

Heterosynaptic LTD of Hippocampal GABAergic Synapses: A Novel Role of Endocannabinoids in Regulating Excitability

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Summary

Neuronal excitability and long-term synaptic plasticity at excitatory synapses are critically dependent on the level of inhibition, and accordingly, changes of inhibitory synaptic efficacy should have great impact on neuronal function and neural network processing. We describe here a form of activity-dependent long-term depression at hippocampal inhibitory synapses that is triggered postsynaptically via glutamate receptor activation but is expressed presynaptically. That is, glutamate released by repetitive activation of Schaffer collaterals activates group I metabotropic glutamate receptors at CA1 pyramidal cells, triggering a persistent reduction of GABA release that is mediated by endocannabinoids. This heterosynaptic form of plasticity is involved in changes of pyramidal cell excitability associated with long-term potentiation at excitatory synapses and could account for the effects of cannabinoids on learning and memory.

Introduction

Activity-dependent changes in synaptic efficacy are essential for neuronal development, learning, and memory formation (Katz and Shatz, 1996; Martin et al., 2000). While most of our knowledge of these changes is derived from studies at excitatory synapses (Malenka and Nicoll, 1999), little is known about synaptic plasticity at inhibitory synapses. Because excitability in the brain is highly dependent on the level of inhibition set by GABAergic interneurons, synaptic plasticity at inhibitory synapses should have important consequences for neural function and pathological states of neuronal excitability such as epilepsy (Dingledine and Gjerstad, 1980; Prince, 1978). Although long-term potentiation (LTP) and long-term depression (LTD) of inhibitory synapses both have been described in different areas of the brain (Gaiarsa et al., 2002), the mechanisms underlying these forms of plasticity are not clear.

A well-established role of inhibition is the regulation of induction of long-term plasticity at excitatory synapses by controlling the level of postsynaptic depolarization (Wigstrom and Gustafsson, 1985). A much less explored but equally interesting aspect of the interaction between excitatory and inhibitory synapses in synaptic plasticity is that excitatory transmission could modulate inhibitory synaptic efficacy. In fact, there is good evidence that glutamate modulates GABAergic transmission by the activation of ionotropic and metabotropic glutamate receptors. However, most of these effects are transient, functioning only *during* the activation of these

receptors (Belan and Kostyuk, 2002). A few studies have reported long-lasting effects *after* transient activation either of the NMDA subtype of glutamate receptors (NMDARs) following high-frequency stimulation (Caillard et al., 1999b; Stelzer et al., 1994; Lu et al., 2000; Ouardouz and Sastry, 2000) or of metabotropic glutamate receptors (mGluRs) activated pharmacologically (Liu et al., 1993). Such long-term change in the balance of excitation and inhibition can have an important functional impact by persistently modifying the overall excitability of neural circuits.

Long-term plasticity at inhibitory and excitatory synapses share common properties. For example, conditioning protocols (i.e., high-frequency stimulation or HFS) like those used to trigger plasticity at excitatory synapses can also trigger long-term plasticity at inhibitory synapses. The usual triggering signal for this plasticity is a rise of postsynaptic Ca^{2+} concentration most commonly through the activation of NMDARs (Caillard et al., 1999b; Lu et al., 2000; Stelzer et al., 1987; Wang and Stelzer, 1996) or voltage-gated Ca^{2+} channels (Caillard et al., 1999a), although in some cases, the release of Ca^{2+} from internal stores may also be required (Caillard et al., 2000; Holmgren and Zilberter, 2001; Komatsu, 1996). While these indirect mechanisms have commonly been predicted to occur postsynaptically, the enduring changes in inhibitory synaptic efficacy could be due to modifications in either the postsynaptic response to GABA (Lu et al., 2000; Stelzer et al., 1994) or in the amount of GABA release (Caillard et al., 1999b; Glaum and Brooks, 1996; Shew et al., 2000). This latter possibility that long-term plasticity at GABAergic synapses may be triggered postsynaptically but expressed presynaptically (Caillard et al., 1999a, 1999b) raises the necessity of a retrograde signal that has not been yet identified.

Here, we described a form of activity-dependent long-term plasticity at hippocampal inhibitory synapses that is triggered by glutamate release but whose induction, in contrast to most frequently described forms of long-term plasticity in the brain, is independent of NMDARs. We found that a stimulating protocol commonly used to induce LTP at excitatory synapses in the CA1 area also triggers a group I mGluR-dependent LTD at inhibitory synapses (I-LTD) that is mediated by retrograde endocannabinoid signaling. Moreover, we provide evidence that I-LTD may underlie changes of pyramidal cell excitability associated with LTP at excitatory synapses. By showing that endocannabinoid retrograde signaling “translates” activity of excitatory synaptic inputs into a persistent disinhibition in the hippocampus, our findings support a novel physiological role of endocannabinoids that could explain the well-known effects of cannabinoids in cognitive functions.

Results

Activity-Dependent Long-Term Depression at Hippocampal GABAergic Synapses

To study inhibitory synaptic transmission, we recorded CA1 pyramidal neurons in whole-cell voltage-clamp

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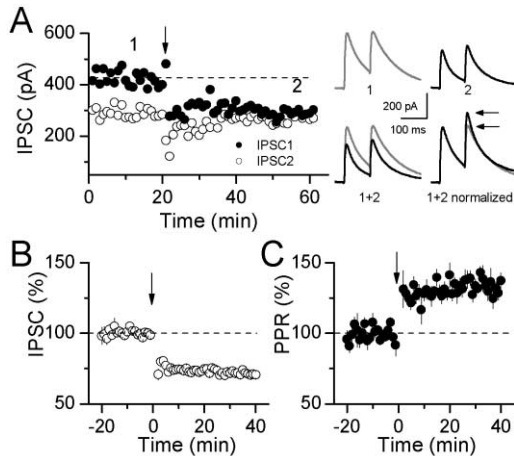


Figure 1. Long-Term Depression at Hippocampal Inhibitory Synapses

(A) Representative experiment in which IPSCs were recorded from a CA1 pyramidal neuron ($V_h = +10$ mV) in presence of $25 \mu\text{M}$ D-APV and $10 \mu\text{M}$ NBQX. Synaptic currents were evoked by paired-pulse stimulation (100 ms apart) and the amplitude of both IPSCs is plotted against time. High-frequency stimulation (HFS) was given at the time indicated by the arrow. Averaged sample traces taken during the experiment (indicated by numbers) are depicted on the right. Traces are superimposed and also normalized (bottom row) to point out the change in PPR (horizontal arrows).

(B) Summary graph of 21 experiments performed as in (A); only the amplitude of the first IPSC is plotted.

(C) Summary graph of the paired-pulse ratio (PPR) percentage change from experiments in (B).

mode in hippocampal slices. Inhibitory postsynaptic currents (IPSCs) were evoked by stimulating in *stratum radiatum* in the continuous presence of the ionotropic glutamate receptor (iGluR) antagonists NBQX ($10 \mu\text{M}$) and D-APV ($25 \mu\text{M}$) to block AMPA/Kainate and NMDA receptors, respectively. After a stable baseline, high-frequency stimulation (HFS; two trains of 100 stimuli at 100 Hz, separated by 20 s) induced long-term depression of the IPSC amplitude (I-LTD) to $72.6\% \pm 1.8\%$ of baseline ($n = 21$, $p < 0.00001$; Figures 1A and 1B). Consistent with a presynaptic locus of expression, I-LTD was associated with a significant change in pair-pulse ratio ($\text{PPR} = \frac{2^{\text{nd}} \text{IPSC}_{\text{amplitude}}}{1^{\text{st}} \text{IPSC}_{\text{amplitude}}}$) of two consecutive IPSCs 100 ms apart (Figures 1A and 1C). PPR was increased to $133.6\% \pm 0.04\%$ ($p < 0.0001$) after HFS, suggesting that I-LTD is due to a persistent reduction of evoked GABA release. In addition, theta burst stimulation (TBS, see Experimental Procedures) also triggered I-LTD ($73.9\% \pm 2.9\%$ of baseline, $n = 5$, $p < 0.001$; data not shown). Finally, we also explored whether I-LTD could be triggered in the absence of antagonists of iGluRs. To minimize the contribution of an iGluR-mediated synaptic component, IPSCs were recorded while holding the membrane potential at the EPSC reversal (0 mV). Although HFS also triggered I-LTD under this conditions, the magnitude of I-LTD was smaller ($86.3\% \pm 1.8\%$ of baseline, $n = 4$, $p < 0.001$; data not shown), which is likely due to the fact that in the absence of iGluR antagonists, IPSCs are contaminated with disynaptic (or polysynaptic) GABAergic inputs that most likely do not express I-LTD. Thus, this initial set of results indicates that two protocols (HFS and TBS)

