Cannabinoid Receptor Modulation of Synapses Received by Cerebellar Purkinje Cells

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Takahashi, K. and Linden D. J., Cannabinoid receptor modulation of synapses received by cerebellar Purkinje cells. J. Neurophysiol. 83:1167–1180, 2000. The high density of cannabinoid receptors in the cerebellum and the degradation of motor coordination produced by cannabinoid intoxication suggest that synaptic transmission in the cerebellum may be strongly regulated by cannabinoid receptors. Therefore the effects of exogenous cannabinoids on synapses received by Purkinje cells were investigated in rat cerebellar slices. Parallel fiber-evoked (PF) excitatory post synaptic currents (EPSCs) were strongly inhibited by bath application of the cannabinoid receptor agonist WIN 55212-2 (5 µM, 12% of baseline EPSC amplitude). This effect was completely blocked by the cannabinoid CB1 receptor antagonist SR 141716. It is unlikely that this was the result of alterations in axonal excitability because fiber volley velocity and kinetics were unchanged and a cannabinoid-induced decrease in fiber volley amplitude was very minor (93% of baseline). WIN 55212-2 had no effect on the amplitude or frequency of spontaneously occurring miniature EPSCs (mEPSCs), suggesting that the effect of CB1 receptor activation on PF EPSCs was presynaptically expressed, but giving no evidence for modulation of release processes after Ca2+ influx. EPSCs evoked by climbing fiber (CF) stimulation were less powerfully attenuated by WIN 55212-2 (5 µM, 74% of baseline). Large, action potential–dependent, spontaneously occurring inhibitory post synaptic currents (sIPSCs) were either severely reduced in amplitude (<25% of baseline) or eliminated. Miniature IPSCs (mIPSCs) were reduced in frequency (52% of baseline) but not in amplitude, demonstrating suppression of presynaptic vesicle release processes after Ca2+ influx and suggesting an absence of postsynaptic modulation. The decrease in mIPSC frequency was not large enough to account for the decrease in sIPSC amplitude, suggesting that presynaptic voltage-gated channel modulation was also involved. Thus, while CB1 receptor activation reduced neurotransmitter release at all major classes of Purkinje cell synapses, this was not accomplished by a single molecular mechanism. At excitatory synapses, cannabinoid suppression of neurotransmitter release was mediated by modulation of voltage-gated channels in the presynaptic axon terminal. At inhibitory synapses, in addition to modulation of presynaptic voltage-gated channels, suppression of the downstream vesicle release machinery also played a large role.

INTRODUCTION

Marijuana has been used by humans since 2700 BC or earlier and is presently one of the most commonly used recreational drugs in the world (reviewed in Adams and Martin 1996). It’s principal active ingredient, (−)-trans-Δ9-tetrahydrocannabinol (Gaoni and Mechoulam 1964), is one of a class of cannabinoid drugs made up of numerous natural and synthetic analogues. The effects of cannabinoid administration are varied and include altered perception of sight and sound, euphoria, anxiolysis, and loosening of associations. Cannabinoids also induce effects such as degraded motor coordination and timing that suggest a possible cerebellar influence.

Cannabinoids act as agonists at specific cannabinoid receptors, two of which, CB1 (Matsuda et al. 1990) and CB2 (Munro et al. 1993), have been cloned. Although both CB1 and CB2 are found in the periphery, only CB1 is found in the central nervous system (CNS) where its density is particularly high and its distribution widespread (Herkenham 1992; Herkenham et al. 1991; Katona et al. 1999; Mailleux and Vanderhaeghen 1992; Matsuda et al. 1993; Pettit et al. 1998; Tsou et al. 1998). The brain regions that have the highest densities of CB1 are the basal ganglia, cerebellum, hippocampus, and parts of the olfactory cortex. The moderate-to-high density of CB1 in the cerebellum, basal ganglia, and motor cortex may largely explain the motor deficits associated with cannabinoid intoxication (reviewed in Herkenham 1992; Loewe 1946).

In the cerebellum, the vast majority of CB1 is found at the presynaptic terminals received by Purkinje cells, the axons of which are the sole output of the cerebellar cortex. GABAergic axon collaterals form a pericellular basket around the Purkinje cell body and a tighter structure, called the pinceau, around the axon initial segment (Palay and Chan-Palay 1974). Basket cell axons and terminals make up this structure, although a small proportion of stellate cells also contribute (Paula-Barbosa et al. 1983). CB1 receptor density is highest by far in the cerebellar pinceau and is moderately high in the pericellular basket (Tsou et al. 1998). This is consistent with the reported lack of cannabinoid receptor mRNA expression by Purkinje cells and the rather high expression in cells of the molecular layer that appear to be stellate and basket cells (Mailleux and Vanderhaeghen 1992; Matsuda et al. 1993). CB1 immunoreactivity is moderately high in the molecular layer and low in the granule cell layer. However, granule cell layer expression of cannabinoid receptor mRNA is uniform and high. This suggests CB1 localization in parallel fibers (PFs), which are the axons of granule cells in the molecular layer. There is low expression of cannabinoid receptor mRNA in the inferior olive (Herkenham 1992; Herkenham et al. 1991; Mailleux and Vanderhaeghen 1992; Matsuda et al. 1993; Pettit et al. 1998; Tsou et al. 1998).

CB1 activation has been widely shown to decrease adenylyl cyclase activity and reduce cAMP accumulation via G1/G0 activation in heterologous expression systems and in neurons including both primary cultures and acute preparations of cerebellar cells (Childers and Deadwyler 1996; Deadwyler et al. 1993; Howlett 1984; Howlett and Fleming 1984; Pacheco et al. 1985).

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CB1 receptors have also been found to modulate voltage-gated ion channels. Using cultured hippocampal neurons, heterologous expression systems, and neuroblastoma and neuroblastoma/glioma cell lines, it has been demonstrated that CB1 activation can decrease N and P/Q type Ca\(^{2+}\) channel activity and increase K\(_{\text{IR}}\) activity through direct interaction with G protein \(\beta\gamma\) subunits. All of these effects are pertussis toxin-sensitive and therefore mediated by G\(_i/G_o\). (Caulfield and Brown 1992; Henry and Chavkin 1995; Mackie and Hille 1992; Mackie et al. 1995; Shen and Thayer 1998; Twitchell et al. 1997). In primary hippocampal cultures, cannabinoid application has been shown to relieve the resting inactivation of K\(_A\) channels, and this may be mediated through a cAMP dependent pathway (Deadwyler et al. 1995; Hampson et al. 1995; reviewed by Childers and Deadwyler 1996).

