Neuron, Vol. 29, 717–727, March, 2001, Copyright ©2001 by Cell Press

Retrograde Inhibition of Presynaptic Calcium Influx by Endogenous Cannabinoids at Excitatory Synapses onto Purkinje Cells

Anatol C. Kreitzer and Wade G. Regehr*
Department of Neurobiology
Harvard Medical School
Boston, Massachusetts 02115

Summary

Brief depolarization of cerebellar Purkinje cells was found to inhibit parallel fiber and climbing fiber EPSCs for tens of seconds. This depolarization-induced suppression of excitation (DSE) is accompanied by altered paired-pulse plasticity, suggesting a presynaptic locus. Fluorometric imaging revealed that postsynaptic depolarization also reduces presynaptic calcium influx. The inhibition of both presynaptic calcium influx and EPSCs is eliminated by buffering postsynaptic calcium with BAPTA. The cannabinoid CB1 receptor antagonist AM251 prevents DSE, and the agonist WIN 55,212-2 occludes DSE. These findings suggest that Purkinje cells release endogenous cannabinoids in response to elevated calcium, thereby inhibiting presynaptic calcium entry and suppressing transmitter release. DSE may provide a way for cells to use their firing rate to dynamically regulate synaptic inputs. Together with previous studies, these findings suggest a widespread role for endogenous cannabinoids in retrograde synaptic inhibition.

Introduction

Retrograde signaling is used by the nervous system to convey information about the activity of neurons back to cells that innervate them. Retrograde messengers play an important role in synapse formation (Fitzsimonds and Poo, 1998) and in the control of synaptic strength on rapid timescales (Alger and Pitler, 1995; Kombian et al., 1997; Zilberter et al., 1999). Short-term retrograde regulation of synapses was first described in the hippocampus and in the cerebellum, where depolarization of either CA1 pyramidal cells or Purkinje cells inhibits presynaptic GABA release onto these cells for tens of seconds (Llano et al., 1991b; Pitler and Alger, 1992, 1994; Vincent and Marty, 1993). Depolarization-induced suppression of inhibition (DSI) depends upon a rise in postsynaptic calcium that triggers the release of a retrograde messenger that in turn acts on the presynaptic nerve terminal through a G protein–dependent mechanism (Pitler and Alger, 1994). However, inhibition of excitatory inputs by the retrograde messenger that gives rise to DSI has not been described. We find a depolarization-induced suppression of excitatory inputs (DSE) in cerebellar Purkinje cells, indicating that both excitatory and inhibitory synapses can be modulated on rapid timescales.

*To whom correspondence should be addressed (e-mail: wade_regehr@hms.harvard.edu).

Studies of DSE in the cerebellum can provide insight into several fundamental issues regarding retrograde synaptic inhibition. A number of retrograde messengers have been identified at various synapses, including glutamate, GABA, and neuropeptides (Glitsch et al., 1996; Kombian et al., 1997; Morishita et al., 1998; Zilberter et al., 1999; Zilberter, 2000), as well as endogenous cannabinoids (R. I. Wilson and R. A. Nicoll, unpublished data). It is not known how widely these messengers serve to retrogradely inhibit synapses throughout the brain. Another unresolved issue is how these retrograde messengers inhibit release from presynaptic terminals. Several possible mechansims include branchpoint failure and incomplete action potential invasion of presynaptic terminals (Hatt and Smith, 1976; Alger et al., 1996), inhibition of presynaptic calcium channels (Anwyl, 1991), or direct effects on the release apparatus (Thompson et al., 1993; Alger and Pitler, 1995; Chen and Regehr, 1997). The excitatory synapses we study are amenable to optical recording methods, which allow us to differentiate between these possibilities.

Here, we find that Purkinje cell depolarization retrogradely inhibits both parallel and climbing fiber inputs. This DSE requires elevated postsynaptic calcium and follows a time course similar to DSI. We find that DSE is due to an inhibition of presynaptic calcium influx. DSE is prevented by blocking cannabinoid CB_1 receptors, which are located in the molecular layer of the cerebellum and can inhibit both parallel fibers and climbing fibers (Mailleux and Vanderhaeghen, 1992; Matsuda et al., 1993; Levenes et al., 1998; Takahashi and Linden, 2000). Thus, a transient elevation of postsynaptic calcium in Purkinje cells results in the release of endogenous cannabinoids, which inhibit afferent excitatory inputs for tens of seconds by modulating presynaptic calcium entry. Taken together with the widespread distribution of CB_1 receptors in the brain and the observation that CB_1 receptors are required for DSI in the hippocampus (R. I. Wilson and R. A. Nicoll, unpublished data), this suggests a general role for endogenous cannabinoids in the retrograde inhibition of both excitatory and inhibitory synapses.

Results

In this study, we examine two excitatory glutamatergic inputs to Purkinje cells (PC) with distinctive properties: parallel fiber (PF) and climbing fiber (CF) synapses (Eccles et al., 1966a, 1966b; Palay and Chan-Palay, 1974; Ito, 1984; Konnerth et al., 1990; Perkel et al., 1990; Silver et al., 1998). PF synapses are the connections between cerebellar granule cells and PCs. Each PC receives tens of thousands of PF synapses from cerebellar granule cells, with most granule cells making only a small number of contacts. PF synapses have a low probability of release and display prominent paired-pulse facilitation. By contrast, PCs are typically innervated by a single CF that originates in the inferior olive. Each CF makes hundreds of synaptic contacts, which have a high probability of release and exhibit paired-pulse depression.

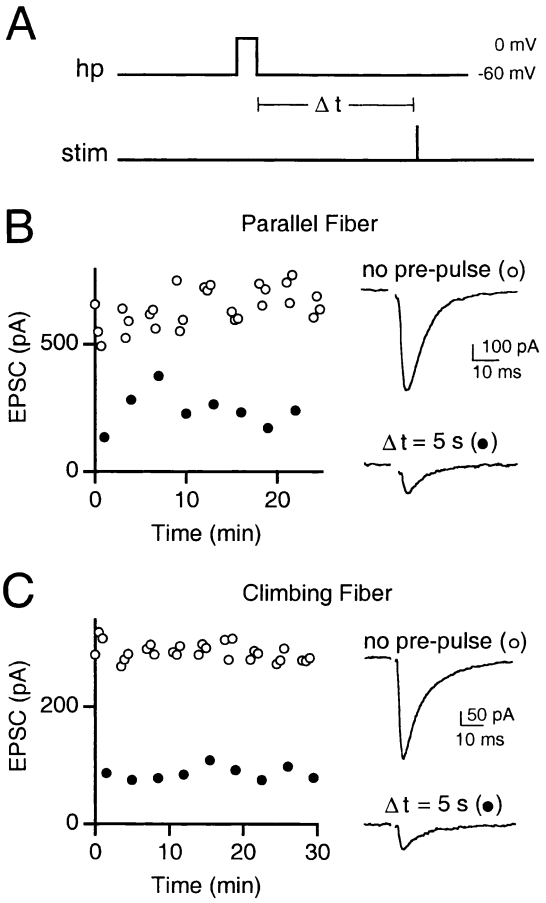

Figure 1. Postsynaptic Depolarization Inhibits Excitatory Purkinje Cell Afferents

(A) Stimulus protocol with the holding potential of the postsynaptic cell (hp; upper) and the stimulation timing (stim; below). Parallel fiber (B) and climbing fiber (C) EPSC amplitudes are plotted over time for control responses with no preceding prepulse to 0mV (open circles) and test responses following Purkinje cell depolarization (closed circles). Average parallel fiber (B) and climbing fiber (C) EPSCs are shown at the right. Stimulus artifacts are blanked for clarity. Parallel fiber and climbing fiber responses are from two representative experiments. The duration of the depolarization to 0mV was 50 ms for parallel fiber experiments and 1 s for climbing fiber responses. The test stimulus followed the depolarization by $\Delta t = 5$ s.

Effects of Postsynaptic Depolarization on Excitatory Synaptic Transmission

The effects of brief postsynaptic depolarizations on excitatory Purkinje cell afferents were studied in transverse rat cerebellar slices. PF EPSCs were evoked with an extracellular electrode placed in the molecular layer. After obtaining a stable synaptic response, we assessed the influence of stepping the voltage of the postsynaptic PC from −60mV to 0mV (Figure 1A). This greatly decreased the PF EPSC amplitude, as shown for a test stimulus that followed the postsynaptic depolarization by a Δt of 5 s (Figure 1B, lower right). This EPSC inhibition recovered within 90 s and was repeatedly and reliably elicited over the duration of an experiment (Figure 1B, left) where EPSCs following depolarization (closed circles) and control EPSCs (open circles) are both plotted. Depolarization of the PC also reliably inhibited CF synapses (Figure 1C). CF synapses appeared to be somewhat less sensitive to postsynaptic depolarization, and, therefore, the duration of the depolarization prior to a test CF EPSC was 1 s, compared to 50 ms for PF stimulation. We refer to this depolarization-induced suppression of excitation as DSE.