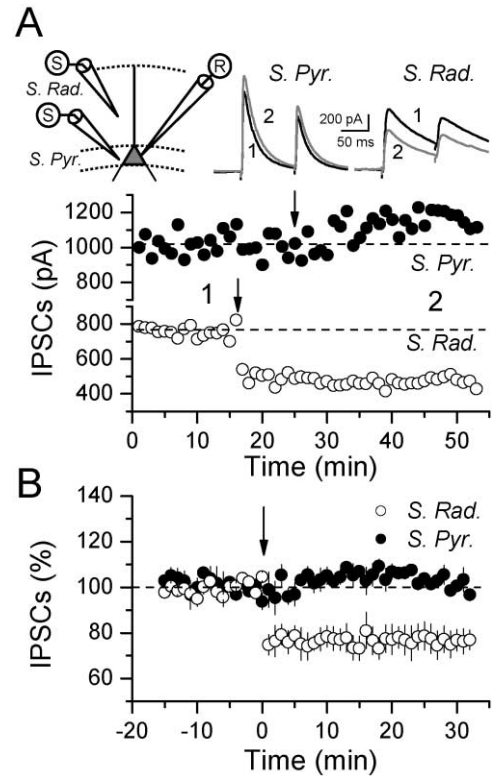


Figure 2. HFS Stimulation in *stratum radiatum*, but not *stratum pyramidale*, Induces LTD of Inhibitory Synapses at CA1 Pyramidal Cells

(A) Representative experiment in which IPSCs evoked by alternate stimulation in *s. radiatum* and *s. pyramidale* were recorded in the same CA1 pyramidal cell. Sample traces taken before and 20–25 min after HFS are shown superimposed on top. Vertical arrows indicate the time point when HFS was delivered.

(B) Summary graph of four cells in which recordings were performed as in (A). The time of HFS for each experiment was aligned and zeroed.

commonly used to trigger LTP at the Schaffer collateral to CA1 pyramidal cell synapse (Sch-CA1) by stimulation in *s. radiatum* concomitantly induce an NMDAR-independent—and most likely presynaptic—form of long-term plasticity of inhibitory synapses on CA1 pyramidal cells.

It has been postulated that perisomatic and dendritic inhibitory synapses onto hippocampal pyramidal cells may have different functional roles (McBain and Fisahn, 2001; Miles et al., 1996). To test whether both sets of synapses exhibited I-LTD under the same conditions, we evoked the corresponding IPSCs by stimulating in *s. pyramidale* and *s. radiatum*, respectively, while recording from the same CA1 pyramidal cells ($n = 4$; Figure 2A). We found that HFS in the *s. pyramidale* failed to induce I-LTD in all cells tested ($104.1\% \pm 2.3\%$ of baseline), whereas I-LTD was normally induced by stimulating in *s. radiatum* ($76.8\% \pm 5.3\%$ of baseline; Figure 2B). These results suggest that I-LTD is a form of synaptic plasticity selectively associated with inhibitory contacts that impinge on the dendritic tree of CA1 pyramidal cells.

Activation of Group I mGluR Is Necessary for I-LTD

We next explored what mechanism could explain a long-term depression of GABA release. We first assessed

the role of GABA_B receptors (GABA_BRs), since these receptors have been previously implicated in long-term plasticity at inhibitory synapses (Komatsu, 1996; Shew et al., 2000) and it is well known that their activation decreases GABA release (Deisz and Prince, 1989). As expected (Davies and Collingridge, 1993), bath application of CGP55845 (5 μM), a specific and potent GABA_BR antagonist, increased PPR in all cells tested (by 38.8% ± 5.2%, $p < 0.05$), but it did not affect the magnitude of I-LTD (to 65.7% ± 3.9% of baseline, $n = 7$, $p > 0.1$; data not shown). Thus, we found that activation of GABA_BRs is not required for I-LTD.

A clue to the mechanism of I-LTD comes from the observation that this form of plasticity is only observed in inhibitory contacts that impinge on the dendritic tree of CA1 pyramidal cells (Figure 2). One important difference between the stimulation in *s. radiatum* and *s. pyramidale* is that the former includes the activation of excitatory fibers (i.e., Schaffer collaterals). In contrast, stimulation in *s. pyramidale* most likely activates inhibitory synapses predominantly since this region is almost devoid of excitatory synapses. Because in our experimental conditions ionotropic glutamate receptors (AMPA/kainate and NMDA receptors) were blocked, we hypothesized that if I-LTD required activation of glutamate receptors, these should be mGluRs.

To test this hypothesis, we first explored whether I-LTD could be induced after pharmacological blockade of mGluR1/5. Bath application of 100 μM LY367385 and 4 μM MPEP, selective antagonists of mGluR subtypes 1 and 5, respectively, had no effect on basal synaptic transmission (not shown) but completely blocked I-LTD (96.8% ± 3.8% of baseline, $n = 5$; Figure 3A), indicating that mGluR1/5 activation is necessary for I-LTD induction. If mGluR1/5 mediate the observed I-LTD, the activation of these receptors with selective agonists should mimic the depression. In agreement with a previous report (Liu et al., 1993), bath application of the selective group I mGluR agonist DHPG (50 μM during 10 min) induced a transient depression followed by a long-lasting depression that remained upon washout (to 72.5% ± 2.2% of baseline, measured 30–40 min after agonist application onset, $n = 7$, $p < 0.0001$; Figure 3B). The DHPG-induced depression also included an increase in PPR (125.3% ± 8.3%, $n = 7$, $p = 0.002$; Figure 3B), which suggests that, as with I-LTD, the DHPG-induced depression is due to a reduction of evoked GABA release. Because DHPG also produces a robust increase in spontaneous IPSCs activity, presumably due to an increase in interneuron excitability (Poncer et al., 1995), this effect could have inhibited synaptic transmission via presynaptic GABA_B receptors activation. This is unlikely because the DHPG-induced depression was unaffected in the presence of 5 μM CGP55845 (to 70.5% ± 2.5% of baseline, $n = 4$, $p > 0.5$). Thus, our experiments indicate that exogenous activation of mGluR1/5 is sufficient to trigger persistent depression of GABA release.