CB1 receptor activation has been found to suppress synaptic transmission at a number of excitatory and inhibitory synapses. CB1 receptor activation acting presynaptically strongly suppressed EPSC amplitude at synapses between hippocampal neurons in dispersed culture. However, in these neurons, IPSC amplitude was unaffected (Shen and Thayer 1998; Shen et al. 1996). Similarly, in acute slices of rat hippocampus, CB1 activation was found to suppress synaptic input from CA3 to CA1 neurons by acting on presynaptic Ca channels (Sullivan 1999). This was sufficient to prevent induction of long-term potentiation and long-term depression by standard protocols (Miscner and Sullivan 1999). In addition, CB1 activation was shown to suppress transmission between cerebellar PFs and Purkinje cells (Lévénes et al. 1998). In rat corpus striatum, recurrent collaterals from the axons of medium spiny neurons form autapses. IPSCs evoked by stimulating these collaterals were strongly suppressed by CB1-mediated presynaptic inhibition (Szabo et al. 1998). CB1 activation was also reported to suppress inhibitory transmission between striatal neurons and their targets in substantia nigra pars reticulata and globus pallidus (Chan et al. 1998; Miller and Walker 1995, 1996). CB1 activation was also shown to suppress IPSCs in the neurons of the rostral ventromedial medulla, a location that participates in the processing of nociceptive information (Vaughn et al. 1999). Finally, CB1 activation reduced the release of \(^{3}H\)-GABA from hippocampal slices stimulated with an electrical field (Katona et al. 1999).

Given the high-density of CB1 on the axons or axon terminals of Purkinje cell afferents and the behavioral deficits produced by cannabinoid intoxication that suggest cerebellar involvement, we examined CB1 receptor-mediated modulation of synapses received by Purkinje cells. This was explored electrophysiologically in both whole-cell patch clamp recordings of Purkinje cells and field potential recordings of PFs.

**METHODS**

**Sagittal preparation and whole-cell patch-clamp recording**

Sagittal slices of cerebellar vermis (200 \(\mu\)m thick) or horizontal cerebellar slices (400 \(\mu\)m thick) were prepared from male and female Sprague-Dawley rats (Harlan) using standard methods (Dittman and Regehr 1996; Konnerth et al. 1990; Perkel et al. 1990) modified as follows. Normal external saline consisted of (in mM) 129 NaCl, 3.0 KCl, 2 CaCl\(_2\), 1.75 MgCl\(_2\), 1 NaH\(_2\)PO\(_4\), 26.3 NaHCO\(_3\), 50 glucose, pH 7.40, 321 mmol/kg, and was gassed with 95% O\(_2\)/5% CO\(_2\). Dissections and sectioning were performed in ice cold external saline in which CaCl\(_2\) had been replaced with MgCl\(_2\). Slices were placed submerged on small slips of nylon mesh supported on a ledge in a custom-built storage chamber containing normal external saline incubated at \(\sim35^\circ\)C and gassed with 95% O\(_2\)/5% CO\(_2\). After all slices were placed in the chamber, solution was removed until an interface condition was achieved and the top of the chamber was covered with parafilm. After incubating for 30–60 min at 35°C, the slices were left at room temperature for at least an additional 30 min before use. Slice health was maximized by storing slices at room temperature in an interface condition. At least 10 min before recording, slices were submerged and secured with a silver wire-nylon thread harp in a recording chamber containing \(\sim2.7\) ml of saline.

Visualized whole-cell patch recordings were conducted at room temperature using standard methods (Konnerth et al. 1990; Perkel et al. 1990). External saline was delivered to the chamber at 3 ml/min with a peristaltic pump. All external saline used in the recording chamber contained either 0.02 or 0.1% DMSO and the concentration of DMSO was always kept constant throughout each recording. When 0.1% DMSO was used, ddH\(_2\)O was also added to compensate for the increase in osmolality. Purkinje cells were visualized using an Axioskop FS upright microscope equipped with a water-immersion 40\(\times\) objective. Patch pipettes were pulled from borosilicate capillary tubing and heat polished. Membrane current was recorded using an Axopatch 200A amplifier controlled with Axodata software and analyzed off-line using Axograph software (Axon Instruments, Burlingame, CA). PFs and climbing fibers (CFs) were stimulated with a monopolar stimulating electrode consisting of a patch pipette filled with filtered external saline. To avoid contamination, stimulating electrodes were changed after every experiment during which a drug was added to the recording chamber.

**Drugs**

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R\left(+\right)-[2,3\text{-dihydro-5\text{-methyl-3\text{-}(\text{morpholinyl})\text{-methyl}}\text{-pyrrolo[1,2,3-de]-1,4\text{-benzoxazin-yl}]-(1\text{-naphthalenyl})methanone \text{mesylate [R}\left(+\right)-\text{WIN 55212-2] and S\left(-\right)-[2,3\text{-dihydro-5\text{-methyl-3\text{-}(4\text{-morpholinyl})\text{methyl}}\text{pyrrolo[1,2,3-de]-1,4\text{-benzoxazin-yl]-(1\text{-naphthalenyl})methanone-meslyate [S\left(-\right)-\text{WIN 55212-3] were obtained from Research Biochemicals Inc. (Natick, MA). Stoks}(10 \text{mM}) \text{ were made in DMSO and stored in 50-\mu l aliquots at } -20^\circ\text{C. A fresh 10-}\text{mM stock of N-piperidino-5\text{-}\text{(4-chlorophenyl)-1\text{-}(2,4\text{-dichlorophenyl)-4\text{-methylpyrazole-3-carboxamide (SR 141716) in DMSO was made on each day of use. As cannabinoids tend to stick to surfaces they contact, most tubing was replaced and all other exposed parts were thoroughly cleansed with ethanol and water after each cannabi-}}
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**PF-evoked EPSC recordings**