We next examined the time course of DSE by systematically varying Δt following a postsynaptic depolarizing prepulse (Figures 2Aa and 2Ba). DSE is small at early times ($\Delta t = 1$ s) and then approaches a maximum at 5–10 s and decays with a $t_{1/2}$ of ~15–20 s. The time course of DSE is similar at both the PF and CF synapses, suggesting that a common mechanism may underlie both phenomena.

To test whether DSE is expressed as a presynaptic or postsynaptic change, we assessed the effects of postsynaptic depolarization on paired-pulse plasticity. Most synapses, including both PF and CF synapses, display prominent short-term synaptic plasticity, which can provide insight into the probability of release. PF and CF synapses behave very differently in response to pairs of stimuli. The ratio of the amplitudes of the EPSC evoked by the second and first stimuli (A2/A1) is typically about 160% for PF synapses, and they are said to facilitate, which is consistent with their low initial probability of neurotransmitter release. CF synapses have a high initial probability of release and display paired-pulse depression, with A2/A1 of about 40%. At both of these synapses, short-term plasticity is thought to be presynaptic in origin (Atluri and Regehr, 1996; Dittman and Regehr, 1998), and a decrease in the initial probability of release increases the ratio A2/A1. Therefore, if the inhibition we observe following depolarization reflects a presynaptic change in the probability of release, A2/A1 should increase following depolarization. Most postsynaptic mechanisms are not consistent with such changes in short-term plasticity.

We found that postsynaptic depolarization affects short-term plasticity at both PF and CF synapses (Figures 2Ab and 2Bb). In the experiment shown, the paired-pulse ratio A2/A1 at the PF synapse increased from 150% in control conditions to 260% at 5 s after postsynaptic depolarization. At the CF synapse, the A2/A1 ratio increased from 30% to 80% at $\Delta t = 5$ s. Moreover, the time course of these changes in A2/A1 at both the PF and CF synapses matches the time course of DSE (Figures 2Aa and 2Ba). These increases in A2/A1 suggest that postsynaptic depolarization decreases the probability of release from both climbing fibers and parallel fibers. Summary data is shown in Figure 3.

The Role of Postsynaptic Calcium in DSE

Because postsynaptic depolarization results in an apparent suppression of presynaptic inputs, we next determined if a rise in postsynaptic calcium is required for DSE. The inclusion of the calcium chelator BAPTA (40 mM) in the postsynaptic recording pipette completely blocked the suppression of EPSCs at both the PF and CF synapses (Figure 3). At $\Delta t = 5$ s, when DSE is maximal in control conditions, there is no sign of either EPSC depression or a change in paired-pulse plasticity with BAPTA in the postsynaptic recording pipette (Figures

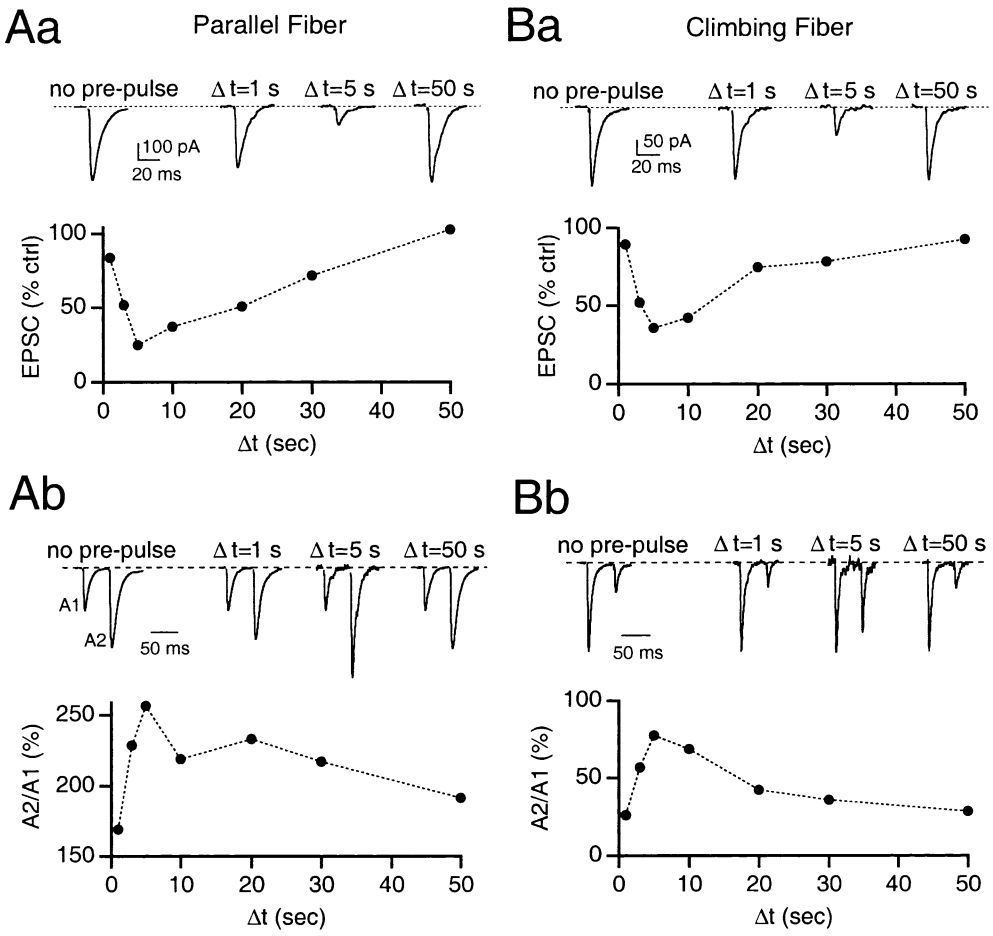

Figure 2. Presynaptic Short-Term Plasticity Is Altered Following Postsynaptic Depolarization

Parallel fiber (Aa) and climbing fiber (Ba) responses to stimuli lacking a postsynaptic prepulse and in response to test stimuli following depolarization to 0mV, during systematic variation of Δt. EPSCs are shown above, and the time course of depression of the test EPSC is below. Short-term plasticity of the parallel fiber (Ab) and climbing fiber (Bb) EPSCs evoked by pairs of stimuli are shown. Traces are normalized to the first EPSC of the control stimulus to aid in comparison. The amplitude of the paired-pulse ratio (A2/A1) as a function of time after the postsynaptic depolarization is plotted below. Data in (Aa) and (Ab) are from a single representative parallel fiber experiment. Data in (Ba) and (Bb) are from a single representative climbing fiber experiment.

3Aa and 3Ba). At PF and CF synapses, postsynaptic BAPTA completely abolished EPSC inhibition following depolarization at each value of Δt tested (Figures 3Ab and 3Bb). The changes in paired-pulse plasticity we observed at the PF and CF synapses during inhibition in control conditions were also eliminated by postsynaptic BAPTA at all time points (Figures 3Ac and 3Bc). These data suggest that elevations in postsynaptic calcium are required for DSE at both PF and CF synapses.

DSE at High Temperature

We next examined the magnitude and time course of DSE at 34°C, which is much closer to physiological temperatures (Figure 4). At PF synapses, both the onset and decay of DSE is faster (Figure 4A). At $\Delta t = 1$ s, a large inhibition is already present, which is maximal at 3 s and completely decayed by 20–30 s. The magnitude of DSE is slightly larger in the parallel fibers at 34°C. In the climbing fiber, a similar increase in the speed of onset and decay of inhibition was observed, while the magnitude of DSE remained similar between 24°C and 34°C (Figure 4B). The prominence of DSE at 34°C suggests that this phenomenon is important at physiological temperatures.

The Mechanism of Presynaptic EPSC Suppression

Changes in paired-pulse plasticity suggest that Purkinje cell depolarization produces a presynaptic suppression of transmitter release. This could arise from an inhibition of presynaptic calcium channels, branchpoint failure, or presynaptic inhibition downstream of calcium influx (Hatt and Smith, 1976; Anwyl, 1991; Thompson et al., 1993; Alger et al., 1996; Chen and Regehr, 1997). We therefore monitored presynaptic calcium influx to distinguish between these possibilities and to further characterize the mechanism responsible for DSE.