If the DHPG-induced long-lasting depression and I-LTD share a common mechanism, these two depressions should occlude each other. As shown in Figure 3C, HFS failed to induce I-LTD in synapses already depressed by DHPG (97.5% ± 2.8% of baseline, $n = 6$). Conversely, DHPG only induced a transient depression in synapses already depressed by HFS (96.1% ± 2.3%, $n = 3$, versus 74.5% ± 3.5% in three interleaved

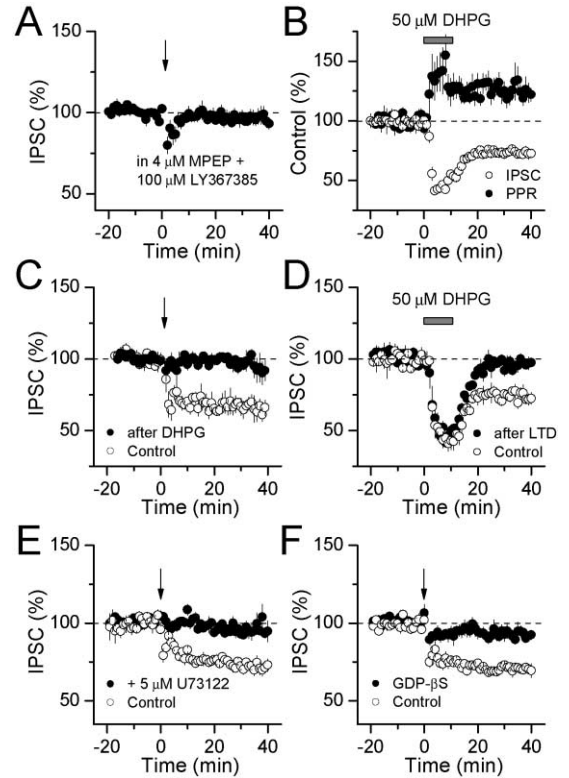


Figure 3. I-LTD Requires Postsynaptic mGluR1/5 Activation

(A) HFS delivered in the continuous presence of group I mGluR antagonists (4 μM MPEP and 100 μM LY367385) failed to induce I-LTD ($n = 5$).

(B) Bath application of the group I mGluR agonist DHPG (50 μM, 10 min, horizontal bar) induced a transient depression of IPSCs amplitude followed by a persistent depression after washout ($n = 7$, white circles). The associated change in PPR magnitude is also plotted against time (black circles).

(C) Once DHPG-induced long-lasting depression was fully developed, HFS was ineffective in inducing I-LTD ($n = 6$, black circles). Control I-LTD (interleaved slices) induced 50–60 min after whole-cell recording is superimposed ($n = 5$, white circles).

(D) Conversely, bath application of 50 μM DHPG 20–30 min after HFS induced only a transient depression ($n = 3$, black circles) but not the long-lasting depression observed in control slices ($n = 3$, white circles) in which DHPG was applied 40–50 min after the beginning of the whole-cell recording.

(E) After 1 hr incubation in the PLC inhibitor (5 μM U73122, also applied during the recording), HFS failed to trigger I-LTD (black circles, $n = 5$), whereas I-LTD was normally induced in control interleaved slices (white circles, $n = 4$).

(F) 2 mM GDP-βS included in the recording pipette significantly reduced I-LTD in six experiments (black circles) as compared to four control experiments (white circles, interleaved slices). HFS was delivered 30–40 min after whole-cell recording in both control and GDP-βS experiments.

control cells; Figure 3D). If the induction of I-LTD requires activation of mGluR1/5, interfering with mGluR signaling should reduce or block I-LTD. We therefore tested whether phospholipase C (PLC), a well-known effector of mGluR1/5, could be involved in I-LTD. Incubation of hippocampal slices in the PLC inhibitor U73122 (5 μM) during 1 hr, which was also bath applied throughout the experiment, completely blocked I-LTD (95.8% ± 3.4%, $n = 5$, versus 71.0% ± 2.0% in four interleaved control cells, $p < 0.001$; Figure 3E). Finally, we wondered

whether I-LTD could be affected by interfering with the mGluR signaling cascade of the postsynaptic neuron exclusively. To test this possibility, we included the irreversible G protein inhibitor GDP- β S (2 mM) in the recording pipette and found that under these conditions I-LTD was strongly reduced ($92.8\% \pm 2.9\%$, $n = 6$, versus $70.9\% \pm 1.8\%$, in four interleaved control cells, $p < 0.001$; Figure 3F). In agreement with their localization on the dendritic tree of CA1 pyramidal cells (Shigemoto et al., 1997), this finding supports a postsynaptic requirement of mGluR1/5 to trigger I-LTD. Because I-LTD induction seems to require the activation of excitatory fibers, it is likely that glutamate released as a result of HFS triggers I-LTD via postsynaptic mGluR1/5 activation. Furthermore, if I-LTD is due to a persistent reduction of GABA release, the effect of mGluR1/5 activation should be mediated by some retrograde signal that feeds back onto GABAergic terminals.

Retrograde Endocannabinoid Signaling Mediates I-LTD

Retrograde endocannabinoid signaling has recently been implicated in transient inhibition of GABA release in the hippocampus (Kreitzer and Regehr, 2002; Wilson and Nicoll, 2002) and could mediate LTD at excitatory synapses in striatum and nucleus accumbens (Gerde-man et al., 2002; Robbe et al., 2002). Cannabinoid receptors (CB₁Rs) also appear to be necessary for LTD at inhibitory synapses in the amygdala (Marsicano et al., 2002). In addition, it has been shown that endocannabinoid release can be triggered via group I mGluR activation (Maejima et al., 2001; Robbe et al., 2002). We therefore hypothesized that endocannabinoids could be the retrograde signal induced by mGluR1/5 activation that triggers I-LTD. To test this possibility, we examined the effect of a CB₁R-selective antagonist AM251 on I-LTD. Bath application of AM251 (2 μ M) had no effect on basal synaptic transmission but completely blocked the induction of I-LTD ($104.7\% \pm 7.0\%$, $n = 7$; Figure 4A). However, when applied 20 min after HFS, AM251 failed to reverse I-LTD ($75.2\% \pm 3.5\%$, $n = 5$; Figure 4B), indicating that CB₁Rs are only required during induction, but not during I-LTD maintenance.

It is known that CB₁R activation depresses inhibitory synaptic transmission by reducing GABA release (Hajos et al., 2000; Katona et al., 1999). If CB₁R-mediated presynaptic depression and I-LTD share common mechanisms, the inhibitory effect of CB₁R agonists should mimic and occlude I-LTD. As previously reported (Hajos et al., 2000; Wilson and Nicoll, 2001), application of the CB₁R agonist WIN512212-2 (500 nM) decreased IPSC amplitude (to $58.1\% \pm 3.4\%$ of baseline, $n = 8$, $p < 0.00001$; not shown). After stabilization of WIN512212-2's inhibitory effect, HFS failed to induce I-LTD (to $96.3\% \pm 1.3\%$ of baseline, $n = 4$; Figure 5A). Conversely, WIN512212-2 had less effect in synapses already expressing I-LTD (to $80.2\% \pm 3.5\%$ of baseline, $n = 4$, $p = 0.001$; Figure 5B), indicating that CB₁R agonist-induced depression and I-LTD occlude each other. Taken together, these results show that CB₁R activation is an essential step in the induction of I-LTD.

Because WIN512212-2 may also depress glutamate release (Misner and Sullivan, 1999; Takahashi and Lin-

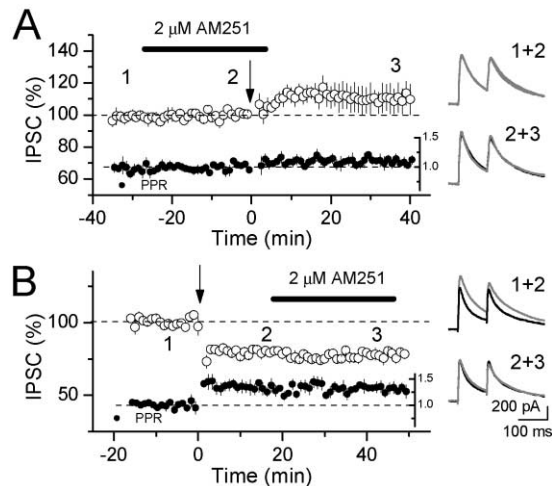


Figure 4. CB₁ Receptors Are Necessary for I-LTD Induction but Not Maintenance

(A) Bath application of the CB₁R antagonist AM251 (2 μ M) for 30 min (horizontal bar) had no effect on inhibitory basal synaptic transmission or PPR but completely blocked I-LTD ($n = 7$). Sample traces from a representative experiment are shown on the right. (B) When bath applied 20 min after I-LTD induction, AM251 had no effect on IPSCs amplitude or PPR ($n = 5$).