Purkinje cells in sagittal sections of postnatal day (P) 15–19 rat cerebellar vermis were whole-cell patch clamped with 3–5.5 M\(\Omega\) pipettes in external saline containing 20 \(\mu\)M bicuculline methiodide. Pipette saline consisted of (in mM) 8 KCl, 125 K-glucuronate, 5 MgCl\(_2\), 10 HEPES, 5 NaOH, 4 Na\(_2\)ATP, 0.4 Na\(_3\)GTP, and sucrose to bring osmolality to 287–290 mmol/kg, pH \(\sim7.23\). Liquid junction potential was \(-12\) mV (pipette negative) and was compensated on-line. Series resistance (\(R_s\)) compensation, used not so much to cancel \(R_s\) as to keep it constant, was set to 2–3 \(\Omega\) \(\text{M}^\circ\), 50% compensation, and 60 \(\mu\)s lag. \(R_s\) was typically 7–10 \(\Omega\) \(\text{M}^\circ\) after compensation and was kept constant throughout each experiment with gentle suction or adjustment of \(R_s\) compensation. PF-EPSCs inevitably contained a voltage-activated component, and large EPSCs evoked Na\(^+\) spikes that escaped voltage clamp. Therefore baseline EPSC amplitude was kept relatively small (typically \(-300\) to \(-500\) pA). \(V_{\text{hold}}\) was set at \(-57\)
mV, which was very close to $V_{\text{rest}}$, to reduce both inactivation and sensitization of voltage-gated currents. With these adjustments, space clamp was poor but adequate. In some pilot experiments, Cs-based internal saline was used to improve space clamp, but slow perfusion of dendrites produced a gradually increasing EPSC amplitude that often took $>30$ min to stabilize. To stimulate PF-evoked EPSCs, the tip of the stimulating electrode was placed in the inner two-thirds of the molecular layer. To obtain stable, spike-free EPSCs, the following

FIG. 1. WIN 55212-2 produced a large dose-dependent suppression of parallel fiber (PF)-evoked excitatory postsynaptic currents (EPSCs) recorded in Purkinje cells. A: PF stimulation protocol and sample trace from voltage-clamped Purkinje cell. This protocol was repeated every 10 s. B: representative experiment. Cannabinoid receptor agonist WIN 55212-2 at 2 μM suppressed PF EPSC amplitude to 19.7% of baseline. Displayed are averaged traces from 0.7–5.7 min before (30 traces) and 10.3–14.3 min after (24 traces) WIN 55212-2 entered the recording chamber. C: PF EPSCs were reduced in amplitude by WIN 55212-2 and eliminated by TTX. Same experiment as in A and B. D: representative experiment. The effect of WIN 55212-2 was completely blocked by preincubation and coapplication of CB1 receptor–specific antagonist SR 141716. E: summary of the effect of WIN 55212-2 and SR 141716 on PF EPSCs (mean ± SE). 1S + 5W = 1.0 μM SR 141716 + 5.0 μM WIN 55212-2. *, not significant, $P = 0.41$. The effect of WIN 55212-2 at all concentrations was significant ($P < 10^{-4}$). F: $R_s$ and $G_m$ were stable and unaffected by WIN 55212-2. These data were taken from the same experiments in E.
protocol was empirically derived (Fig. 1A): every 10 s, \( V_{\text{hold}} \) was stepped from \(-57 \) to \(-80 \) mV. PFs were stimulated 2000 ms after the onset of hyperpolarization. After another 350 ms, \( V_{\text{hold}} \) was stepped from \(-80 \) to \(-85 \) mV for 150 ms to measure \( R_s \) and \( R_h \), and then returned to \(-57 \) mV. In analysis, EPSCs and \(-5 \) mV steps were analyzed separately, with each event baseline to the 100 ms interval that preceded it. Data were filtered at 5 kHz with the four-pole, low-pass Bessel filter integral to the Axopatch 200A and acquired at 5 kHz. Data are presented as mean \( \pm \) SE.

Experiments were conducted as follows (Fig. 1C): after \( \geq 10 \) min of stable baseline recording of PF EPSCs, the external saline reservoir was replaced with either another bottle of control external saline or one containing the cannabinoid receptor agonist WIN 55212-2 for \( \geq 15 \) min. Finally, the reservoir was switched to external saline containing \( 1.0 \) \( \mu \)M TTX, which eliminated the EPSC and isolated the stimulus artifact. Off-line, the stimulus artifact, averaged from \( \geq 10 \) traces in TTX, was subtracted from all traces in the experiment to improve accuracy. Within each experiment, the effect of switching to a second external saline reservoir was calculated as 100% \( X \) (the average of all data 10.3–14.3 min after the second saline entered the recording chamber) divided by the average of all data 0.7–5.7 min before the second saline entered the recording chamber. Experiments in which \( R_s \) or membrane conductance (\( G_m \)) changed by \( >12% \) between these periods were discarded.

**Fiber volley recordings**

These experiments were conducted in 400-\( \mu \)m-thick horizontal sections of cerebellum from P15–16 rats with methods modified from Dittman and Regehr (1996). Submerged recordings were made using saline containing 200 \( \mu \)M CdCl\(_2\) to eliminate Ca\(^{2+}\) currents and Ca\(^{2+}\)-channel-dependent synaptic contributions. Because 200 \( \mu \)M Cd\(^{2+}\) precipitates out of normal NaHCO\(_3\)/NaH\(_2\)PO\(_4\)-based salines, an oxygenated HEPES-based saline was used consisting of (in mM) 149 NaCl, 3.0 KCl, 2 CaCl\(_2\), 1.75 MgCl\(_2\), 20 glucose, 10 HEPES, 0.02 MCd\(_2\) to eliminate Ca\(^{2+}\), and 0.2 bicuculline methiodide, 1 ml/l DMSO, pH 7.40, 322 mmol/kg. All stimulating and recording electrodes consisted of patch-type pipettes with a tip diameter of 1–2 \( \mu \)m filled with filtered external saline. Stimulus intensity was \(-15 \) to \(-100 \) \( \mu \)A (200 \( \mu \)s duration) and was kept well below saturation. A stimulating electrode and two recording electrodes were placed in the vermis in the inner half of the molecular layer, typically 300–500 \( \mu \)m apart (Fig. 2A). Estimated fiber volley velocity was between 15 and 22 cm/s, consistent with previous reports.

**CF-evoked EPSC recordings**

Methods were similar to those used in PF EPSC experiments. To improve voltage clamp, the tip diameter of patch pipettes was increased (\( R_{\text{tipette}} = 3–5 \) M\( \Omega \)). A monopolar stimulating electrode was placed in the granule cell layer and its position and intensity adjusted \((\pm 5 \) to \(-40 \) \( \mu \)A, 200 \( \mu \)s) to elicit a large all-or-none CF EPSC uncontaminated by a PF EPSC component. In many experiments, a second electrode was placed in the molecular layer to elicit pure PF EPSCs. External saline contained 20 \( \mu \)M bicuculline and 0.1% DMSO. After positioning the stimulating electrodes, \( V_{\text{hold}} \) was stepped from \(-57 \) to \(-80 \) mV for 150 ms to measure \( R_s \) and \( R_h \), and then returned to \(-57 \) mV. In analysis, EPSCs and \(-5 \) mV steps were analyzed separately, with each event baseline to the 100 ms interval that preceded it. Data were filtered at 5 kHz with the four-pole, low-pass Bessel filter integral to the Axopatch 200A and acquired at 5 kHz. Data are presented as mean \( \pm \) SE.