Optical measurements of presynaptic calcium levels were made at the climbing fiber. Each PC is innervated by a single CF whose axonal arbor forms a dense cluster of synapses that are contained in a single plane and are

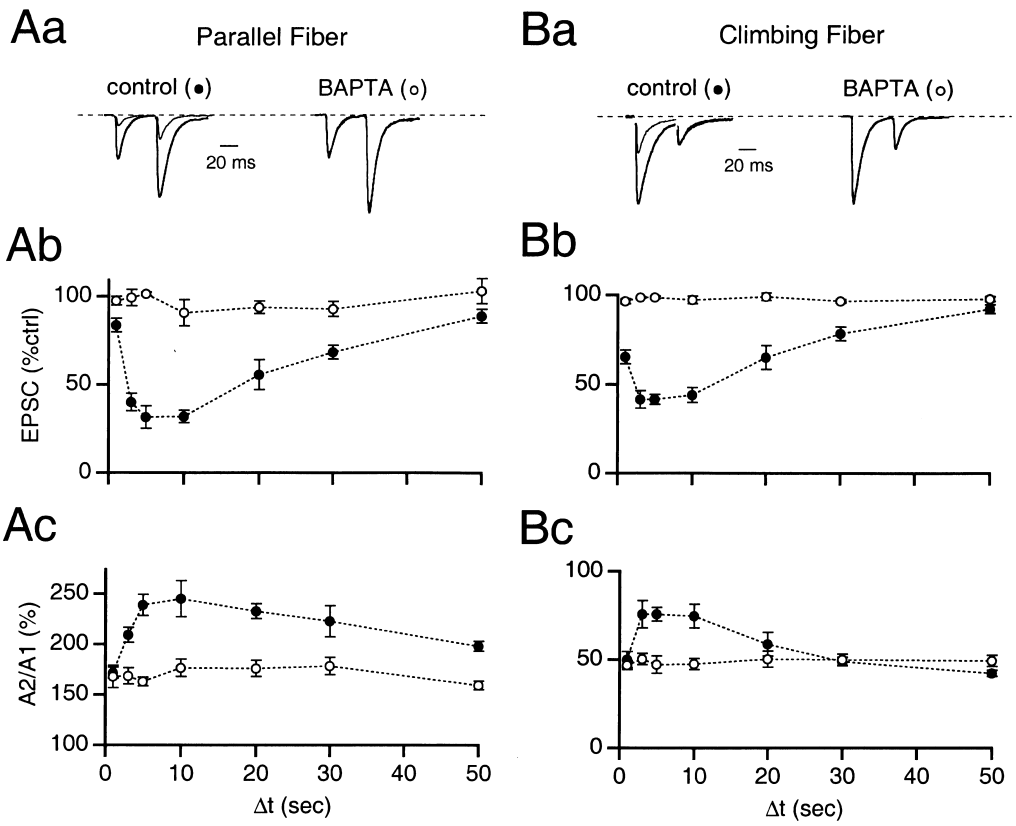

Figure 3. Elevation of Postsynaptic Calcium Is Required for DSE

Parallel fiber (Aa) and climbing fiber (Ba) EPSCs evoked by pairs of stimuli. Responses to stimuli without a preceding prepulse are overlayed on responses to test stimuli, both in control conditions and in the presence of postsynaptic BAPTA (40 mM). Traces are single trials from representative experiments. Summary of the time course of EPSC inhibition following postsynaptic depolarization in control conditions (closed circles) and in the presence of postsynaptic BAPTA (open circles) for parallel fibers (Ab) and climbing fibers (Bb). Summary of paired-pulse plasticity of the parallel fiber (Ac) and climbing fiber (Bc) following a postsynaptic prepulse to 0mV in control conditions (closed circles) and with postsynaptic BAPTA (open circles). Parallel fiber data (control, n = 7; BAPTA, n = 4) and climbing fiber data (control, n = 5; BAPTA, n = 4) are plotted as mean ± SEM.

therefore ideally suited to examining the presynaptic effects of depolarizing a single PC. In contrast, fluorescent signals from the parallel fibers arise from a great number of presynaptic terminals, only a small fraction of which represent synapses onto any given PC. As a result, it is difficult to study in isolation calcium transients in parallel fiber boutons that synapse onto a given Purkinje cell.

To quantify the reduction in CF presynaptic calcium influx following PC depolarization we used fluo-4 dextran (Kreitzer et al., 2000). This low-affinity indicator responds linearly to changes in presynaptic calcium and therefore provides a means for quantifying changes in calcium influx. We coinjected fluo-4 dextran (10,000 MW), which is faint at resting intracellular calcium concentrations, with Texas red dextran (10,000 MW) in order to visualize labeled fibers. Such a fiber is shown in Figure 5A, with a schematic of the recording configuration. Whole-cell voltage-clamp recordings were made from PCs innervated by labeled fibers, and stimulus electrodes were placed near the ascending CF axon. Epifluorescence was measured from the CF axon terminal arborization (Figure 5A, dotted circle). Following postsynaptic depolarization, we found a reduction in presynaptic calcium influx that followed the same time course as the inhibition of the EPSC (Figure 5B). At $\Delta t = 5$ s, when EPSC inhibition is maximal, the inhibition of calcium influx is also greatest. When BAPTA (40 mM) is included in the postsynaptic recording pipette, PC depolarization fails to inhibit CF presynaptic calcium influx at all values of Δt tested (Figure 5C). Therefore, during depolarization of the postsynaptic cell, calcium is required for the generation of a signal that inhibits presynaptic calcium influx for tens of seconds.

It is possible that the decrease in presynaptic calcium influx arises from incomplete action potential invasion of the presynaptic axonal arbor. We tested this hypothesis by using a CCD camera to image the extent of action potential invasion in climbing fibers. Calcium green dextran was used in these studies because it has a high affinity, and fluorescence changes produced by climbing fiber activation are much larger than those obtained with fluo-4 dextran. This makes it well suited to differentiating between a uniform reduction in calcium influx and incomplete spike invasion, although it underestimates changes in calcium entry and thus is poorly suited to quantifying changes in calcium entry.

Climbing fibers were labeled with calcium green dex-

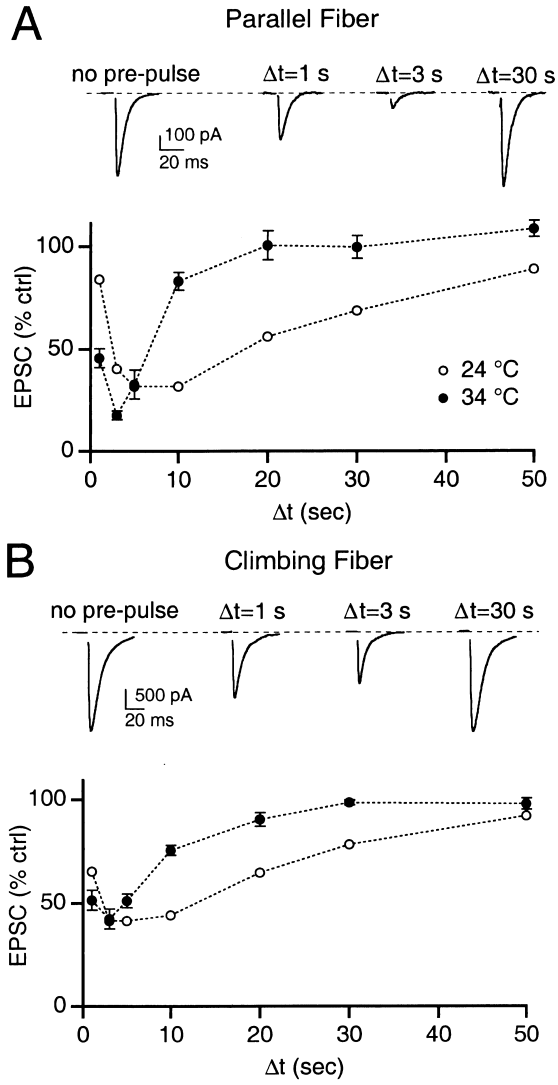

Figure 4. Temperature Dependence of DSE

Parallel fiber (A) and climbing fiber (B) EPSCs recorded in control conditions and at different times after postsynaptic depolarization at 34°C (above). The summary data (below) show the time course of EPSC inhibition at 34°C for the parallel fiber ([A], closed circles, n = 5) and climbing fiber ([B], closed circles, n = 4) inputs. The mean values at 24°C are shown for comparison (open circles). Data are mean ± SEM.