den, 2000), the apparent occlusion we observed (Figure 5A) could reflect a failure to induce I-LTD due to a reduction in glutamate release during tetanus. Similar argument could be raised for the lack of I-LTD after DHPG (Figure 3C), as a transient activation of group I mGluRs may also induce a persistent depression of glutamate release (Anwyl, 1999). To address this possibility, we assessed I-LTD in conditions of low probability of transmitter release by recording in the continuous presence of 10 μ M Cd²⁺. In this experimental condition, although excitatory synaptic transmission can be depressed by more than half (to $36.5\% \pm 3.1\%$ of baseline, estimated by monitoring extracellular field excitatory postsynaptic potential [fEPSP] amplitude in four interleaved slices; not shown), I-LTD was still induced and it had a normal magnitude ($72.2\% \pm 2.8\%$ of baseline, $n = 4$; data not shown), suggesting that I-LTD blockade after WIN512212-2 or DHPG is not due to the reduction of glutamate release.

We reasoned that if mGluR1/5 act upstream from CB₁R activation, the DHPG-induced long-lasting depression should be inhibited by pharmacological blockade of CB₁Rs, as is the case with I-LTD. In agreement with this expectation, 2 μ M AM251 completely abolished long-lasting depression induced by DHPG ($97.6\% \pm 3.8\%$ of baseline, $n = 4$; Figure 5C), and in these conditions, DHPG only induced a transient depression with a magnitude similar to that in the absence of AM251. Thus, the DHPG-induced depression includes a transient CB₁R-independent depression and a long-lasting component that is entirely dependent on the activation of CB₁Rs.

Endogenous cannabinoids are also known to mediate depolarization-induced suppression of inhibition (DSI), a transient depression of IPSCs induced by a brief depolarization of CA1 pyramidal neurons (Kreitzer and Re-

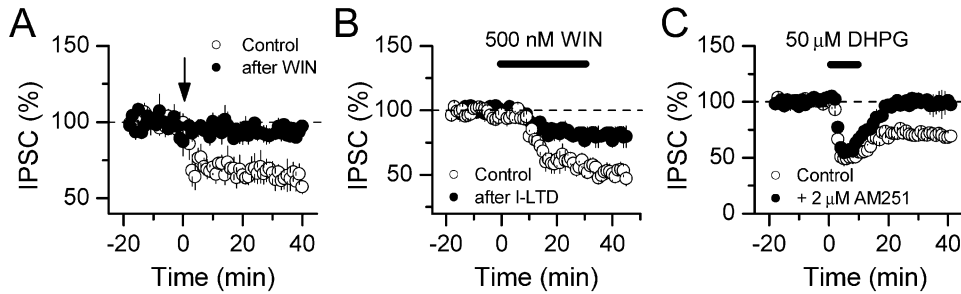


Figure 5. CB₁R_s Mediate I-LTD- and DHPG-Induced Depression of IPSCs

(A) Application of the CB₁R agonist WIN (500 nM, 30 min) induced an irreversible depression of IPSCs (not shown, $n = 8$). Subsequent HFS provided after stabilization of WIN effects induced no further depression (black circles, $n = 4$). As a control, HFS was delivered 50–60 min after whole-cell recording in five interleaved slices.

(B) Conversely, WIN induced a smaller depression when applied 30–40 min after I-LTD induction (black circles, $n = 4$) than that induced when applied 50–60 min after whole-cell recording in four interleaved control slices (white circles).

(C) The long-term depression of IPSCs induced by 50 μ M DHPG (white circles, $n = 4$) was blocked by 2 μ M AM251, whereas the transient depression during DHPG application remained unchanged ($n = 4$).

gehr, 2002; Wilson and Nicoll, 2002). It is believed that membrane depolarization promotes endocannabinoid release, which in turn inhibits GABA release via presynaptic CB₁R_s. Hence, we wondered whether the same populations of inhibitory inputs are subjected to both DSI and I-LTD. We found that all cells that exhibited IPSCs evoked by stimulation in *s. radiatum* and expressed I-LTD also expressed DSI (Figure 6A). Moreover, the magnitudes of DSI and I-LTD were correlated ($r = 0.85$; Figure 6B), suggesting that the same population of cannabinoid-sensitive presynaptic inhibitory fibers express DSI and I-LTD. Finally, while I-LTD was absent when IPSCs were evoked by stimulation in *s.*

pyramidale (Figure 6C), DSI could be induced by stimulation in either region and its average magnitude was comparable ($DSI_{pyramidal} 53.3\% \pm 7.8\%$, $n = 10$, $DSI_{radiatum} 54.6\% \pm 5.3\%$, $n = 14$, $p = 0.92$; Figure 6D). In addition, 10 min bath application of 50 μ M DHPG induced LTD in both *s. radiatum*- and *s. pyramidale*-evoked IPSCs ($71.8\% \pm 4.4\%$ in *s. radiatum* and $77.5\% \pm 3.7\%$ in *s. pyramidale*, $p = 0.34$, $n = 6$; not shown). These findings make it unlikely that a differential sensitivity to endocannabinoids between perisomatic and dendritic inhibitory synapses could explain the lack of I-LTD of IPSCs evoked by stimulating in *s. pyramidale* (Figure 2) and support the idea that the I-LTD at dendritic inhibitory synapses is presumably triggered as a result of glutamate released from Schaffer collaterals.

Taken together, our results strongly suggest that glutamate released as a result of HFS of Schaffer collaterals activates postsynaptic mGluR1/5, which in turn promotes release of endocannabinoids. By activating presynaptic CB₁R_s, endocannabinoids may trigger a persistent depression of evoked GABA release by inhibitory terminals synapsing nearby to Schaffer collateral inputs.

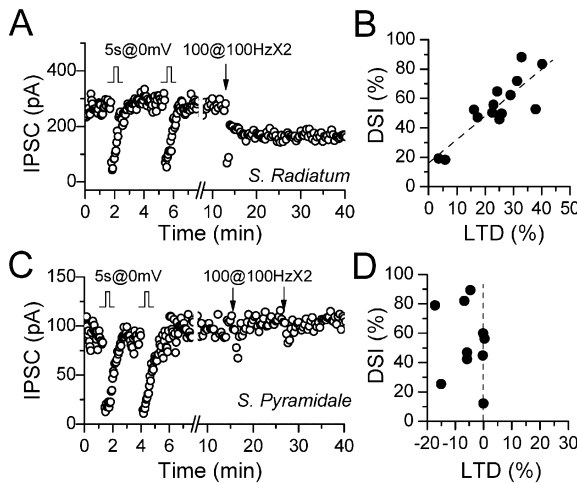


Figure 6. I-LTD versus DSI

(A) Representative experiment in which IPSCs were evoked by stimulation in *stratum radiatum* and two successive episodes of DSI were followed by I-LTD.

(B) Summary plot of 14 experiments as in (A) showing the magnitude of DSI versus I-LTD.

(C and D) Representative experiment (C) and summary plot (D) (as in A and B, respectively) in which IPSCs were instead evoked by stimulation in *stratum pyramidale* ($n = 10$ pyramidal cells). Membrane potential was voltage-clamped at -60 mV in all cells analyzed in this figure.