**FIG. 2.** WIN 55212-2 produced a small decrease in fiber volley amplitude and no change in fiber volley velocity and kinetics. **A:** fiber volleys recorded from PFs in horizontal cerebellar slices. **B:** perfusion with WIN 55212-2 produced a small decrease in fiber volley amplitude about half of the time but never a change in velocity. Baseline (solid line) is the average of all traces 0.7–5.7 min before the second saline entered the recording chamber. The other trace (dotted line) is the average of all traces 24.3–29.3 min after WIN 55212-2 entered the recording chamber. **Inset:** even when a decrease in fiber volley amplitude was seen, there was no effect on fiber volley kinetics. Here, average “before” and “after” traces from recording electrode 1 are scaled.

**C:** summary of the effect of WIN 55212-2 and the inactive enantiomer WIN 55212-3 on fiber volley amplitude and velocity. When compared by two-tailed, heteroscedastic \( t \)-test to the effect of 0 \( \mu \)M WIN 55212-2, only the 7% decrease in amplitude in 5.0 \( \mu \)M WIN 55212-2 was significant (*, \( P = 0.017 \)).
changed to \(-20\) mV. This caused the amplitude of both CF-evoked and PF-evoked EPSC amplitudes to decrease several-fold, caused spiking to disappear, and caused \(G_m\) to greatly increase. The cell was allowed to accommodate to this new \(V_{\text{hold}}\) as long as necessary (\(\geq10\) min) before data was collected. Thereafter, CF EPSC amplitude and \(R_s\) were stable, although \(G_m\) had a tendency to drift a little over time. Every 20 s, the following stimulation protocol was applied (see Fig. 3A): after collecting a 100-ms baseline, a pair of stimuli 100 ms apart were delivered to the CF. PFs were stimulated 1 s after the second CF stimulation. After an additional 500 ms, a \(-5\) mV, 150 ms voltage step was imposed on the cell to measure \(R_s\) and \(R_i\). In some experiments, PF stimulation was omitted and the \(-5\) mV voltage step occurred 400 ms after the second CF stimulation. After recording a stable baseline for \(\geq15\) min, the external saline reservoir was replaced with saline containing 5.0 \(\mu\)M WIN 55212-2 and recordings were continued for \(\geq15\) min before isolation of stimulus artifact with 1.0 \(\mu\)M TTX for subsequent digital subtraction. In control experiments, each cerebellar slice was incubated in SR 141716 for \(\geq15\) min before recording commenced. Within each experiment, the effect of switching to external saline containing WIN 55212-2 was calculated from the average of all data 0.7–5.7 min before and 10.3–14.3 min after WIN 55212-2 entered the recording chamber. Experiments in which \(R_s\) changed more than 10% or \(G_m\) changed more than 20% were excluded.

**mEPSC experiments**

mEPSCs were recorded in whole-cell patched Purkinje cells using methods similar to those used in PF-evoked EPSC experiments. \(V_{\text{hold}} = -80\) mV. To decrease \(R_s\), increase space clamp, and improve detection of small currents, we used P10–11 rats and patch pipettes with large tip diameters (1.8–2.5 MΩ) filled with (in mM) 35.3 CsOH, 95 Cs₂SO₄, 4 MgSO₄·7H₂O, 10 EGTA, 4 CaCl₂, 10 HEPES, 4 Na₂ATP, 0.4 Na₃GTP, 287–290 mmol/kg, pH \(\sim 7.23\). Liquid

![FIG. 3. WIN 55212-2 produced a moderate suppression of CF-evoked EPSCs recorded in Purkinje cells. A: CF stimulation protocol and sample trace from voltage-clamped Purkinje cell. This protocol was repeated every 20 s. B: representative experiment. 5.0 \(\mu\)M WIN 55212-2 produced a decrease in climbing fiber (CF) EPSC amplitude. Solid line: average of 15 traces 0.7–5.7 minutes before WIN 55212-2 entered the recording chamber. Dotted line: average of 12 traces 10.3–14.3 min after WIN 55212-2 entered the recording chamber. All traces were normalized to 0 pA and stimulus artifacts in TTX were subtracted. C: compiled CF EPSC recordings (mean \(\pm\) SE). Note the small recovery from maximal suppression over time. Peak suppression, measured at 5–6.75 min in WIN 55212-2, was 67.1 \(\pm\) 5.5% of baseline, and this recovered to 74.5 \(\pm\) 5.4% at 15–16.75 min \((P = 0.054\) by paired, two-tailed \(t\)-test). Preincubation in SR 141716 completely blocked the effect of WIN 55212-2. 1.0 \(\mu\)M TTX eliminated all EPSCs. Data points after 15 min in WIN 55212-2 and before TTX application were deleted for purposes of alignment. D: compiled CF paired-pulse ratios from same recordings as in C (mean \(\pm\) SE). 5.0 \(\mu\)M WIN 55212-2 produced an increase in CF EPSC2/EPSC1 (relaxation of paired pulse depression). Preincubation in SR 141716 completely blocked the effect of WIN 55212-2.**
juncture potential was measured to be approximately $-8$ mV (pipette negative) and was compensated on-line. Data were filtered extensively at 5 kHz with the four-pole Bessel filter intrinsic to the Axopatch 200A, and externally at 200 Hz with an additional eight-pole, low-pass Bessel filter. Recordings were performed in external saline containing 20 $\mu$M bicuculline, 1.0 $\mu$M TTX, and 1 ml/l DMSO. Every 5 s, 10 times per minute, 3200 ms of data were acquired at 5 kHz. At 3050 ms, a $-5$ mV, 150 ms step was applied from which $R_s$ and $G_m$ were derived. After recording for $\geq20$ min in normal external saline, the reservoir was switched to one containing 5.0 $\mu$M WIN 55212-2 for another 16 min. Finally, 10 $\mu$M 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)quinoxaline (NBQX) was applied to confirm that detected events were mEPSCs and not noise or mIPSCs. To allow Cs$^+$ in the Purkinje cell time to equilibrate, an interval of $\geq60$ min was imposed between the time the cell was whole-cell patch clamped and when WIN 55212-2 first entered the recording chamber. Within each experiment, the effect of switching to external saline containing WIN 55212-2 was calculated from the average of all data 1–6 min before and 11–15 min after WIN 55212-2 entered the recording chamber. Data from each cell were analyzed off-line using Axograph 3.5 software that used template-based detection. Events smaller than $-5$ pA were discarded. A separate template was created for each recording by averaging a large number of hand-selected unambiguous mEPSCs. Detection thresholds were adjusted such that in the presence of 10 $\mu$M NBQX the total frequency of detected events was $<0.05$ Hz. There was a wide range of basal mEPSC frequencies among the cells, and recordings with very low frequencies were not continued. Average $R_s$ did not change by more than 4% in any of the accepted recordings. Each 3200-ms trace was visually inspected and traces contaminated with transient noise were deleted. If $>3$ in 10 traces were rejected in any minute-long bin, the entire minute was deleted. Average mEPSC frequency and amplitude were calculated at 1 min intervals.