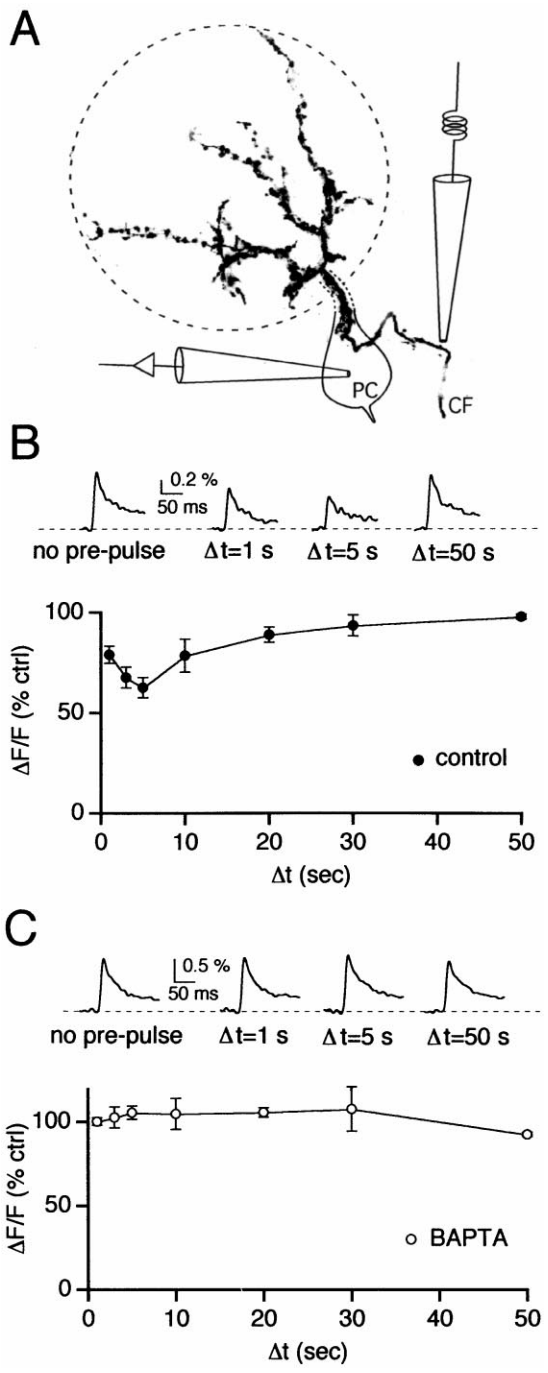

Figure 5. Postsynaptic Depolarization Inhibits Presynaptic Calcium Influx

(A) A confocal image stack of a climbing fiber colabeled with Texas red dextran and fluo-4 dextran is shown with a schematic of the recording configuration. The photodiode spot measurement area is delineated by the dotted circle. CFs were stimulated in control conditions and at varying times following postsynaptic depolarization. Fluo-4 fluorescence transients from a representative experiment are shown ([B], top). Traces are averages of five trials. A summary of the inhibition of presynaptic calcium influx following PC depolarization is shown ([B], solid circles, n = 5 CF-PC pairs). Fluo-4 transients were also measured in the presence of postsynaptic BAPTA (40 mM) (C). Traces from a representative experiment are shown above, while the summary data is plotted below (open circles, n = 3 CF-PC pairs). Data are mean ± SEM.

tran as described previously (Kreitzer et al., 2000). Whole-cell recordings were then obtained from Purkinje cells innervated by labeled fibers. Each climbing fiber was imaged at rest and following stimulation. The complete arborization of a young climbing fiber is shown in Figure 6A, and the stimulus electrode is located to the upper right outside the field of view. A computation of the difference in CF fluorescence (ΔF) at rest and following a single stimulus with no prepulse showed that stimulation evoked an increase of calcium throughout the climbing fiber under normal conditions (Figure 6Ca). The ΔF/F signal following a single stimulus with no prepulse is shown in Figure 6Cb, and the simultaneously recorded CF EPSC is displayed in Figure 6Cc for com-

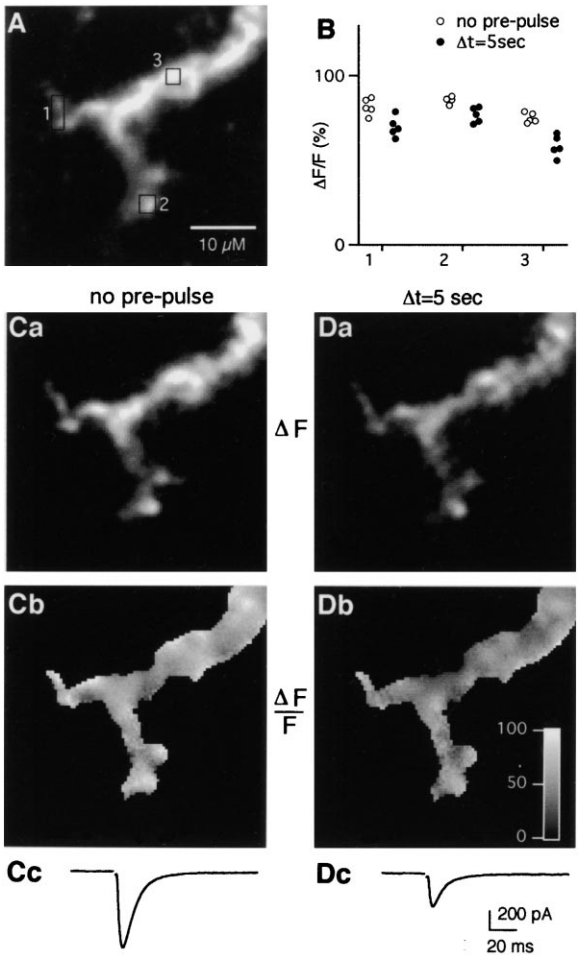

Figure 6. Postsynaptic Depolarization Does Not Result in Presynaptic Branchpoint Failure

(A) A CCD image of a calcium green dextran–labeled climbing fiber is shown. Image represents an average of ten frames taken without prior stimulation. Subregions used in the analysis in (B) are shown overlayed on the fiber. (B) The ΔF/F responses following stimulation either with a prepulse (closed circles) or without a prepulse (open circles). Responses to individual trials are plotted for each of the three subregions outlined in (A). (Ca) ΔF following a single stimulus without a preceding prepulse. (Da) ΔF following a test stimulus 5 seconds after depolarization of the postsynaptic cell innervated by the labeled climbing fiber. The ΔF/F signals following a single stimulus without a prepulse (Cb) and 5 s following postsynaptic depolarization (Db) are shown with the background masked for clarity. The calibration bar in (Db) is the percent ΔF/F and applies to both (Cb) and (Db). The average of the simultaneously recorded EPSCs without a prepulse (Cc) and the EPSCs after postsynaptic depolarization (Dc) are shown for comparison.

parison. Following postsynaptic depolarization (Δt = 5 s), a single stimulus evokes a weaker ΔF signal that extends throughout the climbing fiber (Figure 6Da). The ΔF/F signal (Figure 6Db) shows a uniform inhibition of calcium influx in the CF axonal arbor, while the simultaneously recorded EPSC is inhibited by ~50% (Figure 6Dc).

We quantified the change in the ΔF/F signal in the main axon and on secondary branches. As shown in Figure 6A, the ΔF/F signal was calculated from three separate regions of the CF arbor (labeled in Figure 6A), both for stimuli lacking a prepulse (Figure 6B, open circles) and for stimuli following a postsynaptic depolarization (Figure 6B, closed circles). During each trial, stimulation evoked a consistent ΔF/F signal in all subregions. In this example, during DSE the ΔF/F signal was 84% ± 5% in the main branch (box 3) and 88% ± 4% and 78% ± 5% in the secondary branches (boxes 2 and 3, respectively). For experiments conducted on two additional fibers, a similar analysis showed that the ΔF/F signal during DSE (percent of control ± SD) was 67% ± 15% in the main axon compared to 65% ± 32% and 75% ± 29% in the side branches for one fiber and 51% ± 7% in the main axon compared to 43% ± 12% and 40% ± 7% in side branches for another. These relatively uniform reductions in calcium influx throughout the axonal arbor, which were observed in all CF-PC pairs examined, suggest that the reduction of calcium influx into the CF presynaptic terminals is not a result of branchpoint failure.

Identification of the Receptors Targeted by the Retrograde Messenger

The suppression of release from presynaptic terminals by a calcium increase in the postsynaptic cell suggests that a retrograde messenger is involved. We started by considering the possibility that DSE requires activation of a metabotropic receptor that can mediate presynaptic inhibition at CF and PF synapses. These include metabotropic glutamate receptors (mGluRs), adenosine receptors, $GABA_B$ receptors, and cannabinoid receptors (Dittman and Regehr, 1996; Pekhletski et al., 1996; Levenes et al., 1998; Takahashi and Linden, 2000).

In the cerebellum, glutamate has been suggested to mediate DSI through activation of presynaptic mGluRs (Glitsch et al., 1996). Therefore, we first tested the involvement of metabotropic glutamate receptors in DSE. At parallel fiber presynaptic terminals, the group III mGluR agonist L-AP4 potently inhibits transmitter release (Figure 7A). We interleaved stimuli lacking a preceding prepulse (Figure 7A, open circles) with test stimuli following PC depolarization (Δt = 5 s) (Figure 7A, closed circles), while washing in L-AP4 (5 μM). We then reversed the effects of L-AP4 with the broad spectrum mGluR antagonist LY 341495 (100 μM), which inhibits all cloned mGluR subtypes (Fitzjohn et al., 1998). DSE persisted throughout the experiment, even in the presence of L-AP4 and LY 341495 (Figure 7A). The ratio of the magnitudes of EPSCs following a prepulse to those without a prepulse was 17% ± 5% for control conditions and 22% ± 5% after wash-in of 100 μM LY 341495 (n = 3).