Release of Endocannabinoid Pathways Associated with I-LTD and DSI Differ

We next investigated the mechanism downstream from mGluR activation and G proteins involved in endocannabinoid release. Several previous studies pointed out the relevance of intracellular Ca^{2+} rises for endocannabinoid synthesis. For example, it has been reported that endocannabinoid-dependent forms of short-term (Kreitzer and Regehr, 2001; Wilson and Nicoll, 2001) and long-term (Gerdeman et al., 2002; Robbe et al., 2002) depression are blocked in the presence of an intracellular Ca^{2+} chelator. However, endocannabinoids can also be released via group I mGluR activation independently of any postsynaptic Ca^{2+} rise (Maejima et al., 2001; Ohno-Shosaku et al., 2002). To investigate whether a rise in postsynaptic Ca^{2+} is required for inducing I-LTD, we included the calcium chelator BAPTA (20 mM) in the recording pipette solution. We found that HFS still induced an I-LTD of normal magnitude ($75.8\% \pm 2.5\%$ of baseline, $n = 5$;

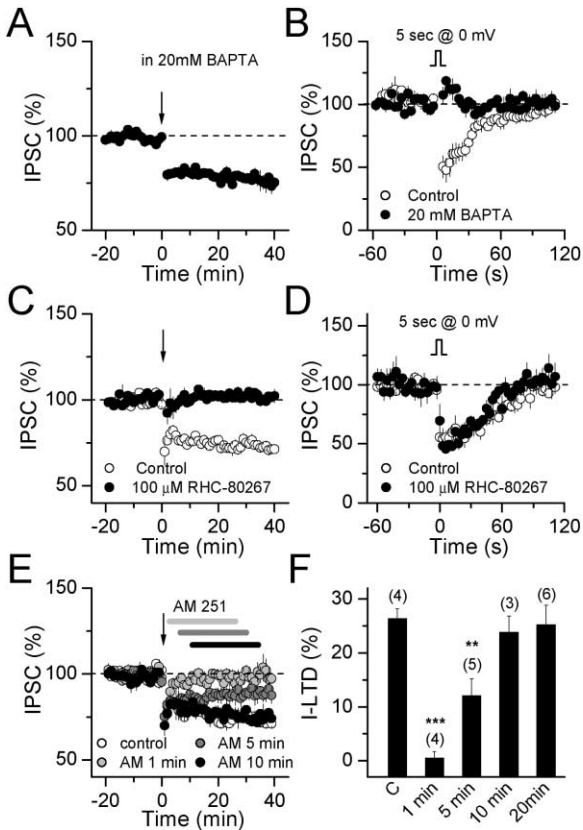


Figure 7. Cannabinoid Release and Duration of CB₁R Activation Differ between I-LTD and DSI
(A) I-LTD magnitude was normal in all cells (n = 5) recorded with 20 mM BAPTA in the recording pipette.
(B) DSI was blocked by 20 mM BAPTA (black circles, n = 5). DSI recorded in control conditions (n = 4) is superimposed for comparison (white circles).
(C) The DAG-lipase inhibitor RHC-80267 completely blocked I-LTD in four experiments (black circles). Slices were incubated during at least 1 hr in 100 μM RHC-80267 and also continuously applied at 30 μM during the experiment.
(D) No difference was observed between DSI induced in control condition (white circles, n = 3) or in presence of RHC-80267 (black circles, n = 3).
(E) Effect of 4 μM AM251 on I-LTD when applied at 1, 5, and 10 min after HFS. Control I-LTD (white circles) is also superimposed for comparison purposes.
(F) Summary graph of the magnitude of I-LTD in control conditions (C) and after AM251 application at different intervals after HFS. Numbers on top of each bar indicate the number of experiments.

Figure 7A), whereas in a group of interleaved cells, BAPTA blocked DSI, as reported previously (Pitler and Alger, 1992) ($DSI_{control} = 50.6\% \pm 9.6\%$, n = 4; $DSI_{BAPTA} = 111.3\% \pm 2.6\%$, n = 5; Figure 7B). These results clearly suggest that in contrast to DSI, the release of endocannabinoids during I-LTD does not require a rise in intracellular Ca^{2+} .

If Ca^{2+} is not the triggering signal for I-LTD, the endocannabinoids may be alternatively produced via diacylglycerol (DAG) formation and its subsequent conversion into 2-arachidonoyl glycerol (2-AG). While several putative ligands for cannabinoid receptors, including anandamide, 2-AG, and 2-arachidonoyl glyceryl ether (noladin ether), have been identified (Howlett et al., 2002; Mech-

oulam et al., 1998; Pertwee and Ross, 2002), evidence is accumulating that 2-AG is the most efficacious endogenous natural ligand for cannabinoid receptors in the brain. It is notable that a HFS protocol identical to that used in this study to induce I-LTD has been reported to produce a selective enhancement of 2-AG, but not of anandamide, in hippocampal slices (Stella et al., 1997). Thus, we predicted that blockade of 2-AG production should also block I-LTD. As shown in Figure 7C, we found that I-LTD was abolished when HFS was delivered in the presence of 50 μM RHC-80267 ($102.1\% \pm 0.7\%$ of control, n = 4), an inhibitor of the enzyme DAG lipase that converts DAG into 2-AG (Stella et al., 1997). In contrast, DSI was unaffected by RHC-80267 ($54.9\% \pm 7.2\%$ in control, n = 3, and $53.2\% \pm 3.7\%$ in RHC-80267, n = 4; Figure 7D). Consistent with this observation, we found that DSI was unaffected by 5 μM U73122 ($51.5\% \pm 7.1\%$, n = 4; not shown), whereas in the same cells this PLC inhibitor blocked I-LTD (Figure 3E). Taken together, these results suggest that 2-AG, produced via DAG lipase, is the natural endocannabinoid that mediates I-LTD but not DSI.

Although the nature of the endogenous cannabinoids that mediate DSI and I-LTD may differ, one may still wonder how these molecules, probably acting on the same presynaptic receptor, could trigger short- or long-lasting depression of GABAergic synaptic transmission. One possibility is that the different time course of these two CB₁R-dependent phenomena is based on the duration of CB₁R activation during induction. DSI only lasts around 1 min, and because CB₁R does not desensitize at this time scale, it is likely that endocannabinoids are only released during a few seconds after postsynaptic depolarization. In contrast, it has been suggested that 2-AG levels remain elevated for several minutes as a result of HFS of hippocampal slices (Stella et al., 1997). We reasoned that if I-LTD induction requires CB₁R activation during certain period of time, pharmacological blockade of these receptors within this time window should reduce or block I-LTD. To test this possibility, we applied 4 μM AM251 at different time points immediately after HFS. As shown in Figure 6E, bath application of the CB₁R antagonist 1 min after HFS virtually blocked I-LTD ($99.5\% \pm 0.8\%$ of baseline), whereas I-LTD was normally induced when the antagonist was applied 10 min after HFS ($78.1\% \pm 4.1\%$ of baseline). It is remarkable, however, that AM251 reduced I-LTD magnitude by half when applied 5 min after HFS ($87.0\% \pm 3.3\%$ of baseline). A summary graph of the I-LTD magnitudes obtained after CB₁R blockade at different intervals post-HFS is shown in Figure 6F. Thus, these results strongly suggest that in contrast to DSI, CB₁R need to be activated for several minutes in order to trigger I-LTD.

I-LTD Is Involved in E-S Coupling Potentiation

Changes in GABAergic tone can indirectly regulate induction of synaptic plasticity at excitatory synapses and can also directly affect neuronal excitability. From the first description of LTP at excitatory synapses (Bliss and Lomo, 1973), it was suggested that LTP has two components: (1) an increase in the excitatory postsynaptic potential (EPSP), and (2) an increase in the ability of an equal-sized EPSP to fire an action potential. The latter is the so-called E-S coupling component of LTP.