**sIPSC experiments**

Methods were similar to those used in mEPSC experiments. P12–13 rats were used. $V_{hold} = -70$ mV. Pipette saline consisted of (in mM) 35.9 CsOH, 121 CsCl, 4 MgCl$_2$, 10 EGTA, 4 CaCl$_2$, 10 HEPES, 4 Na$_2$ATP, 0.4 Na$_3$GTP, pH 7.22, 290 mmol/kg. Liquid junction potential was measured to be approximately $-4$ mV with kynurenate-containing external saline and was compensated on-line. To allow CsCl to efficiently perfuse the cell, large-tipped patch pipettes were used (1.6–2.5 MΩ). The four-pole, low-pass Bessel filter intrinsic to the Axopatch 200A was set to 5 kHz, and in most cases current was additionally filtered at 1 kHz with an external eight-pole, low-pass Bessel filter. At the end of each experiment, 20 $\mu$M bicuculline was applied to confirm that detected events were mIPSCs and not noise or mEPSCs. Detection thresholds were adjusted such that detection frequency in bicuculline was $<0.05$ Hz. In mixed sIPSC/mIPSC experiments (without TTX), in the absence of kynurenate, an amplitude cutoff of $-50$ pA was used and smaller events, including almost all mEPSCs, were discarded. In the presence of 2 $\mu$M kynurenate, an amplitude cutoff of $-20$ pA was used. The few IPSCs that elicited action potentials were included in calculations of total frequency but omitted from calculations of average amplitude. In mIPSC experiments, 1.0 $\mu$M TTX and 2.0 mM kynurenate were added to all external salines. After recording a baseline of $\geq15$ min, the external saline reservoir was switched to one containing either control saline, 5.0 $\mu$M WIN 55212-2, or 100 $\mu$M CdCl$_2$ for at least 17 min, and was then switched to 20 $\mu$M bicuculline. Recordings in which $R_s$ was not constant or holding current changed unusually were discarded. Because of the large size and high frequency of mIPSCs, $R_s$ could not be determined accurately, and all capacitative transients used to calculate $R_s$ that were contaminated by IPSCs were discarded. In no experiment did $R_s$ change by $>7\%$ after experimental manipulation.

**RESULTS**

**PF-evoked EPSCs**

Whole-cell patch clamp recordings were made from Purkinje cells in acute sagittal sections of cerebellar vermis from P15–19 rats. Regular stimulation of PFs (Fig. 1A and METHODS) produced EPSCs similar to those seen previously (Konnerth et al. 1990; Llano et al. 1991; Perkel et al. 1990) and that were stable for over an hour. In control experiments, switching the external saline reservoir to a second bottle of normal external saline had no effect on PF-evoked EPSC (PF EPSC) amplitude (103 $\pm$ 3.7% of baseline, $n = 5$). Switching to external saline containing WIN 55212-2 resulted rapidly in a steep reduction in PF EPSC amplitude that would typically continue for $\sim10$ min before stabilizing. Subsequent application of 1.0 $\mu$M TTX completely eliminated the PF EPSC. A typical cannabinoid experiment is depicted in Fig. 1, A–C. The effect of TTX could be washed out, but in almost all cases the effect of WIN 55212-2 could not be washed out (data not shown), probably because of its high lipophilicity. Preincubation with 1.0 $\mu$M SR 141716, a CB1-selective antagonist, completely blocked the effect of 5.0 $\mu$M WIN 55212-2 (Fig. 1D). This suggests that the effect of WIN 55212-2 is fully CB1 receptor–mediated and not the result of a direct membrane effect, a direct effect on Ca channels (Shen and Thayer 1998), or other non-CB1-receptor–mediated processes. WIN 55212-2 reduced PF EPSC amplitude in a dose-dependent manner with the effect appearing to approach saturation at high concentrations (5.0 $\mu$M: 12.3 $\pm$ 2.2% of baseline, $n = 3$; see Fig. 1E). The effect of WIN 55212-2 at all concentrations differed significantly from the no-drug control by two-tailed, heteroscedastic t-test, $P < 10^{-4}$. In the presence of 1.0 $\mu$M SR 141716, 5.0 $\mu$M WIN 55212-2 had no effect (107 $\pm$ 2.60% of baseline, $n = 4$, $P = 0.41$). $R_s$ and $G_m$ were stable and always unaffected by WIN 55212-2 (Fig. 1F).

**PF-fiber volley**

There are several mechanisms by which CB1 activation could produce a decrease in PF EPSC amplitude. Two possibilities are CB1-mediated reduction in PF excitability or action potential duration. These possible mechanisms were assessed by examining the effect of WIN 55212-2 on fiber volley recorded in stimulated bundles of PFs. Slices containing long tracts of PFs were prepared by horizontal sectioning of P15–16 cerebella. Two recording electrodes were placed along the path of the stimulated axons so that fiber volley velocity could be measured (Fig. 2A). Because perhaps 40% of the PF in the rat is made up of synaptic varicosities (Ito 1984), it is theoretically possible that synaptic $Ca^{2+}$ channels might contribute to the fiber volley. To isolate the fiber volley from any potential contribution from $Ca^{2+}$ channels or field excitatory postsynaptic potentials (EPSPs), all experiments were performed in external saline containing 200 $\mu$M CdCl$_2$, which was determined to be a saturating concentration (data not shown). WIN 55212-2 had a variable effect on fiber volley amplitude. In about half of the experiments, 5.0 $\mu$M WIN 55212-2 had no effect. In the remaining experiments, it caused a small decrease in amplitude (Fig. 2B). Fiber volley velocity and kinetics were never affected, even in those recordings in which there was a decrease in fiber volley amplitude. On average, the effect of
WIN 55212-2 on fiber volley amplitude was very small (5.0 μM: 93 ± 2% of baseline, n = 9; see Fig. 2C). But this was significantly different (P = 0.017) from switching the external saline reservoir to a second bottle of control saline (104 ± 3%, n = 5). S(-)-WIN 55212-3, the inactive enantiomer of R(+)-WIN 55212-2, had no effect (105 ± 6%, n = 3), suggesting that the small effect of WIN 55212-2 was receptor mediated. However, this small and inconsistent effect on fiber volley amplitude cannot account for the very large and extremely consistent PF EPSC results.