Similar studies were performed on the CF synapse. The CF EPSC is inhibited by the group II mGluR agonist L-CCG-1 (1 μM) (Figure 7B) but is unaffected by L-AP4 (A C. K. and W. G. R., unpublished data). This inhibition is reversed by LY 341495 (100 μM), while DSE is unaffected by either L-CCG-1 or LY 341495 (100 μM).

The ratio of the magnitudes of EPSCs following a prepulse to those without a prepulse was 42% ± 3% (n = 5) for control conditions, compared to 42% ± 14% (n = 3) in 100 μM LY 341495, 35% ± 10% (n = 4) for 300 μM of the mGluR antagonist CPPG, and 23% (n =

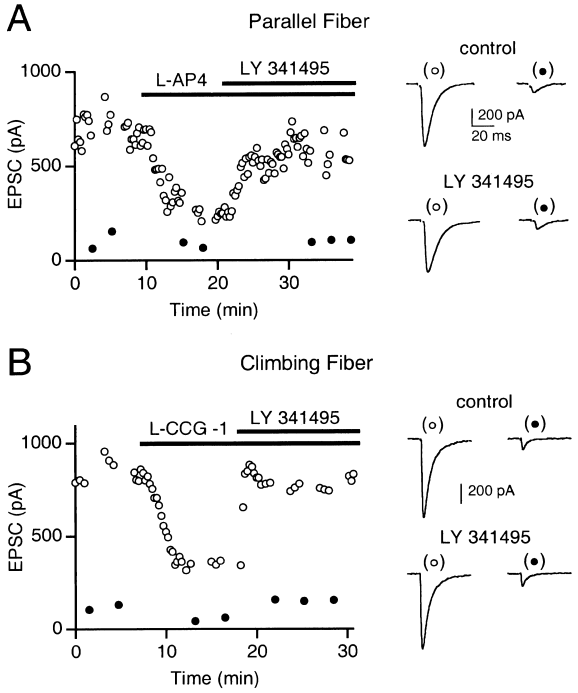

Figure 7. Metabotropic Glutamate Receptors Are Not Involved in DSE

Parallel fiber (A) and climbing fiber (B) EPSCs were evoked in response to stimuli without a prepulse (open circles) and test stimuli (closed circles) following postsynaptic depolarization ($\Delta t = 5$ s). In (A), the mGluR group III agonist L-AP4 (5 μM) was applied during parallel fiber stimulation, followed by the broad spectrum mGluR antagonist LY 341495 (100 μM). In (B), The mGluR group II agonist L-CCG-1 (1 μM) was applied during climbing fiber stimulation, followed by LY 341495 (100 μM). The average responses for stimuli with (closed circles) and without (open circles) a prepulse are shown at the right, both before and after application of mGluR agonists and LY 341495.

2) for 5 mM of the broad spectrum mGluR antagonist MCPG. These results demonstrate that metabotropic glutamate receptors are not involved in DSE of either CF or PF EPSCs.

Additional experiments revealed that $GABA_B$ receptors and adenosine A_1 receptors are also not involved in DSE. Application of the high-affinity $GABA_B$ antagonist CGP55845a and the adenosine A_1 antagonist DPCPX failed to block DSE. The ratio of the magnitudes of EPSCs following a prepulse to those without a prepulse was 14% $\pm$ 2% for control conditions and 11% $\pm$ 4% after wash-in of 2 μM CGP55845a and 5 μM DPCPX ($n = 3$).

We then tested the involvement of cannabinoid CB_1 receptors in DSE. This was a promising possibility because activation of CB_1 receptors can inhibit both PF and CF synapses (Levenes et al., 1998; Takahashi and Linden, 2000) and CB_1 receptors are involved in hippocampal DSI (R. I. Wilson and R. A. Nicoll, unpublished data). The high-affinity CB_1 receptor antagonist AM251 (Gatley et al., 1996) greatly reduced the amount of DSE at PF synapses without affecting the magnitude of EPSCs lacking a prepulse (Figure 8A). A 1 s depolarizing prepulse was used to elicit maximal DSE. We were unable to reverse the effects of AM251, which likely reflects the difficulty of washing this lipophilic drug from the slice. AM251 also greatly reduced DSE for CF synapses (Figure 8B). The ratio of the magnitudes of EPSCs following a prepulse ($\Delta t = 5$ s) to those without a prepulse increased from 15% $\pm$ 8% in control conditions to 90% $\pm$ 4% in the presence of AM251 for PF synapses ($n = 5$) and increased from to 40% $\pm$ 14% to 89% $\pm$ 2% in the presence of AM251 for CF synapses ($n = 3$). AM251 also eliminated retrograde inhibition of calcium influx (Figure 8C). In three such experiments, the ratio of the CF $\Delta F/F$ signal following PC depolarization to that measured without depolarization was 63% $\pm$ 4% in control conditions and 99% $\pm$ 1% in the presence of AM251. These results indicate that the CB_1 receptor is involved in DSE at both the CF and PF synapses.

We next determined whether cannabinoid receptor agonists occlude DSE at parallel fiber and climbing fiber synapses. Bath application of the cannabinoid receptor agonist WIN 55,212-2 (5 μM) inhibited PF EPSCs without a depolarizing prepulse (Figure 9A, open circles) but had little effect on stimuli following PC depolarization ($\Delta t = 5$ s) (Figure 9A, closed circles). In WIN 55,212-2, the inhibition of the EPSC produced by PC depolarization was reduced, and DSE was not prominent (Figure 9A, open squares). Similar results were found at the CF synapse (Figure 9B). Because EPSC inhibition by this lipophilic cannabinoid receptor agonist was often slow to develop during acute applications, we also preincubated slices in cannabinoid agonists for 1–3 hr prior to recording. The ratio of the magnitudes of EPSCs following a prepulse to those without a prepulse increased from 15% $\pm$ 8% ($n = 5$) in control conditions to 90% $\pm$ 4% ($n = 6$) in WIN 55,212-2 (5 μM) at PF synapses and went from 42% $\pm$ 3% ($n = 5$) to 83% $\pm$ 5% ($n = 5$) in WIN 55,212-2 (5 μM) at CF synapses. This occlusion of DSE by cannabinoid receptor agonists further supports the involvement of cannabinoid receptors in DSE.

Discussion

We found that Purkinje cell depolarization retrogradely inhibits the excitatory synapses that it receives—a phenomenon we term DSE. DSE requires a rise in postsynaptic calcium, which ultimately leads to a decrease in the probability of neurotransmitter release by inhibiting presynaptic calcium entry. Inclusion of a cannabinoid CB_1 receptor antagonist prevents DSE, suggesting that the retrograde messenger is an endogenous cannabinoid. Together with previous studies on DSI in the cerebellum (Llano et al., 1991b; Vincent and Marty, 1993), our data indicate that elevation of postsynaptic calcium levels in a Purkinje cell suppresses both excitatory and inhibitory inputs. This retrograde suppression provides a way for a cell to use its own rate of firing to dynamically adjust the weight of synapses that it receives on the tens of seconds timescale.

DSE Reflects a Change in Presynaptic Calcium Influx

A remarkable aspect of DSE is that postsynaptic depolarization rapidly inhibits transmission through a presynaptic mechanism. One piece of evidence for a presynap-

