s) or muscarinic acetylcholine receptors (mAChRs) on postsynaptic pyramidal cells causes the mobilization of endocannabinoids (eCBs, green circles), which activate the cannabinoid-1 receptor (CB1R) on presynaptic terminals. Activation of mGlur1s or mAChRs may mobilize eCBs in the postsynaptic cell through the phospholipase C (PLC)–diacylglycerol lipase (DAG) pathway. A hypothetical factor released by mGlur1 activation (necessary for eCB–iLTD induction) is also shown. CB1R activation ultimately leads to a reduction in the release of γ-aminobutyric acid (GABA) in the short term (depolarization-induced suppression of inhibition [DSI]) through βγ-mediated suppression of the N-type voltage-gated Ca channel (VGCC), or in the long term via the protein kinase A (PKA) pathway. CB1R activation acts in concert with calcineurin (CaN), although its downstream targets are yet unknown. It is also possible that CB1R acts through a yet unknown biochemical cascade to modulate Rab3-interacting-molecule-α (RIM1α; its phosphorylation site at Ser-413 is shown as a red triangle). Other putative molecules that may be involved in eCB–iLTD, such as Munc13 (blue rectangle) and Rab3 (blue circle), are also shown. Presynaptic mGlur1 activation could also be part of the mechanism leading to eCB–iLTD. Dashed arrows indicate putative pathways. Solid arrows indicate pathways that are supported by experimental evidence. Yellow arrows indicate pathways that could lead to eCB–iLTD induction.
have the same effect as the knock out; that is, eCB–iLTD should be prevented. A study by Lonart et al. (2003) concluded that PKA could phosphorylate RIM1α in two sites, but of these two, only one (Ser-413) was crucial for the expression of long-term synaptic plasticity in cultured cerebellar neurons. It would be expected then, in light of the results from Lonart et al. (2003) and Chevaleyre et al. (2007), that mutating this site would prevent the induction of eCB–iLTD. However, when Kaenser et al. (2008) “knocked in” a RIM1α mutant that rendered RIM1α unphosphorylatable by a serine-to-alanine substitution (S413A), they observed no change in eCB–iLTD. Although they confirmed that Serine 413 is the PKA phosphorylation site crucial for the binding of adaptor proteins to RIM1α, the S413A mutant failed to prevent eCB–iLTD, implying that PKA must act via a different target. It remains unclear how RIM1α is regulated during presynaptic long-term plasticity.

The regulation of synaptic neurotransmitter release is complex and involves many proteins besides RIM1α (for review see Schoch and Gundelfinger 2006). Other proteins studied for their role in long-term synaptic plasticity are Munc 13-1 and its isoform Munc 13-2. Munc isoforms are also located in the presynaptic active zone and are necessary for the docking of synaptic vesicles. Although Munc 13-1 is thought to be primarily localized to glutamatergic synapses, indirect evidence suggests it mediates GABA release as well. In cultured Munc 13-1−/− neurons there is a significant increase of GABergic currents after a 10-Hz action potential train compared with those recorded in wild-type cells, suggesting proper priming mechanisms are absent (Rosenmund et al. 2002). In RIM1α−/−, expression of Munc 13-1 and Munc 13-2 was decreased (Andrews-Zwilling et al. 2006). Therefore the absence of eCB–iLTD in RIM1α−/− mice may be attributable to lowered expression of Munc 13-1 and Munc 13-2 in these animals. This would be consistent with the observation that, in the RIM1α S413A knock-in mice, Munc isoform levels are normal, as is eCB–iLTD expression. Low levels of Munc proteins would be likely to have a profound effect on the induction of eCB–iLTD. In fact, there is a complete lack of excitatory and inhibitory synaptic transmission (spontaneous and evoked) in primary hippocampal neurons from mice that are double knockouts for Munc 13-1 and Munc13-2 (Varoqueaux et al. 2002). Although the double mutation is often lethal, primary hippocampal neurons from these mice exhibit normal morphology. The importance of Munc isoforms for eCB–iLTD could be directly tested using Munc13-1 and Munc13-2 knockout mice, or by “rescue” experiments (by viral insertion of a construct with the desired protein) to increase Munc levels in the RIM1α−/− animals.

As we try to identify key players out of the hundreds of proteins downstream of the CB1R on the presynaptic side of the synapse, many questions remain unanswered about the mechanisms needed to induce iLTD. For example, is activating CB1R by any mechanism sufficient to cause short-term (DSI) or long-term (eCB–iLTD) plasticity? Does varying the length of time that the CB1R is activated determine whether DSI or iLTD is observed? Do all methods of mobilizing eCBs have the potential to cause eCB–iLTD? The answer to these questions remains controversial. The view that long-term activation of CB1R by eCBs is sufficient to trigger eCB–iLTD is challenged by the observation that the activation of mACHRs by the agonist carbachol (CCh) for 20 min fails to produce eCB–iLTD (Edwards et al. 2006). The coincident activation of the CB1R and the presynaptic activity of the Ca2+−activated phosphatase calcineurin are needed for the induction of eCB–iLTD (Heifets et al. 2008). Calcineurin is activated by the rise in [Ca2+]i, that occurs during interneuron firing. It is therefore surprising that, even when mACHR agonists release eCBs and induce the continuous firing of CB1R-containing interneurons—therefore fulfilling both prerequisites for the induction of eCB–iLTD suggested by Heifets et al. (2008)—no long-term plasticity is observed. This may imply the existence of a yet unidentified cofactor that is synthesized or released only by mGlur1, but not mACHR, activation. This cofactor would presumably act in concert with eCBs to induce eCB–iLTD (Edwards et al. 2006). Alternatively, mACHR activation might suppress the eCB–iLTD induction mechanism while still releasing eCBs. This last possibility could be easily tested by applying both CCh and the mGlur1 agonist (S)-3,5-dihydroxyphenylglycine (DHPG) simultaneously for 10 min. If CCh suppresses eCB–iLTD it should also prevent the long-term depression induced by DHPG. If, on the other hand, eCB–iLTD is not inhibited by CCh, a more likely explanation would be the release of the hypothetical factor by mGlur1 activation. It would also be of great interest to replicate the experiments by Heifets et al. (2008) with CCh instead of DHPG. Yet another possibility is that coincident activation of both pre- and postsynaptic group I mGlur is necessary to induce eCB–iLTD. Indeed, whereas mGlur5 is localized mainly on principal neurons, mGlur1 is prominent in different types of interneurons. Boscia et al. (2008) report that mGlur1 can be colocalized with CB1R in cholecystokinin-containing interneurons. High-frequency stimulation patterns or bath application of mGlur1 agonists could therefore be simultaneously activating many of these receptors. If presynaptic mGlur5s play an important role, this may add a whole new level of complexity to the way eCB–iLTD is regulated. A model that incorporates these many different pathways is shown in Fig. 1.

In summary, eCB-mediated long-term synaptic plasticity is a widely distributed process in the brain and elucidating the complex cascade of events leading to its induction is a field of study that has just begun. Given that eCBs have been implicated in many physiological and pathophysiological conditions of the CNS, discovering how they work will no doubt give us great insight into the physiology of the brain and a better understanding of some of its disorders.

REFERENCES