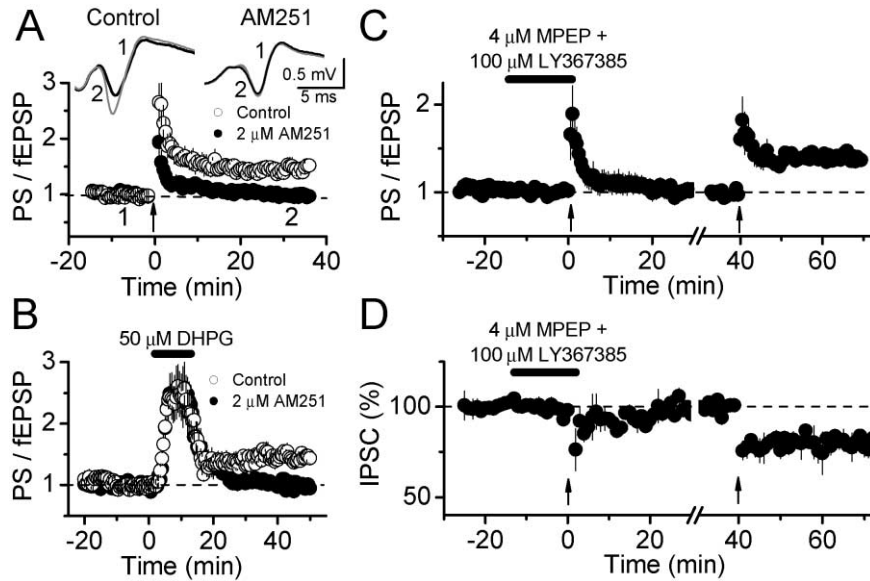


Figure 8. I-LTD Is Involved in E-S Coupling Potentiation

(A) Effect of AM251 on the E-S coupling potentiation induced by HFS. E-S coupling is plotted from control slices (white circles, $n = 7$) and from slices in the continuous presence of $2 \mu\text{M}$ AM251 (black circles, $n = 6$). Sample traces of population spike (PS) extracellular potentials taken at the time indicated by numbers are superimposed on top.

(B) Time course of the effect of $50 \mu\text{M}$ DHPG bath application (horizontal bar) on E-S coupling in control slices (white circles, $n = 5$ slices) and in slices in the continuous presence of $2 \mu\text{M}$ AM251 (black circles, $n = 5$ slices).

(C) E-S coupling potentiation induced by HFS was evaluated in presence of $4 \mu\text{M}$ MPEP and $100 \mu\text{M}$ LY367385 (bath application indicated by the horizontal bar) and after 40 min washout of these antagonists ($n = 5$ slices).

(D) I-LTD induction in presence of MPEP and LY367385 and upon 40 min washout was assessed in the same slice ($n = 3$) using similar experimental protocol as in (C).

It has been postulated that the E-S coupling could be mediated by a persistent reduction of inhibitory drive (Abraham et al., 1987; Chavez-Noriega et al., 1989; Lu et al., 2000); however, the cause of this reduction is as yet unclear. To test whether I-LTD could explain E-S coupling potentiation, we first assessed the effect of CB_1R blockade on the magnitude of E-S coupling (see Experimental Procedures). We found that E-S coupling potentiation was blocked when HFS was delivered in presence of AM251 ($98.5\% \pm 6.9\%$ in AM251, $n = 6$; $142.2\% \pm 8.8\%$ in seven interleaved control slices, $p = 0.002$; measurements were taken 30–35 min after HFS; Figure 8A), whereas AM251 on its own had no effect on E-S coupling ($96.1\% \pm 7.4\%$; 35–45 min after application, $n = 7$; not shown). Similarly to I-LTD, E-S coupling potentiation was observed in presence of $25 \mu\text{M}$ D-APV ($135.1\% \pm 8.2\%$, $n = 5$), and under these conditions, it was also blocked by AM251 ($98.8\% \pm 3.9\%$ in AM251, $n = 5$). These results indicate that E-S coupling potentiation is mostly NMDAR independent and mediated by the activation of CB_1Rs .

Because mGluR1/5 activation is an essential step for the induction of I-LTD, we predicted that if I-LTD underlies E-S coupling potentiation, activation or blockade of mGluRs1/5 should also affect the magnitude of this potentiation. DHPG bath application induced a transient increase in E-S coupling that was followed by a long-lasting potentiation upon washout ($145.1\% \pm 10.2\%$ of baseline, $n = 5$, $p = 0.003$, 30–35 min after onset DHPG application; Figure 8B). This result is a mirror image of the time course of IPSC depression induced by DHPG application, as seen in Figure 3B, and is also in agree-

ment with a previous report in which a long-lasting increase in E-S coupling was described after transient application of the nonspecific mGluR agonist ACPD (Breakwell et al., 1996). Moreover, DHPG application in the presence of AM251 induced only a transient effect, while the long-term effect was abolished ($101.5\% \pm 4.3\%$, $n = 5$; Figure 8B). This result is consistent with the DHPG-induced effects on IPSCs as shown in Figure 5C and clearly suggests that DHPG-induced persistent potentiation of E-S coupling was mediated by CB_1Rs .

Finally, potentiation of E-S coupling was abolished in the presence of the mGluR1/5 antagonists ($4 \mu\text{M}$ MPEP and $100 \mu\text{M}$ LY367385), whereas in the same slices a subsequent HFS delivered 40 min after washout of the antagonists induced virtually normal E-S coupling potentiation ($138.1\% \pm 2.5\%$, $n = 5$, $p < 0.001$; Figure 8C). Consistent with the hypothesis that I-LTD is involved in E-S coupling potentiation, we verified that HFS also induced I-LTD upon washout of the mGluR1/5 antagonists (Figure 8D, $n = 3$). Taken together, our results indicate that the persistent disinhibition that occurs during I-LTD can account for the enhancement in CA1 pyramidal cell excitability associated with LTP at the Sch-Ca1 synapse.

Discussion

The interaction between excitatory and inhibitory synapses is central in the control of neuronal excitability and synaptic plasticity. It is known that inhibitory synapses, by controlling the level of postsynaptic depolarization, modulate the induction of activity-dependent

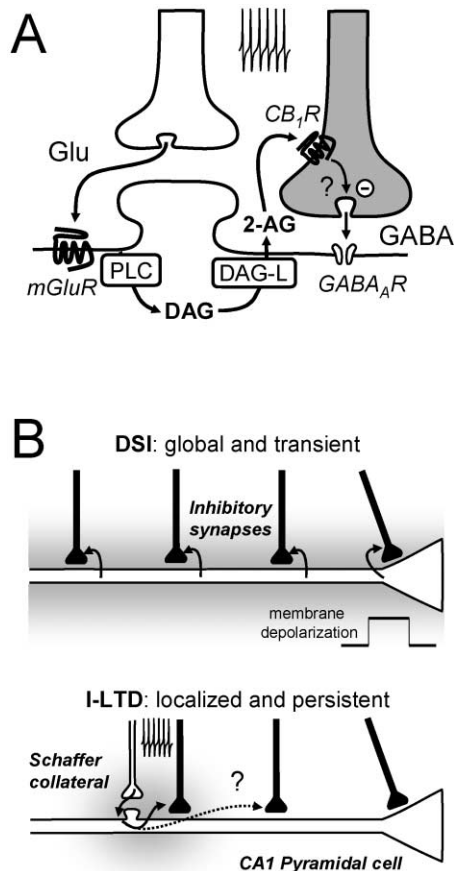


Figure 9. I-LTD: Mechanism of Induction and Functional Properties (A) Glutamate release from Schaffer collaterals as a result of high-frequency stimulation activates postsynaptic mGluR1/5, leading to PLC activation and DAG formation. DAG is then converted by the DAG lipase (DAG-L) into 2-AG, which inhibits GABA release by acting on presynaptic CB₁Rs. The mechanisms downstream from CB₁R underlying I-LTD are still unknown. (B) Temporal and spatial differences between I-LTD and DSI. While DSI produces a generalized but transient depression of cannabinoid-sensitive GABAergic inputs (in black), I-LTD is more localized and long lasting. DSI is commonly triggered by a brief (~5 s) postsynaptic depolarization of the CA1 pyramidal cell, whereas I-LTD induction requires glutamate release from Schaffer collaterals and CB₁R activation during several minutes.

long-term synaptic plasticity at glutamatergic excitatory synapses (Wigstrom and Gustafsson, 1985). Here we show that, in addition to this well-established mechanism, glutamate effects can feedback onto inhibitory synapses and induce long-term changes in GABAergic synaptic efficacy. Specifically, we found that repetitive activation of presynaptic glutamatergic fibers can induce long-term depression of hippocampal inhibitory synapses via retrograde cannabinoid signaling (Figure 9A).