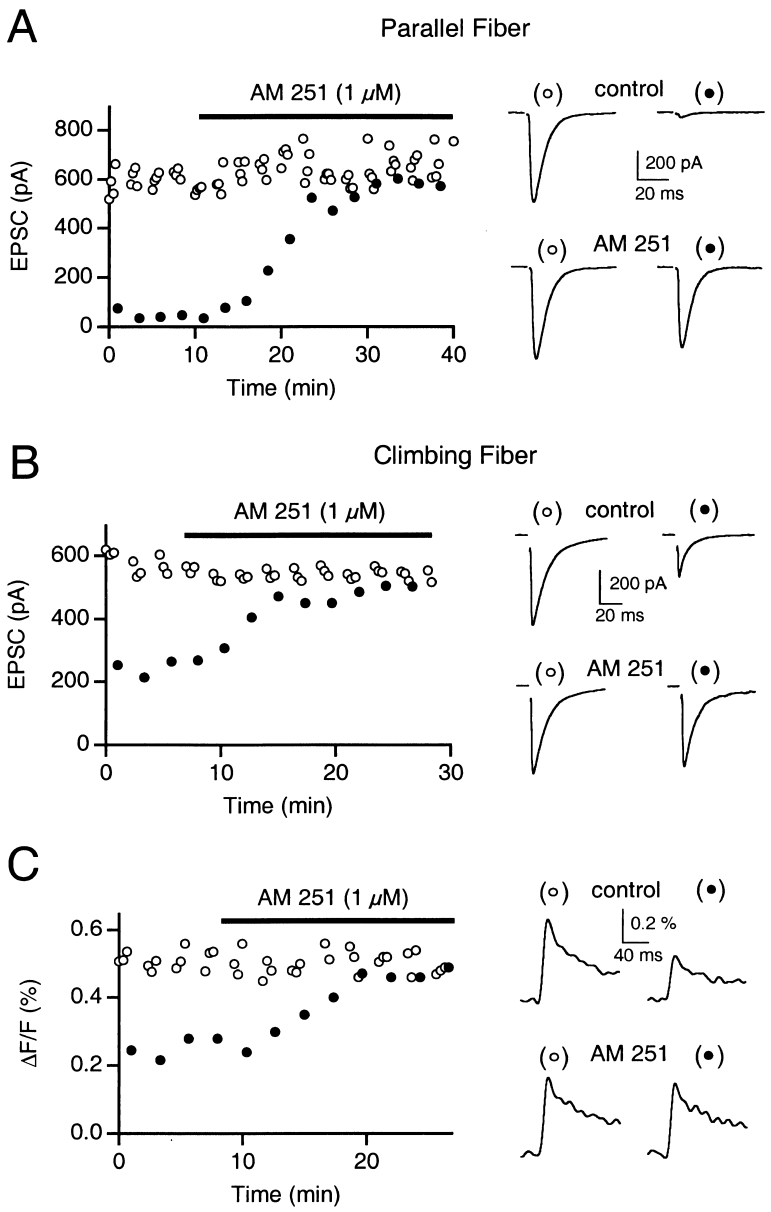

Figure 8. Cannabinoid CB_1 Receptor Antagonists Block DSE

Parallel fiber (A) and climbing fiber (B) EPSCs, as well as climbing fiber presynaptic calcium transients (C), were measured in response to stimuli without a prepulse (open circles) and test stimuli (closed circles) following a 1 s postsynaptic depolarization to 0mV ($\Delta t = 5$ s). In (A)–(C), the CB_1 receptor antagonist AM251 (1 μM) was bath applied during the time marked by the bar. The average responses are shown at the right, before AM251 application (top) and after AM251 application (bottom), in (A)–(C).

tic effect was the increase in the paired-pulse ratio at both PF and CF synapses, which is a hallmark of a decrease in the probability of release. Because CB_1 receptors are not found in Purkinje cells but are located in presynaptic fibers throughout the molecular layer (Mailleux and Vanderhaeghen, 1992; Matsuda et al., 1993; Egertova et al., 1998), the elimination of presynaptic inhibition by CB_1 receptor antagonists further supports a presynaptic locus. At the CF synapse, our most compelling evidence for a presynaptic alteration is the decrease in action potential–evoked presynaptic calcium influx that accompanies retrograde inhibition.

The most likely mechanism for cannabinoid modulation of presynaptic calcium influx is through activation of a G protein–coupled receptor, which can directly modulate presynaptic calcium channels. Direct calcium channel modulation by activation of CB_1 receptors has been demonstrated previously (Twitchell et al., 1997), and the time course of the inhibition we observe is consistent with such a mechanism. The relationship between calcium influx and EPSC amplitude is consistent with DSE arising primarily from the inhibition of calcium influx for both PF and CF synapses. At CF synapses, decreasing synaptic strength by reducing calcium entry had comparable effects on influx and release as those observed during DSE. For DSE ($\Delta t = 5$ s, 1 s depolarization), calcium influx and EPSCs are reduced to 63% and 42% of control, respectively, compared to 58% and 43% when extracellular calcium was lowered from 2 mM to 1 mM (A. C. K. and W. G. R., unpublished data). At PF synapses, we are unable to measure the reduction in calcium entry during DSE, but we have found that WIN 55,212-2 reduces calcium entry to 50% of control (A. C. K. and W. G. R., unpublished data). Based on the power law relationship between calcium influx and release at this synapse of ~2.5 (Mintz et al., 1995; Ditt-

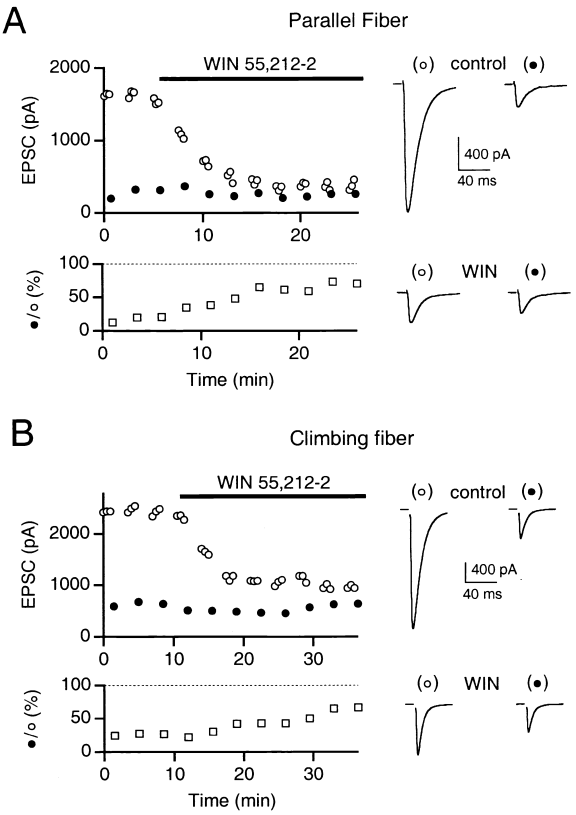

Figure 9. Cannabinoid Receptor Agonists Occlude DSE

Parallel fiber (A) and climbing fiber (B) EPSCs were evoked in response to stimuli without a prepulse (open circles) and test stimuli (closed circles) following a 1 s postsynaptic depolarization to 0mV ($\Delta t = 5$ s). In (A) and (B), the cannabinoid receptor agonist WIN 55,212-2 (5 μM) was bath applied during the time marked by the bar. The ratio of the amplitudes of EPSCs following a prepulse to EPSCs without a prepulse is plotted below on a separate graph (open squares) for the experiments in (A) and (B). At right are shown the average responses before drug application (control, top) and after washing in WIN 55,212-2 (WIN, bottom) in both (A) and (B).

man and Regehr, 1996), this would reduce the PF EPSC amplitude to 17% of control, which is similar to the EPSC amplitude 5 s after a 1 s PC depolarization (15% of control). In addition, WIN 55,212-2 does not affect the amplitude or frequency of mEPSCs in Purkinje cells or the presynaptic waveform (Takahashi and Linden, 2000). Taken together, these results suggest that at both CF and PF synapses DSE is primarily a consequence of inhibiting presynaptic calcium channels and that the higher sensitivity of PF synapses to changes in calcium entry contributes to the more pronounced DSE at PF synapses compared to CF synapses.

DSE Is Mediated by Endogenous Cannabinoids

The elimination of DSE by antagonists of the CB_1 receptor and the occlusion of DSE by cannabinoid receptor agonists together indicate that the retrograde messenger is an endogenous cannabinoid released by the Purkinje cell in a calcium-dependent manner, which then activates presynaptic CB_1 receptors to inhibit neurotransmitter release. A number of previous findings support this scheme. The synthesis and release of the endogenous cannabinoids anandamide and 2-AG is calcium dependent (Di Marzo et al., 1994; Stella et al., 1997), consistent with the elimination of DSE by BAPTA in the postsynaptic cell. It is also known that CB_1 receptors are expressed at high levels in the molecular layer, where the climbing fiber and parallel fiber synapses are located, but they are not found in Purkinje cells (Herkenham et al., 1990; Egertova et al., 1998). Activation of CB_1 receptors inhibits neurotransmitter release from both PF and CF synapses (Levenes et al., 1998; Takahashi and Linden, 2000). Finally, in the cerebellum, fatty acid amide hydrolase (FAAH) (Cravatt et al., 1996), the enzyme that degrades endogenous cannabinoids, is found only in Purkinje cells (Egertova et al., 1998). Taken together, these results are consistent with a calcium-dependent formation and release of endogenous cannabinoids from Purkinje cells; following release and activation of presynaptic metabotropic receptors on the PF and CF terminals, the cannabinoids are taken up by the Purkinje cell, where they are degraded.

Retrograde Signaling: A General Role for Endogenous Cannabinoids

Our results suggest a general role for endogenous cannabinoids. Recent work demonstrates that cannabinoids mediate DSI in the hippocampus (R. I. Wilson and R. A. Nicoll, unpublished data). Here, we demonstrate a similar role for endogenous cannabinoids in the regulation of excitatory synapses in the cerebellum. These results, combined with the widespread distribution of CB_1 receptors in the brain (Herkenham et al., 1990; Mailleux and Vanderhaeghen, 1992; Matsuda et al., 1993; Pettit et al., 1998), suggest that cannabinoids may be generally involved in the retrograde regulation of both inhibitory and excitatory synapses by postsynaptic neurons throughout the brain.

