Persistent Synaptic Activity Produces Long-Lasting Enhancement of Endocannabinoid Modulation and Alters Long-Term Synaptic Plasticity

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Zhu PJ, Lovinger DM. Persistent synaptic activity produces long-lasting enhancement of endocannabinoid modulation and alters long-term synaptic plasticity. J Neurophysiol 97: 4386–4389, 2007. First published March 28, 2007; doi:10.1152/jn.01228.2006. Learning and memory are thought to involve activity-dependent changes in synaptic efficacy such as long-term potentiation (LTP) and long-term depression (LTD). Recent studies have indicated that endocannabinoid-dependent modulation of inhibitory transmission facilitates induction of hippocampal LTP and that endocannabinoids play a key role in certain forms of LTD. Here, we show that repetitive low-frequency synaptic stimulation (LFS) produces persistent up-regulation of endocannabinoid signaling at hippocampal CA1 GABAergic synapses. This LFS also produces LTD of inhibitory synapses and facilitates LTP at excitatory, glutamatergic synapses. These endocannabinoid-mediated plastic changes could contribute to information storage within the brain.

INTRODUCTION

Endogenous cannabinoids (endocannabinoids) facilitate memory and synaptic plasticity (Carlson et al. 2002; Marsicano et al. 2002; Sullivan 2000) through activation of their cognate G protein–coupled cannabinoid receptor-1 (CB1) (Matsuda et al. 1990). In the CA1 region of hippocampus, endocannabinoids are released postsynaptically from the pyramidal neurons during depolarization or activation of metabotropic receptors and act presynaptically on a subset of GABAergic neurons (Herkenham et al. 1990; Katona et al. 1999; Tsou et al. 1999). This retrograde endocannabinoid signaling produces short-term depression of GABAergic synaptic transmission termed depolarization-induced suppression of inhibition (DSI), as well as long-term depression (LTD) at hippocampal inhibitory synapses (Chevaleyre and Castillo 2003; Llanó et al. 1991; Ohno-Shosaku et al. 2001; Pitler and Alger 1992; Wilson and Nicoll 2001). Endocannabinoid-induced presynaptic depression of GABAergic transmission can also facilitate induction of long-term potentiation (LTP) in the hippocampal CA1 region (Carlson et al. 2002; Chevaleyre and Castillo 2004). Although these studies prove evidence that endocannabinoids participate in short- and long-term depression of transmission mediated by other neurotransmitters, it is not clear if endocannabinoid retrograde signaling itself undergoes long-term plasticity.

METHODS

Brain slices (400 μm thickness) containing the hippocampus were obtained from Sprague-Dawley rats (Charles River, Wilmington, MA) or mGluR5+/+ mice and mGluR5−/− mice (P16–P20). The composition of the external recording buffer was (in mM) 124 NaCl, 3 KCl, 1.3 MgSO4, 2 CaCl2, 1.2 NaH2PO4, 25 NaHCO3, and 10 glucose. Slices were maintained in external buffer at room temperature for ≥1 h before recording. All experiments were performed at room temperature, and drugs were bath-applied. Whole cell patch-clamp recordings were made in the presence of 25 μM D-AP5 (Sigma, St. Louis, MO) and 5 μM NBQX (Tocris, Ellisville, MO) to block fast glutamatergic transmission. Patch pipettes had resistances of 4–6 MΩ after filling with a solution containing (in mM) 140 CsCl, 10 HEPES, 0.2 BaPTA, 2 MgCl2, 2 Mg-ATP, 0.3 GTP, and 5 QX314.

Inhibitory postsynaptic currents (IPSCs) were evoked with bipolar electrodes (Teflon coated platinum and iridium wire, 75 μm diam) placed in or near CA1 stratum radiatum. DSI was tested every 4 min and consisted of 33 stimuli at 0.33 Hz with depolarization from −60 to −10 mV for 4 s inserted after the seventh stimulus. For LTD analysis, the means of the five evoked IPSCs (eIPSCs) evoked just before depolarization and the three eIPSCs just after the depolarization were used as Ampbaseline and Amptest, respectively. DSI magnitude (%) = 100 [1 – (Amptest/Ampbaseline)]. Field excitatory postsynaptic potentials (fEPSPs) were recorded using artificial cerebrospinal fluid (ACSF)-filled micropipettes placed in the s. radiatum. The stimulus strength needed to elicit an fEPSP ~30% of maximum amplitude was estimated using input/output analysis, and this stimulus magnitude was used in the weak theta burst stimulation (TBS) protocol. The strong TBS protocol was given at 50% maximal stimulus intensity. TBS consisted of a series of 10 bursts of five stimuli (100 Hz within-burst, 200-ms interburst interval), which was repeated four times (5 s apart). The stimulating and recording pipettes were placed close to each other (within 100 μm).