Heterosynaptic I-LTD Requires Activation of mGluRs1/5 on Pyramidal Cells and CB₁Rs on GABAergic Terminals

We have found one means by which glutamate can induce long-term plasticity at GABAergic synapses. Because all our experiments included AMPA/kainate and NMDA receptor antagonists, it is unlikely that ionotropic

glutamate receptors localized either on CA1 pyramidal cells or on inhibitory interneurons play a major role in the I-LTD studied here. We postulate instead that I-LTD is triggered by glutamate activation of mGluRs because I-LTD was absent after blockade of group I mGluRs and could be mimicked by exogenous activation of these receptors (Figures 3A and 3B). Glutamate could have triggered this plasticity by activating postsynaptic mGluRs on CA1 pyramidal cells and/or presynaptic mGluRs on GABAergic terminals. We favor the first alternative because I-LTD was also blocked by interfering postsynaptically with G protein activity (Figure 3D). In further support of this postulate, mGluRs subtypes 1 and 5 are expressed on the dendritic tree of CA1 pyramidal cells but have been difficult to find at GABAergic terminals (Shigemoto et al., 1997). We thus conclude that I-LTD is a glutamate-induced and therefore a heterosynaptic form of plasticity at GABAergic synapses that is triggered by postsynaptic activation of group I mGluRs.

Our results indicate that induction of I-LTD also requires activation of CB₁Rs (Figure 4). Immunocytochemical studies in the hippocampus have shown that CB₁Rs are mostly localized at presynaptic terminals of GABAergic interneurons that innervate pyramidal cells (Hajos et al., 2000; Irving et al., 2000; Katona et al., 1999; Tsou et al., 1999). Moreover, virtually all hippocampal CB₁R-immunoreactive neurons have so far proven to be cholecystokinin-containing GABAergic interneurons (Tsou et al., 1999). Some of these interneurons project to the soma (i.e., basket cells), whereas some others project to the dendritic tree (i.e., bistratified cells) of the CA1 pyramidal cell (Cope et al., 2002; Pawelzik et al., 2002). Because of the proximity of the stimulating electrode to the main dendritic shaft (<100 μm), it is likely that our stimulation method mostly recruited axons from bistratified cells rather than basket cells. We hypothesize that because of the close interaction of excitatory synapses with GABAergic terminals that synapse on the dendritic tree, these synapses can undergo I-LTD as a result of postsynaptic activation of mGluR1/5 and retrograde release of 2-AG.

The specific presynaptic localization of CB₁Rs (Irving et al., 2000; Katona et al., 1999; Tsou et al., 1999) and the associated change in PPR during I-LTD (Figure 1) strongly suggests that the expression of I-LTD is presynaptic. The mechanism by which CB₁R activation triggers a persistent reduction of GABA release remains to be determined. Diverse signal transduction pathways downstream from CB₁R activation could be involved. For example, the CB₁R is coupled to Pertussis toxin-sensitive G proteins (G_{i/o}) and has been shown to inhibit adenylyl cyclase, activate mitogen-activated protein kinases, reduce voltage-dependent Ca²⁺ currents, and modulate several K⁺ conductances (Howlett et al., 2002; Schlicker and Kathmann, 2001). Finally, there is also evidence that the inhibitory effect of CB₁R agonists on transmitter release may involve sites downstream from Ca²⁺ entry, i.e., the release machinery (Takahashi and Linden, 2000; Vaughan et al., 1999). Future studies should identify which of these pathway(s) and target(s) underlie the persistent reduction of GABA release during I-LTD.

Endocannabinoids are synthesized and released upon stimulation (Mechoulam et al., 1998; Wilson and

Nicoll, 2002). Thus, both anandamide and 2-AG are produced in an activity-dependent manner in different areas of the brain (Di Marzo et al., 1994; Giuffrida et al., 1999; Stella et al., 1997). However, to our knowledge, no specific function has been attributed to an identified endocannabinoid. Because I-LTD is blocked by inhibiting both PLC (which promotes the generation of DAG) and DAG-lipase (which converts DAG into 2-AG) (Prescott and Majerus, 1983; Sugiura et al., 2002), we propose that 2-AG is the endogenous cannabinoid involved in hippocampal I-LTD. This notion is consistent with the fact that the same HFS of Schaffer collaterals we used to trigger I-LTD was also reported to trigger a 4-fold enhancement of 2-AG levels, but not anandamide, in hippocampal slices (Stella et al., 1997).

Finally, it has been reported that a postsynaptic rise of Ca^{2+} is required to induce endocannabinoid-dependent long-term depression in the striatum (Gerdeman et al., 2002) and nucleus accumbens (Robbe et al., 2002). In our study, however, buffering postsynaptic Ca^{2+} did not block I-LTD but was sufficient to block DSI. This discrepancy could reflect different mechanisms of endocannabinoid release at these synapses. Our findings are in agreement with previous observations in that endocannabinoid release via group I mGluR activation may not require a Ca^{2+} rise in both cerebellum (Maejima et al., 2001) and hippocampus (Ohno-Shosaku et al., 2002). Thus, our study is consistent with the notion that endocannabinoids can be released independently of any elevation of Ca^{2+} concentration.

Endocannabinoid Signaling Mediating I-LTD Is Localized

An interesting scenario arises from our observation that GDP- β S blocks I-LTD, presumably by preventing the G protein-dependent synthesis of 2-AG (Figure 3F). This finding strongly suggests that the source of 2-AG for I-LTD induction is from the recorded postsynaptic CA1 pyramidal cell itself. Because our extracellular stimulation method also activates fibers that synapse on neighboring cells, it is expected that endocannabinoids could have diffused from those cells that were not filled with GDP- β S. However, our results indicate that the contribution of 2-AG released from nearby CA1 pyramidal cells is unlikely and support the idea that endocannabinoid retrograde signaling is a highly localized process (Gerdeman et al., 2002; Maejima et al., 2001; Robbe et al., 2002). It is still possible that some spread of the mGluR-induced synthesis of 2-AG may occur as a result of intradendritic diffusion of signaling molecules such as DAG.

DSI and I-LTD: Short-Term versus Long-Term Endocannabinoid-Mediated Phenomena

Although both DSI and I-LTD are mediated by endocannabinoid retrograde signaling, their durations are remarkably different (Figure 4). The different time courses of these two processes may reflect either the amount, duration, or nature of endocannabinoid release. While DSI is presumably triggered by a brief release of endocannabinoids, our results indicate that to trigger I-LTD, CB₁Rs need to be activated for several minutes (Figure 7F). Thus, the duration of endocannabinoid release and

consequent CB₁R activation may contribute to determine whether the inhibition of GABA release will be transient or long lasting.

In contrast to I-LTD, DSI was unaffected by the blockade of PLC or DAG-lipase, suggesting that DSI would be mediated by another endocannabinoid than 2-AG. Alternatively, it has been postulated that the biosynthetic pathways of 2-AG appear to differ, depending on the cells and types of stimuli (Sugiura et al., 2002), and if 2-AG indeed mediates DSI, it is most likely produced through a different pathway.