Significance of Retrograde Regulation of Synaptic Inputs

Calcium signaling in Purkinje cell dendrites and the calcium dependence of endogenous cannabinoid release both contribute to the manner in which DSE regulates synapses in physiological conditions. We have found, for example, that the extent of PF inhibition by CF activation depends crucially on the internal solution: CF activation reliably inhibits PF synapses when the recording electrode contains cesium, but, when it contains potassium, the magnitude and reliability of DSE is greatly diminished (A. C. K. and W. G. R., unpublished data). These findings suggest that dendritic potassium channels have a major impact on the extent of calcium elevations in PC dendrites, thereby influencing the magnitude and spatial extent of DSE. Many other factors, such as internal calcium stores (Llano et al., 1994) and the activation of inhibitory inputs onto Purkinje cells (Callaway et al., 1995), will affect the magnitude and spatial extent of calcium signals, thereby controlling whether DSE globally decreases synaptic efficacy or locally inhibits specific synapses.

Our data, together with previous studies, suggest that Purkinje cells are capable of inhibiting both excitatory and inhibitory inputs following depolarization through a

presynaptic mechanism. While DSI would paradoxically silence inhibition during periods of high activity and lead to even greater excitation, DSE provides a feedback mechanism by which a postsynaptic cell can regulate the strength of excitatory inputs on a scale of tens of seconds. This homeostatic regulation of synaptic strength is reminiscent of postsynaptic changes that occur on longer timescales to regulate neuronal excitability (Turrigiano et al., 1998). However, by acting rapidly through a presynaptic mechanism, the postsynaptic cell does not simply scale its inputs but also changes short-term synaptic plasticity (Figure 2). Therefore, retrograde inhibition provides a means for altering both the strength and the properties of presynaptic inputs for tens of seconds during periods of high postsynaptic activity.

Experimental Procedures

Electrophysiology

Transverse and sagittal slices (300 μM thick) were cut from the cerebellar vermis of 11- to 14-day-old Sprague Dawley rats. Slices were superfused with an external saline solution containing (in mM) NaCl, 125; KCl, 2.5; $CaCl_2$, 2; $MgCl_2$, 1; $NaHCO_3$, 26; NaH_2PO_4, 1.25; and glucose, 25; bubbled with 95% O_2/5% CO_2. For experiments examining paired-pulse facilitation (PPF), the external calcium concentration was increased to 4 mM in order to reduce the amplitude of baseline PPF. Bicuculline (20 μM) was added to the external solution to suppress synaptic currents mediated by $GABA_A$ receptors. For climbing fiber experiments, CNQX (1–2 μM) was included to reduce the amplitude of the climbing fiber EPSC. LY 341495, L-AP4, L-CCG-I, and AM251 were purchased from Tocris. WIN 55,212-2 was purchased from RBI.

Whole-cell recordings of Purkinje cells were obtained as described previously (Llano et al., 1991a; Regehr and Mintz, 1994). Glass electrodes (2–4 MΩ) were filled with one of two internal solutions. Solution 1 consisted of (in mM) CsGlu, 120; CsCl, 15; NaCl, 8; EGTA, 0.2; HEPES, 10; Mg-ATP, 2; and Na-GTP, 0.3; adjusted to pH 7.3 with CsOH. Solution 2 consisted of (in mM) $CsMeSO_3$, 100; CsCl, 50; EGTA, 0.2; HEPES, 10; $MgCl_2$, 1; Mg-ATP, 2; and Na-GTP, 0.3; adjusted to pH 7.3 with CsOH. Internal solutions also contained 5 mM QX-314. Solution 1 provided better seals, while solution 2 often gave more stable recordings. The magnitude and time course of retrograde inhibition were identical for both solutions. For experiments that included postsynaptic calcium buffer, the internal solution consisted of (in mM) $CsMeSO_3$, 40; CsCl, 20; Cs-BAPTA, 40; $CaCl_2$, 4; HEPES, 10; $MgCl_2$, 1; Mg-ATP, 2; and Na-GTP, 0.3; adjusted to pH 7.3 with CsOH. Synaptic currents were monitored with a holding potential of −60mV. Access resistance and leak currents were monitored continuously, and experiments were rejected if these parameters changed significantly during recording.

Parallel fibers were stimulated with a glass electrode filled with external saline and placed in the molecular layer. The interstimulus interval for parallel fiber stimulation was 10 s (single stimulus) or 15 s (two stimuli). Climbing fibers were stimulated with a glass electrode placed in the granule cell layer. The interstimulus interval for climbing fiber stimulation was 20 s (single stimulus) or 30 s (two stimuli). Following postsynaptic depolarizations and test stimuli, the interval was 80 s (single stimulus) or 90 s (two stimuli) for both parallel fiber and climbing fiber experiments.

Inferior Olive Injections

Injections were performed as described previously (Kreitzer et al., 2000). In brief, rats (P9-P12) were anesthetized with a continuous flow of a 0.75%–1.5% isoflurane/oxygen mixture and placed in a stereotaxic device. The brain stem was exposed by a dorsal/caudal approach. The calcium indicators fluo-4 dextran (10,000 MW) and calcium green dextran (3,000 and 10,000 MW) were dissolved in water, at a concentration of 20%–25%. These solutions were then mixed, in a 1:1 ratio, with a similar solution of Texas red dextran of the same molecular weight and injected into the inferior olive, using a Hamilton syringe.

Detecting Presynaptic Calcium Transients

Injected animals were sacrificed 2–4 days following injections, and sagittal slices of the cerebellar vermis were cut. Climbing fibers labeled with dextran-conjugated calcium indicators were stimulated with a glass electrode placed near their ascending axon in the granule cell layer. Fluorescence was measured from the climbing fiber arborization either with a photodiode or with a CCD camera (Cooke SensiCam) using Xenon illumination.

The filter set for calcium green dextran and fluo-4 dextran was 490DF10 for excitation, 510DRLP dichroic, and OG530 for emission. The filter set for Texas red dextran was 580DF15 for excitation, 600DRLP dichroic, and OG610 for emission. (Omega Optical, Brattleboro, VT).

Data Acquisition and Analysis

EPSCs were filtered at 1 kHz with a 4-pole Bessel filter. All signals were digitized at 5 kHz with a 16 bit D/A converter (Instrutech, Great Neck, NY), with Pulse Control software (Herrington and Bookman, 1995). Photodiode currents were digitally filtered offline. Analysis was performed using Igor Pro software (Wavemetrics, Lake Oswego, OR). CCD images were acquired using IP Lab software (Scanalytics, Fairfax, VA).

Acknowledgments

We thank Dawn Blitz, Adam Carter, Chinfei Chen, Kelly Foster, Kaspar Vogt, and Matthew Xu-Friedman for comments on the manuscript. This work was supported by the National Institutes of Health R01-NS32405-01.

Received November 21, 2000; revised January 28, 2001.

References

Alger, B.E., and Pitler, T.A. (1995). Retrograde signaling at $GABA_A$-receptor synapses in the mammalian CNS. Trends Neurosci. *18*, 333–340.

Alger, B.E., Pitler, T.A., Wagner, J.J., Martin, L.A., Morishita, W., Kirov, S.A., and Lenz, R.A. (1996). Retrograde signalling in depolarization-induced suppression of inhibition in rat hippocampal CA1 cells. J. Physiol. *496*, 197–209.

Anwyl, R. (1991). Modulation of vertebrate neuronal calcium channels by transmitters. Brain Res. Rev. *16*, 265–281.

Atluri, P.P., and Regehr, W.G. (1996). Determinants of the time course of facilitation at the granule cell to Purkinje cell synapse. J. Neurosci. *16*, 5661–5671.

Callaway, J.C., Lasser-Ross, N., and Ross, W.N. (1995). IPSPs strongly inhibit climbing fiber-activated $[Ca2+]_i$ increases in the dendrites of cerebellar Purkinje neurons. J. Neurosci. *15*, 2777–2787.

Chen, C., and Regehr, W.G. (1997). The mechanism of cAMP-mediated enhancement at a cerebellar synapse. J. Neurosci. *17*, 8687–8694.

Cravatt, B.F., Giang, D.K., Mayfield, S.P., Boger, D.L., Lerner, R.A., and Gilula, N.B. (1996). Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature *384*, 83–87.

Di Marzo, V., Fontana, A., Cadas, H., Schinelli, S., Cimino, G., Schwartz, J.C., and Piomelli, D. (1994). Formation and inactivation of endogenous cannabinoid anandamide in central neurons. Nature *372*, 686–691.

Dittman, J.S., and Regehr, W.G. (1996). Contributions of calcium-dependent and calcium-independent mechanisms to presynaptic inhibition at a cerebellar synapse. J. Neurosci. *16*, 1623–1633.