Whole cell and extracellular recordings were performed with an Axopatch 200B amplifier (Axon Instruments, Foster City, CA), and data were stored using pClamp8 software (Axon Instruments). Data are expressed as mean ± SE. Student’s t-test was performed to determine statistical significance. All drugs were bath-applied. NBQX, 6-methyl-2-(phenylethynyl)-pyridine (MPEP), and SR141716 were obtained from Tocris-Cookson. The mGluR5 knockout mice were provided by Dr. Alan I. Faden (Georgetown University).

RESULTS

To study whether the retrograde signaling itself is subject to long-term modulation, eIPSCs were recorded in visually identified CA1 pyramidal neurons in hippocampal slices. A 4-s depolarization step (from −60 to 0 mV) caused a 60 ± 6.4% (n = 7) inhibition of eIPSCs from the control level, consistent

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with DSI observed in previous studies (Wilson and Nicoll, 2001). SR141716 (2 μM), a CB1 receptor antagonist, prevented the depolarization-induced inhibition (data not shown). Reducing the duration of the depolarization step (250 ms to 1 s) yielded a smaller magnitude DSI (15 ± 7.2% decrease; n = 7; Fig. 1, A and C). This submaximal DSI was examined in subsequent experiments.

Subsequent to measuring basal DSI magnitude, LFS (1 Hz for 5 min) was applied to afferents in the slice. We found that LFS led to enhancement of the magnitude of DSI in response to the same short depolarizing step (Fig. 1, A and B). The magnitude of DSI produced by the 250-ms-to-1-s pulse was 52 ± 4.4% after LFS priming ($P < 0.01$ relative to DSI without priming in Fig. 1C). The magnitude of DSI induced by a 4-s step was 70 ± 10.4% (n = 7) and not significantly different from that observed before LFS priming ($P > 0.05$, t-test). This stimulus-induced increase in DSI magnitude persisted over pre-LFS levels for >30 min after the stimulus train (46 ± 4.9% reduction in eIPSC amplitude baseline in Fig. 1B). This LFS “priming” stimulation also produced an LTD of eIPSC amplitude (40 ± 4.2% reduction; Fig. 1E).

It was reported previously that activation of metabotropic glutamate receptors (mGluRs) enhances endocannabinoid signaling (Varma et al. 2001). Biochemical studies also indicate that endocannabinoid biosynthesis can be triggered by activation of metabotropic receptors coupled to Gq/Gi family G proteins (Maejima et al. 2005; van der Stelt et al. 2005; Wettchureck et al. 2006). Thus mGluR activation at glutamatergic synapses during LFS may contribute to the priming of DSI and the LTD of eIPSCs.

Blockade of mGluR5 by MPEP did indeed prevent the LFS-enhanced DSI and attenuated the LTD induced by LFS priming (Fig. 1, D–F). In the presence of MPEP (10 μM), DSI magnitude averaged 17 ± 8% before LFS priming and only 12 ± 9% after 1-Hz priming ($P > 0.05$, t-test); the LFS itself produced only a 15 ± 1.3% reduction in eIPSC amplitude ($P < 0.05$ compared with LFS alone, t-test). MPEP itself had no significant effect on magnitude of DSI produced by a 4-s depolarization step ($P > 0.05$; Fig. 1, C and D). All data were obtained in the presence of antagonists of ionotropic glutamate receptors, and thus these receptors do not play a role in LFS enhancement of DSI. The CB1 antagonist SR141716 (2 μM) abolished the LFS-induced reduction in eIPSC amplitude (Fig. 1, E and F). In the presence SR141716, eIPSC amplitude was 95 ± 2.7% of the control level after LFS priming.

Decreased synaptic inhibition enhances LTP at glutamatergic synapses (Wigström and Gustafsson 1985). Thus the LFS-induced and mGluR- and CB1-mediated inhibition of GABAergic transmission may facilitate induction of LTP. To examine this possibility, field EPSPs (fEPSPs) resulting from glutamatergic transmission were recorded in the CA1 s. radiatum (Fig. 2Aa). Theta burst–induced LTP was examined in the absence of ionotropic glutamate receptor antagonists. Delivery of a strong theta burst stimulus (see METHODS) reliably produced LTP in the absence of LFS priming (26 ± 7% increase in fEPSP slope; Fig. 2, Ab and Ac). Without LFS priming, a weak TBS, however, produced no significant change in field EPSP slope (8 ± 6% increase from preburst baseline; $P > 0.05$, paired-t-test; Fig. 2Ba). In contrast, in slices previously given the LFS priming stimulation, the same weak theta burst protocol produced a long-term potentiation of fEPSP slope (21 ± 8% increase above the baseline, $P < 0.01$, t-test compared with no priming condition). Priming stimulation alone produced only a short-term inhibition of the fEPSP slope (Fig. 2Bb). The LFS priming failed to facilitate induction of theta burst LTP when the CB1 receptor antagonist, SR141716, was present throughout the experiment (Fig. 3A). Blockade of mGluR5 throughout the experiment also prevented the LFS-facilitated theta burst LTP (Fig. 3B).