The different properties of I-LTD and DSI strongly suggest that their functional roles may also differ. While DSI is a transient depression that globally affects most GABAergic inputs, I-LTD is a more localized and persistent reduction of synaptic efficacy (Figure 9B). Indeed, I-LTD was only observed at GABAergic inputs on the dendritic tree, but not on the soma, whereas DSI was observed in both input areas. The degree to which the effect of glutamate released by Schaffer collaterals is restricted within the dendritic tree remains to be determined. Nevertheless, these two endocannabinoid-mediated phenomena with their different temporal and spatial impacts on synaptic inhibitory efficacy greatly enlarge the repertoire of effects that can be mediated by endocannabinoids.

Physiological Relevance of I-LTD in the Hippocampus

GABAergic transmission plays a critical role in the regulation of neuronal excitability. Thus, changes in the level of inhibition can mediate the so-called E-S coupling potentiation; that is, a persistent increase in the ability of an EPSP to trigger an action potential following HFS (Bliss and Lomo, 1973). Although the most accepted mechanism underlying E-S coupling potentiation is a reduction of the inhibitory drive (Abraham et al., 1987; Chavez-Noriega et al., 1989), the cause of this disinhibition is still unclear. It was recently postulated that a calcineurin-mediated and NMDAR-dependent LTD of GABAergic inhibition, presumably due to postsynaptic changes in the sensitivity to GABA, could explain E-S coupling potentiation (Lu et al., 2000). However, a previous report postulated that E-S coupling potentiation is independent of the activation of NMDARs (Bernard and Wheal, 1995). Interestingly, the I-LTD described in our study is NMDAR independent and most likely due to a persistent reduction of GABA release. Moreover, we found that mGluR1/5 and CB₁R activation are both required for the increase in E-S coupling after HFS. In conclusion, our findings strongly suggest that the excitability change associated with I-LTD may underlie E-S coupling potentiation.

Disruptive effects of cannabinoids on memory and cognition are among the most frequently described findings (Lichtman et al., 2002; Sullivan, 2000), and it is believed that changes in hippocampal synaptic plasticity may underlie these effects. Several studies indicate that induction of LTP at hippocampal excitatory synapses is impaired after exogenous cannabinoid application (Collins et al., 1995; Misner and Sullivan, 1999; Stella et al., 1997; Terranova et al., 1995). This action may result from a decrease in glutamate release (Misner and Sullivan, 1999), presumably mediated by a cannabinoid

receptor other than CB₁R. In contrast to these effects on excitatory transmission, an opposite effect on LTP can be predicted by taking into account the endocannabinoid-mediated reduction of GABA release. In fact, it has been recently postulated that endocannabinoids released following depolarization of CA1 pyramidal cells, i.e., DSI, may actually facilitate the induction of LTP (Carlson et al., 2002). In addition to this transient disinhibitory action, we have now shown that endocannabinoids also mediate a persistent depression of inhibitory synaptic transmission, i.e., I-LTD. This form of plasticity not only may underlie changes in CA1 pyramidal cell excitability but may also exert long-lasting modulatory actions on the induction of LTP at excitatory synapses. Finally, it has been shown that chronic treatment with cannabinoid decreases the number of CB₁Rs in the hippocampus (Breivogel et al., 1999; Romero et al., 1995). In that way, cannabinoids could affect the ability of inhibitory synapses to express I-LTD and consequently impair excitatory synaptic plasticity and memory formation.

Conclusions

We found that excitatory inputs in the hippocampus can feedback onto inhibitory synapses and induce long-term changes in GABAergic synaptic efficacy via retrograde endocannabinoid signaling. This long-lasting disinhibitory effect can account for changes in excitability associated with synaptic plasticity at excitatory synapses. Our study supports a physiological role of endocannabinoids in the control of activity-dependent forms of plasticity and may explain the well-known effects of cannabinoids in cognitive functions.

Experimental Procedures

Transverse hippocampal slices (400 μ M thickness) were prepared from 3- to 4-week-old Sprague Dawley rats. Animals were killed by decapitation in accordance with local regulations. Slices were cut on a vibratome (Dosaka, Kyoto, Japan) in ice-cold extracellular solution containing (in mM) 238 sucrose, 2.5 KCl, 10 glucose, 25 NaHCO₃, 1.25 NaH₂PO₄, 2.5 CaCl₂, and 1.3 MgCl₂. The cutting medium was gradually switched to the recording solution (ACSF) that contained (in mM) 124 NaCl, 2.5 KCl, 10 glucose, 25 NaHCO₃, 1.25 NaH₂PO₄, 2.5 CaCl₂, and 1.3 MgCl₂. Cutting and recording solutions were both saturated with 95% O₂ and 5% CO₂ (pH 7.4). The slices were kept at room temperature for at least 1.5 hr before transfer to the recording chamber.

Whole-cell recordings of IPSCs were performed in CA1 pyramidal neurons voltage clamped in most experiments at +10 mV with a pipette (3–5 M Ω) containing (in mM) 123 cesium gluconate, 8 NaCl, 1 CaCl₂, 10 EGTA, 10 HEPES, 10 glucose, 5 ATP, 0.4 GTP (pH 7.2; 280–290 mOsm). DSI was evoked in neurons voltage clamped at –60 mV with a patch pipette containing (in mM) 131 cesium gluconate, 1 CaCl₂, 10 EGTA, 10 HEPES, 10 glucose, 5 ATP, 0.4 GTP (pH 7.2; 280–290 mOsm). In experiments including postsynaptic calcium buffer, 20 mM cesium gluconate were replaced by 20 mM BAPTA. Series resistance (typically 10–20 M Ω) was monitored throughout each experiment with a –4 mV, 80 ms pulse, and cells with more than 10% change in series resistance were excluded from analysis.

IPSCs were evoked by monopolar stimulation with a patch pipette filled with ACSF and placed in the middle third of *s. radiatum* or in *s. pyramidale*. Recordings were regularly performed in the continuous presence of NMDA and AMPA/KA receptor antagonists (50 μ M D-APV and 10 μ M NBQX) otherwise stated. I-LTD was commonly induced after 20 min of stable baseline by high-frequency stimulation (HFS), which consisted of 2 trains (20 s apart), each containing 100 pulses at 100 Hz. Theta burst stimulation (TBS) consisted of a

series of 10 bursts of 5 stimuli (100 Hz within the burst, 200 ms interburst interval), which was repeated 2 or 4 times (5 s apart). The magnitude of I-LTD was estimated by comparing averaged responses 35–40 min after HFS with baseline-averaged responses before induction protocol. DSI was evoked by a 5 s voltage step from –60 to 0 mV. IPSCs were monitored every 3 s for DSI and every 20 s for I-LTD. DSI magnitude was measured as the percentage of change between the mean of the ten consecutive IPSCs preceding the depolarization and the mean of three IPSCs immediately following depolarization (acquired 3–12 s after the pulse).

For extracellular recordings, two patch pipettes filled with 1 M NaCl were used to record simultaneously field excitatory synaptic potentials (fEPSPs) from *s. radiatum* and population spike (PS) extracellular potential from *s. pyramidale*. The stimulation strength was adjusted to give 30%–40% of maximal PS amplitude. The E-S coupling was calculated as the ratio between PS amplitude and fEPSP slope and its magnitude was estimated as the percentage of change before and 30–35 min after HFS.

All experiments were performed at 25.0°C \pm 0.1°C. Recordings were performed with a MultiClamp 700A (Axon Instruments Inc., Union City, CA) and output signals were filtered at 3 KHz. Data were digitized (5 kHz) and analyzed online using a macro written in IgorPro (Wavemetrics Inc., Lake Oswego, OR). Results are reported as mean \pm SEM. Statistical comparisons were performed using Student's *t* test. All drugs were bath applied following dilution into the external solution from concentrated stock solutions. D-APV, NBQX, (S)-3,5-DHPG, CGP 55845, MPEP, LY367385, U53122, AM251, and WIN 55,212-2 were obtained from Tocris-Cookson. RHC-80267 and GDP- β S were obtained from Biomol and all other chemicals and drugs were from Sigma-Aldrich.

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