Dittman, J.S., and Regehr, W.G. (1998). Calcium dependence and recovery kinetics of presynaptic depression at the climbing fiber to Purkinje cell synapse. J. Neurosci. *18*, 6147–6162.

Eccles, J.C., Llinas, R., and Sasaki, K. (1966a). Parallel fiber stimulation and the responses induced thereby in the Purkinje cells of the cerebellum. Exp. Brain. Res. *1*, 17–39.

Eccles, J.C., Llinas, R., and Sasaki, K. (1966b). The excitatory synaptic action of climbing fibers on the Purkinje cells of the cerebellum. J. Physiol. *182*, 268–296.

Egertova, M., Giang, D.K., Cravatt, B.F., and Elphick, M.R. (1998). A new perspective on cannabinoid signalling: complementary localization of fatty acid amide hydrolase and the CB1 receptor in rat brain. Proc. R. Soc. Lond. B Biol. Sci. *265*, 2081–2085.

Fitzjohn, S.M., Bortolotto, Z.A., Palmer, M.J., Doherty, A.J., Ornstein, P.L., Schoepp, D.D., Kingston, A.E., Lodge, D., and Collingridge, G.L. (1998). The potent mGlu receptor antagonist LY341495 identifies roles for both cloned and novel mGlu receptors in hippocampal synaptic plasticity. Neuropharmacology *37*, 1445–1458.

Fitzsimonds, R.M., and Poo, M.M. (1998). Retrograde signaling in the development and modification of synapses. Physiol. Rev. *78*, 143–170.

Gatley, S.J., Gifford, A.N., Volkow, N.D., Lan, R., and Makriyannis, A. (1996). 123I-labeled AM251: a radioiodinated ligand which binds in vivo to mouse brain cannabinoid CB1 receptors. Eur. J. Pharmacol. *307*, 331–338.

Glitsch, M., Llano, I., and Marty, A. (1996). Glutamate as a candidate retrograde messenger at interneurone-Purkinje cell synapses of rat cerebellum. J. Physiol. *497*, 531–537.

Hatt, H., and Smith, D.O. (1976). Synaptic depression related to presynaptic axon conduction block. J. Physiol. *259*, 367–393.

Herkenham, M., Lynn, A.B., Little, M.D., Johnson, M.R., Melvin, L.S., de Costa, B.R., and Rice, K.C. (1990). Cannabinoid receptor localization in brain. Proc. Natl. Acad. Sci. USA *87*, 1932–1936.

Herrington, J., and Bookman, R.J. (1995). Pulse Control V4.5: IGOR XOPs for Patch Clamp Data Acquisition (Miami, FL: University of Miami).

Ito, M. (1984). The Cerebellum and Neural Control (New York: Raven Press).

Kombian, S.B., Mouginot, D., and Pittman, Q.J. (1997). Dendritically released peptides act as retrograde modulators of afferent excitation in the supraoptic nucleus in vitro. Neuron *19*, 903–912.

Konnerth, A., Llano, I., and Armstrong, C.M. (1990). Synaptic currents in cerebellar Purkinje cells. Proc. Natl. Acad. Sci. USA *87*, 2662–2665.

Kreitzer, A.C., Gee, K.R., Archer, E.A., and Regehr, W.G. (2000). Monitoring presynaptic calcium dynamics in projection fibers by in vivo loading of a novel calcium indicator. Neuron *27*, 25–32.

Levenes, C., Daniel, H., Soubrie, P., and Crepel, F. (1998). Cannabinoids decrease excitatory synaptic transmission and impair long-term depression in rat cerebellar Purkinje cells. J. Physiol. *510*, 876–879.

Llano, I., Marty, A., Armstrong, C.M., and Konnerth, A. (1991a). Synaptic- and agonist-induced excitatory currents of Purkinje cells in rat cerebellar slices. J. Physiol. *434*, 183–213.

Llano, I., Leresche, N., and Marty, A. (1991b). Calcium entry increases the sensitivity of cerebellar Purkinje cells to applied GABA and decreases inhibitory synaptic currents. Neuron *6*, 565–574.

Llano, I., DiPolo, R., and Marty, A. (1994). Calcium-induced calcium release in cerebellar Purkinje cells. Neuron *12*, 663–673.

Mailleux, P., and Vanderhaeghen, J.J. (1992). Distribution of neuronal cannabinoid receptor in the adult rat brain: a comparative receptor binding radioautography and in situ hybridization histochemistry. Neuroscience *48*, 655–668.

Matsuda, L.A., Bonner, T.I., and Lolait, S.J. (1993). Localization of cannabinoid receptor mRNA in rat brain. J. Comp. Neurol. *327*, 535–550.

Mintz, I.M., Sabatini, B.L., and Regehr, W.G. (1995). Calcium control of transmitter release at a cerebellar synapse. Neuron *15*, 675–688.

Morishita, W., Kirov, S.A., and Alger, B.E. (1998). Evidence for metabotropic glutamate receptor activation in the induction of depolarization-induced suppression of inhibition in hippocampal CA1. J. Neurosci. *18*, 4870–4882.

Palay, S.L., and Chan-Palay, V. (1974). Cerebellar Cortex (New York: Springer-Verlag).

Pekhletski, R., Gerlai, R., Overstreet, L.S., Huang, X.P., Agopyan, N., Slater, N.T., Abramow-Newerly, W., Roder, J.C., and Hampson, D.R. (1996). Impaired cerebellar synaptic plasticity and motor performance in mice lacking the mGluR4 subtype of metabotropic glutamate receptor. J. Neurosci. *16*, 6364–6373.

Perkel, D.J., Hestrin, S., Sah, P., and Nicoll, R.A. (1990). Excitatory synaptic currents in Purkinje cells. Proc. R. Soc. Lond. B Biol. Sci. *241*, 116–121.

Pettit, D.A., Harrison, M.P., Olson, J.M., Spencer, R.F., and Cabral, G.A. (1998). Immunohistochemical localization of the neural cannabinoid receptor in rat brain. J. Neurosci. Res. *51*, 391–402.

Pitler, T.A., and Alger, B.E. (1992). Postsynaptic spike firing reduces synaptic $GABA_A$ responses in hippocampal pyramidal cells. J. Neurosci. *12*, 4122–4132.

Pitler, T.A., and Alger, B.E. (1994). Depolarization-induced suppression of GABAergic inhibition in rat hippocampal pyramidal cells: G protein involvement in a presynaptic mechanism. Neuron *13*, 1447–1455.

Regehr, W.G., and Mintz, I.M. (1994). Participation of multiple calcium channel types in transmission at single climbing fiber to Purkinje cell synapses. Neuron *12*, 605–613.

Silver, R.A., Momiyama, A., and Cull-Candy, S.G. (1998). Locus of frequency-dependent depression identified with multiple-probability fluctuation analysis in rat climbing fibre-Purkinje cell synapses. J. Physiol. *510*, 881–902.

Stella, N., Schweitzer, P., and Piomelli, D. (1997). A second endogenous cannabinoid that modulates long-term potentiation. Nature *388*, 773–778.

Takahashi, K.A., and Linden, D.J. (2000). Cannabinoid receptor modulation of synapses received by cerebellar Purkinje cells. J. Neurophysiol. *83*, 1167–1180.

Thompson, S.M., Capogna, M., and Scanziani, M. (1993). Presynaptic inhibition in the hippocampus. TINS *16*, 222–227.

Turrigiano, G.G., Leslie, K.R., Desai, N.S., Rutherford, L.C., and Nelson, S.B. (1998). Activity-dependent scaling of quantal amplitude in neocortical pyramidal cells. Nature *391*, 892–896.

Twitchell, W., Brown, S., and Mackie, K. (1997). Cannabinoids inhibit N- and P/Q-type calcium channels in cultured rat hippocampal neurons. J. Neurophysiol. *78*, 43–50.

Vincent, P., and Marty, A. (1993). Neighboring cerebellar Purkinje cells communicate via retrograde inhibition of common presynaptic interneurons. Neuron *11*, 885–893.

Zilberter, Y. (2000). Dendritic release of glutamate suppresses synaptic inhibition of pyramidal neurons in rat neocortex. J. Physiol. *528*, 489–496.

Zilberter, Y., Kaiser, K.M., and Sakmann, B. (1999). Dendritic GABA release depresses excitatory transmission between layer 2/3 pyramidal and bitufted neurons in rat neocortex. Neuron *24*, 979–988.

Note Added in Proof

Two papers are in press showing that hippocampal DSI is a consequence of retrograde signaling by endogenous cannabinoids: Wilson, R.I., and Nicoll, R.A. (2001). Endogenous cannabinoids mediate retrograde signaling at hippocampal synapses. Nature *410*, 588–592; Ohno-Shosaku, T., Maejima, T., and Kano, M. (2001). Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. Neuron *29*, 729–738.