The role of mGluR5 in LFS-priming enhanced LTP is also supported by studies in mGluR5−/− mice. In wild-type mice, weak TBS produced a significant potentiation of fEPSP slope after LFS priming stimulation (18 ± 2% increase; $P < 0.05$, t-test; Fig. 3C). In mGluR5 knockout mice, however, LFS priming failed to facilitate weak theta burst–induced LTP. The TBS produced an 8 ± 5.2% reduction in fEPSP slope (Fig. 3C) after priming in slices from mGluR5−/− mice.

To determine if DSI enhancement or I-LTD is responsible for the facilitation of LTP, SR141517 was applied after 10 min of LFS but before and during TBS. Application of SR141716 at this time did not abolish the LFS-facilitated theta burst LTP (Fig. 3D). The fEPSP slope after TBS increased to 112 ± 3.8% of baseline in this experiment ($P < 0.01$, n = 5, t-test).
DISCUSSION

We report evidence for persistent enhancement of endocannabinoid-mediated DSI at inhibitory synapses in the hippocampal CA1 region after repetitive synaptic stimulation. Activity-dependent long-lasting enhancement of retrograde endocannabinoid signaling has not been previously reported, although retrograde endocannabinoid signaling is up-regulated following past experience of early febrile seizures (Chen et al. 2003, 2007), because of an increase in CB1 receptors on GABAergic terminals. It remains to be determined if the enhancement of retrograde endocannabinoid signaling that we have observed is caused by increased endocannabinoid release, increased CB1 receptor numbers, or increased signal transduction downstream of the CB1 receptor. LFS stimulation did not enhance strong DSI produced by prolonged depolarization, indicating that the maximal effect of endocannabinoids is not altered. Activation of mGluR5 is needed for DSI up-regulation, and this receptor has been suggested to enhance endocannabinoid production in CA1 pyramidal neurons (Chevaleyre and Castillo 2004), but not in amygdala (Azad et al. 2004). Thus it is tempting to speculate that enhanced endocannabinoid production and/or release underlies the long-lasting effects of LFS observed in this study. The persistent increase in DSI could produce a long-lasting increase in responsiveness of CA1 pyramidal neurons to intrinsic or synaptic excitation.

We also observed that LFS produced LTD of inhibitory transmission (I-LTD) and enhanced LTP of excitatory transmission in hippocampal CA1. The I-LTD is similar to that observed by Chevaleyre and Castillo (2003). Enhancement of LTP likely results from mechanisms similar to those previously implicated in priming of LTP during endocannabinoid-mediated DSI and I-LTD (Carlson et al. 2002; Chevaleyre and Castillo 2004). Facilitation of LTP induction by mGluR activation was also observed in a previous study (Miura et al. 2002), and similar endocannabinoid actions may underlie this enhancement. It is likely that mGluR5 and CB1 blockade prevent stimulus-primed LTP by interfering with endocannabinoid signaling during LFS. MPEP itself does not prevent induction of LTP (Auclair et al. 2000; Bortolotto et al. 2005; Lu et al. 1997). Thus it is unlikely that mGluR5 activation during TBS is responsible for enhanced LTP. Enhancement of LTP could be directly caused by enhanced DSI or endocannabinoid-mediated I-LTD because both mechanisms would lead to enhancement of glutamatergic transmission. However, application of SR141716 after LFS but during TBS does not abolish the LFS facilitation of LTP. Thus CB1 activation is not directly involved in LTD induction. Because endocannabinoid signaling is blocked when the CB1 antagonist is present during TBS, no DSI would take place during LTD induction, and thus LTD-enhanced DSI could not contribute to LTD enhancement. For this reason, we favor the idea that the I-LTD establishment during LFS most likely underlies enhanced LTD. This finding is similar to that reported by Chevaleyre and Castillo (2004), although we used a lower frequency for priming stimulation than that used in this previous study.

The endocannabinoid system has broad functions in the brain. Endocannabinoid release contributes to tonic modulation of GABAergic transmission (Hentges et al. 2005) and protects dopaminergic neurons against ischemic injury (Melis et al. 2006). The brain endocannabinoid system also regulates food intake and energy balance (Di Marzo and Matias 2005). We propose here that enduring up-regulation of retrograde endocannabinoid signaling and I-LTD contribute to long-lasting enhancement of glutamatergic transmission through inhibition of GABAergic transmission. These processes might thus contribute to endocannabinoid enhancement of learning and memory.

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GRANTS

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REFERENCES