**CF-evoked EPSCs.**

CF stimulation elicited characteristically large, all-or-nothing EPSCs that exhibited paired-pulse depression (Konnerth et al. 1990; Llano et al. 1991; Perkel et al. 1990) (Fig. 3, A and B). 5.0 μM WIN 55212-2 produced a moderate and somewhat variable decrease in CF-evoked EPSC (CF EPSC) amplitude, which tended to recover slightly with continued agonist application (Fig. 3C). In control experiments, slices were incubated in 1.0 μM SR 141716 for 30 min before switching to 5.0 μM WIN 55212-2 + 1.0 μM SR 141716. In the presence of this CB1 antagonist, WIN 55212-2 had no effect on EPSCs. On average, 5.0 μM WIN 55212-2 reduced the amplitude of the first CF EPSC of each pair to 78.6 ± 5% of baseline (n = 6; P = 0.0029). In addition, 5.0 μM WIN 55212-2 increased EPSC2/EPSC1 to 109 ± 1.3% of baseline (changed paired-pulse depression from 78.6 ± 1.0% to 85.5 ± 1.7%) compared with 99.9 ± 0.8% of baseline in controls (P = 0.0002; see Fig. 3D). When recorded concurrently as an internal control, as before, PF EPSCs were severely reduced by WIN 55212-2 and this reduction was completely blocked by SR 141716 (data not shown). Rm was stable and unaffected by WIN 55212-2 in both test recordings (99.3 ± 1.3% of baseline) and control recordings with SR 141716 pretreatment (99.6 ± 0.8% of baseline). Because Gm tended to drift unpredictably in CF EPSC experiments at −20 mV (see METHODS), inspection of individual records was the most reliable way to look for an effect of WIN 55212-2 on Gm. No effect was seen. On average, ΔGm was 97.5 ± 2.6% of baseline in test recordings and 92.1 ± 2.7% of baseline in control recordings.

**mEPSCs**

In a further effort to determine the mechanism by which CB1 receptor activation suppresses EPSCs, miniature EPSCs (mEPSCs) were recorded. mEPSCs result from spontaneous fusion of neurotransmitter-containing vesicles to the presynaptic terminal membrane. mEPSCs recorded in Purkinje cells were found by Chen and Regh (1997) to be unaffected by externally applied Cd2+, demonstrating that they are independent of Ca2+ influx. A change in the frequency of these spontaneous fusion events is taken to be evidence of modulation of the synaptic vesicle release machinery downstream from Ca2+ entry. A change in the amplitude of mEPSCs is taken to be evidence of postsynaptic modulation. Most mEPSCs probably reflect spontaneous vesicular release from PFs although some may result from release from CFs (see DISCUSSION). In pilot experiments (data not shown), asynchronous vesicular release from CFs and PFs was induced by stimulating these afferents in external saline in which CaCl2 was replaced with an equal amount of SrCl2 (Goda and Stevens 1994; Lévénes et al. 1998). There were no differences between CF-evoked and PF-evoked asynchronous EPSCs that were sufficient to allow mEPSCs from these sources to be discriminated from each other.

P10–11 rats were used. mEPSCs were very difficult to record in Purkinje cells from rats older than P11. At all ages, on first breaking into whole-cell mode, there was a great amount of noise of up to −15 pA, which probably reflected the activity of intrinsic conductances in the Purkinje cell. This noise was impossible to distinguish from mEPSCs with certainty. In very young Purkinje cells, this noise dissipated rapidly, probably because of perfusion of the dendrites with Cs+ from the pipette saline. In older cells, it took much longer for this noise to dissipate. Additionally, as age increased, the frequency of mEPSCs appeared to decrease, possibly because of increased dendritic filtering. mEPSCs were detected in TTX under low-noise recording conditions as very small currents that could be blocked by the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptor antagonist, NBQX (Fig. 4A). The average baseline mEPSC frequency and amplitude were 4.8 ± 1.7 Hz and −19.6 ± 1.3 pA (mean ± SE, n = 4), respectively. The baseline distribution of mEPSCs in one Purkinje cell is plotted in Fig. 4B. A representative experiment is shown in Fig. 4C. After collecting a long, stable baseline, the external saline reservoir was switched to 5.0 μM WIN 55212-2 for 16 min. There was absolutely no effect on either mEPSC frequency or amplitude. Subsequent application of 10 μM NBQX eliminated the mEPSCs, confirming them to be true AMPA/kainate receptor–mediated events.

The combined experiments plotted in Fig. 4, D and E, show that WIN 55212-2 had no effect. On average (n = 4), mEPSC frequency and amplitude in 5.0 μM WIN 55212-2 were 103 ± 4.0% and 97.1 ± 3.9% of baseline, respectively. To address the possibility that WIN 55212-2 might be altering a subpopulation of mEPSCs, mEPSCs within each recording were segregated into several bins by amplitude. Each bin was normalized to its own baseline and then combined with the corresponding bins of the other experiments. The binwidths used for this analysis were −5 to −10 pA, −10 to −20 pA, −20 to −30 pA, −30 to −50 pA, and greater than −50 pA. In none of these amplitude ranges did 5.0 μM WIN 55212-2 have any effect on mEPSC frequency. Recordings were stable; on average, Rm was 102 ± 0.64% of baseline and Rm was 112 ± 2.0% of baseline in WIN 55212-2.

It is possible that no effect was seen on mEPSCs because P10–11 rats were used whereas P15–19 rats were used in all other PF and CF experiments. These ages span a period of rapid growth; cannabinoid binding/mg of cerebellar homogenate (Bmax) was reported to increase ~2.4-fold between P7 and P14 and another ~1.7-fold between P14 and P21 (Belue et al. 1994). Therefore the effect of 5.0 μM WIN 55212-2 on mEPSCs was reexamined in three Purkinje cells from P10–11 rats using the same CsSO4-based internal saline used for mEPSC recording and the protocol shown in Fig. 2A at Vhold = −30 to −40 mV. The effect in P10–11 rats was even larger than that seen in P15–19 rats, perhaps because there was less glial membrane in these young slices to impede WIN 55212-2 access to the relevant synapses. Results in WIN 55212-2 were as follows (% of baseline): PF EPSC, 10.0 ± 2.8; CF EPSC1, 67.3 ± 7.1; CF EPSC2/CF EPSC1, 116 ± 8.3; Rm 104 ± 0.5; Gm 100.7 ± 8.1.
In conclusion, no evidence was found that CB1 receptor activation modulates the synaptic vesicle release machinery downstream of Ca\(^{2+}\) entry at excitatory synapses impinging on Purkinje cells. Furthermore, the absence of any effect on mEPSC amplitude suggests that CB1 does not act postsynaptically at these synapses.

**IPSCs**

Purkinje cells receive powerful inhibitory input that comes mainly from local GABAergic interneurons. There are about six basket cells and 17 stellate cells/Purkinje cell in cat (reviewed in Ito 1984). Another minor inhibitory input to Purkinje cells...
cell dendrites comes from Purkinje cell axon collaterals (reviewed in Ito 1984). Basket cells, stellate cells, and Purkinje cells fire action potentials spontaneously (Llano and Gershenfeld 1993; Llínás and Sugimori 1980; Pouzat and Hestrin 1997). Basket and stellate cells express CB1 mRNA, but Purkinje cells do not (Mailleux and Vanderhaeghen 1992; Matsuda et al. 1993). CB1 immunoreactivity colocalizes with the pericellular basket and is especially intense on the pinceau (Tsou et al. 1998). To determine the functional consequences of this localization, the effect of CB1 activation on inhibitory currents was examined. Purkinje cells were whole-cell patched and held at −70 mV with a high [Cl−] pipette saline that shifted the GABA_A reversal potential to about 0 mV. Under these conditions, GABA_A activation produced inward currents. Spontaneous inhibitory currents were then recorded and analyzed in much the same way as mEPSCs. Recordings of spontaneous IPSCs were similar to those reported previously (Farrant and Cull-Candy 1991; Konnerth et al. 1990; Vincent et al. 1992). Spontaneous IPSCs were numerous and much larger than mEPSCs. Average baseline IPSC frequency and amplitude were 7.1 ± 2.3 Hz and −362 ± 31 pA (n = 3 cells). Run-down of IPSC amplitude over time was prominent (see also Kano 1992), especially after 35–50 min of recording, and appeared to correlate positively with the size of the pipette tip. IPSCs fell into two classes, action potential–dependent and mIPSCs (Fig. 5A). Very large TTX-sensitive, action potential–dependent IPSCs reflect spontaneously arising action potentials in basket and stellate cells. Most action potential–dependent IPSCs were between −1 and −5 nA in amplitude and were sometimes large enough to escape space clamp and evoke a

![Figure 5](http://jn.physiology.org/)

**Fig. 5.** CB1 receptor activation greatly reduced the amplitude of action potential–dependent inhibitory postsynaptic currents (IPSCs) and greatly reduced the frequency of miniature IPSCs (mIPSCs). Representative experiment. A: sample traces. 5.0 μM WIN 55212-2 reduced the amplitude of the large events by >75% and greatly reduced total IPSC frequency. These effects were reversed by subsequent addition of 1.0 μM SR 141716. In 1.0 μM TTX, the large events were again eliminated, showing them to be action potential–dependent. The amplitude of the small events was unaffected by TTX, revealing them to be mIPSCs. 20 μM bicuculline eliminated all events. Traces are numbered to correspond to numbers in parentheses in (B). B: amplitude of every IPSC is plotted at 1 min intervals. Events greater than −5.5 nA (15 IPSCs of which 9 induced spikes) are not shown. Data for the 39- and 67-min time points were deleted because of transient noise (x). C: total IPSC frequency and average IPSC amplitude are plotted vs. time. Note that although WIN 55212-2 greatly reduced total IPSC frequency, TTX had little effect, suggesting that there were far more mIPSCs than action potential–dependent IPSCs and that CB1 activation greatly reduced mIPSC frequency. Although infrequent, TTX-sensitive IPSCs were large in amplitude and contributed significantly to average IPSC amplitude. Therefore, suppressing action potential–dependent IPSCs resulted in a large decrease in average IPSC amplitude. This experiment was repeated twice in the presence of 2 mM kynurenate. All results were consistent.
postsynaptic action potential. Smaller TTX-insensitive mIPSCs reflect spontaneous vesicle fusion events at inhibitory terminals. mIPSCs were generally less than −1 nA in amplitude. mIPSC frequency was much greater than that of action potential–dependent IPSCs.

To get an overall view of the effect of CB1 activation on IPSCs, experiments were conducted as follows (Fig. 5, A–C). After recording a baseline of mixed large and small IPSCs, WIN 55212-2 was added to the recording chamber. In 5.0 μM WIN 55212-2, the large IPSCs disappeared and total spontaneous IPSC frequency decreased by more than half. This effect was completely reversed by subsequent application of 1.0 μM SR 141716 + 5.0 μM WIN 55212-2, indicating that it was CB1 receptor–mediated. To identify which IPSCs were dependent on spontaneous action potentials and which were mIPSCs, 1.0 μM TTX (or 1.0 μM TTX + 1.0 μM SR 141716 in repeated experiments) was then applied. In TTX, the large IPSCs again disappeared whereas small IPSCs remained. On the whole, TTX produced only a small decrease in the total frequency of IPSCs, indicating that mIPSCs far outnumbered action potential–dependent IPSCs. Because WIN 55212-2 produced a much larger decrease in total IPSC frequency than TTX, WIN 55212-2 must have substantially reduced mIPSC frequency. Bicuculline (20 μM) eliminated the IPSCs, proving them to be GABA A–mediated. Because spontaneously occurring IPSCs were studied, and because the cannabinoid–induced elimination of large TTX-sensitive IPSCs was observed on a background of unaffected mIPSC amplitude, it could not be determined whether action potential–dependent IPSCs were reduced in amplitude to within the range of mIPSCs, or whether they were eliminated altogether. Action potential–dependent IPSC amplitude must have been reduced by >75% because they were sometimes more than four-fold larger than the largest mIPSCs. This experiment was performed three times. In two recordings, 2 mM kynurenate was present throughout the experiment. All three results were consistent.

mIPSCs

In the previous experiment, the effects of WIN 55212-2 on a mixture of action potential–dependent IPSCs and mIPSCs were studied. To study mIPSCs in isolation, we recorded from Purkinje cells in the presence of 1.0 μM TTX and 2.0 mM kynurenate (Fig. 6A). Average baseline frequency and amplitude were 3.1 ± 0.4 Hz and −128 ± 9 pA, respectively. A typical baseline frequency versus amplitude distribution is shown in Fig. 6B. The great majority of mIPSCs were smaller than −200 pA, though some were larger than −700 pA. After recording a stable baseline, either more control saline, 5.0 μM WIN 55212-2, or 100 μM CdCl2 was bath-applied (Fig. 6, C and D). In controls, mIPSC frequency spontaneously increased late in two of four recordings, driving the average change to 126 ± 15.6% of baseline (n = 4). This increase was not accompanied by any change in recording stability, and all events detected as mIPSCs were eliminated by 20 μM bicuculline. In contrast, 5.0 μM WIN 55212-2 produced a large and rapid decrease in mIPSC frequency to 52.3 ± 8.6% of baseline (n = 4), which was significantly different from control (P = 0.018). 100 μM CdCl2 had no effect on IPSC frequency (101 ± 4.4%; n = 3; P = 0.26), suggesting that WIN 55212-2–mediated suppression of mIPSC frequency is not mediated by Ca2+ channel inhibition (Fig. 6C). Because mIPSC amplitude tended to run down over time, it was important to be sure that any decrease in amplitude was statistically significant. Neither WIN 55212-2 or CdCl2 had a significant effect on IPSC amplitude compared with control (Fig. 6D). R S remained constant (control, 101 ± 0.8%; WIN 55212-2, 101 ± 1.0%; CdCl2, 96.3 ± 1.1%).

DISCUSSION

CB1 activation was found to presynaptically suppress all major classes of synaptic input to cerebellar Purkinje cells. Cannabinoid suppression of PF-evoked EPSCs was large, consistent, and dose-dependent. Several possible mechanisms were investigated. Cannabinoid–induced decreases in fiber volley amplitude were too small and inconsistent to account for the effect on PF EPSCs. In addition, fiber volley velocity and kinetics were completely unaffected, suggesting that voltage–gated Na+ channels and delayed rectifier K+ channels were not involved. If Na+ channels had been inhibited, a slowing of action potential velocity and kinetics would have been expected to result. A significant increase in delayed rectifier K+ channel activity would also have been expected to change fiber volley kinetics. It is more likely that the observed cannabinoid–induced decrease in excitability is caused by a small increase in resting KIR activity. If the increase in KIR were small enough, it might decrease axonal excitability without affecting kinetics and velocity, and the decrease in fiber volley amplitude would reflect a proportional decrease in the number of activated PFs. CB1 activation has been shown to increase KIR channel activity in other systems (Garcia et al. 1998; Henry and Chavkin 1995).

The fact that WIN 55212-2 had no effect on mEPSC amplitude suggests that CB1 receptor activation suppresses EPSCs recorded in Purkinje cells presynaptically. This conclusion is supported by the work of others who found no CB1 mRNA in Purkinje cells (Mailleux and Vanderhaeghen 1992; Matsuda et al. 1993). It should be noted that mEPSCs recorded in Purkinje cells could be the result of spontaneous release at either granule cell axon or CF terminals. There is no reliable way to discriminate between them. However, because the ratio of granule cell synapses to CF synapses in the Purkinje cell is on the order of 150:1 (reviewed in Strata and Rossi 1998), it is generally assumed that most mEPSCs reflect granule cell axon terminal release. However, because CF synapses tend to be closer to the Purkinje cell body than most PF synapses, the amplitude of CF mEPSCs may tend to be larger than that of PFs, making them more likely to be detected by somatic whole-cell recording. In contrast, PF, single-vesicle release events occurring in the tertiary branches of distal dendrites may often be attenuated by dendritic filtering to the point of undetectability. Release probability after an action potential is unusually high in CFs (Silver et al. 1998); however, it is not known whether spontaneous release probability in the absence of action potentials is larger at CF terminals than at PF terminals. Thus while most mEPSCs recorded in Purkinje cells result from PF events, it is possible that a measurable fraction comes from CF terminals as well.

To summarize the CB1 effects on PF EPSCs, receptor activation greatly suppressed PF EPSC amplitude while producing only a weak and inconsistent decrease in PF
excitability and no change in postsynaptic sensitivity or membrane properties. We suggest, by elimination, that CB1 receptor activation inhibits neurotransmitter release at PFs by inhibiting Ca\(^{2+}\) channels and/or increasing the activity of K\(^{+}\) channels at the axon terminal. CB1 activation was previously reported to decrease Ca\(^{2+}\) channel activity and increase K\(^{+}\) channel activity; however these effects were evaluated in somata and not at axon terminals. K\(^{+}\) channel modulation is not well established to be a general mechanism of modulation of release at vertebrate synapses (Miller 1998). Moreover, presynaptic modulation of PF–Purkinje cell transmission via GABAB or adenosine A1 receptors does not appear to involve presynaptic K\(^{+}\) channels (Dittman and Regehr 1996; Wu and Saggau 1997). Therefore inhibition of axonal Ca channels seems the more likely mechanism of CB1-mediated suppression of PF EPSC amplitude.

These results differ in some ways from those of Lévénez et al. (1998). 1 µM WIN 55212-2 reduced PF EPSC amplitude to 28.8% of baseline in the present experiments, but only to 44.4% of baseline in their experiments. In addition, although we report an average 7% decrease in fiber volley amplitude, Lévénez et al. (1998) saw no change, although this difference is minor. More important, although we observed no change in mEPSC frequency, Lévénez et al. (1998) reported a 23% decrease. However, basal mEPSC frequency was extremely low in their research (0.26 Hz at P15–21) compared with our work (~4.8 Hz at P10–11) and the work of others also recording from Purkinje cells in acute slices of rat cerebellum [3–5 Hz at P9–14 (Dittman and Regehr 1996), 5–8 Hz at P9–15 (Chen and Regehr 1997), and ~2 Hz at P10 (Barbour 1993)]. This discrepancy, which is probably caused by their use of older Purkinje cells, makes the mEPSC results of Lévénez et al. (1998) difficult to compare with the present findings.

CF EPSCs were suppressed by CB1 receptor activation, but to a lesser extent and with much more variability than at PF synapses. This is the first report of cannabinoid-induced suppression of this synapse. It is likely that inhibition of CF EPSCs results largely from modulation of axon terminal Ca\(^{2+}\) or K\(^{+}\) channels as indicated for PFs. Even though some component of the mEPSCs recorded in Purkinje cells is probably caused by spontaneous vesicle release from CF terminals, that fraction is likely to be small and indistinguishable from PF
Suppression of PF EPSC amplitude, could add to the inhibition of inhibitory interneurons. It is plausible that such channel modulation of voltage-gated channels in the axon terminals of PF excitatory synapses received by the Purkinje cell is mediated largely by modulation of voltage-gated channels in the axon terminal. In contrast, at inhibitory synapses, modulation of vesicle release machinery after presynaptic Ca\textsuperscript{2+} influx also plays a significant role. All of these actions have the potential to contribute to the cerebellar behavioral symptoms of cannabinoid intoxication.

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