Cerebellar Depolarization-Induced Suppression of Inhibition Is Mediated by Endogenous Cannabinoids

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

Depolarization of cerebellar Purkinje neurons transiently suppresses IPSCs through a process known as depolarization-induced suppression of inhibition (DSI). This IPSC suppression occurs presynaptically and results from an unknown retrograde signal released from Purkinje cells. We recorded IPSCs from voltage-clamped Purkinje cells in cerebellar brain slices to identify the retrograde signal for cerebellar DSI. We find that DSI persists in the presence of the broad-spectrum metabotropic glutamate receptor antagonist LY341495 and the GABA<sub>B</sub> receptor antagonist CGP55845, suggesting that the retrograde signal is not acting through these receptors. However, an antagonist of the cannabinoid CB1 receptor AM251 completely blocked cerebellar DSI. Additionally, the cannabinoid receptor agonist WIN55,212-2 suppressed IPSCs and occluded any additional IPSC reduction by DSI. These results indicate that cannabinoids released from Purkinje cells after depolarization activate CB1 receptors on inhibitory neurons and suppress IPSCs for tens of seconds. Cerebellar DSI thus shares a common retrograde messenger with DSI in the hippocampus and depolarization-induced suppression of excitation in the cerebellum, suggesting that retrograde synaptic suppression by endogenous cannabinoids represents a widespread signaling mechanism.

Key words: cannabinoid; DSI; cerebellum; Purkinje cell; stellate cell; basket cell

However, other evidence suggests a potential role for GABA or glutamate as the retrograde signal in cerebellar DSI. In the cortex, dendritic release of GABA from inhibitory neurons results in a retrograde inhibition of synaptic inputs through activation of GABA<sub>B</sub> receptors on presynaptic neurons (Zilberter et al., 1999); similar mechanisms could exist at synapses onto Purkinje cells, which are also GABAergic. In the hippocampus, such a mechanism is less likely because the CA1 pyramidal neurons that elicit DSI are glutamatergic. Additionally, it has been proposed that cerebellar DSI is mediated by glutamate release from Purkinje cells, which could act at presynaptic group II metabotropic glutamate receptors (mGluRs) on interneurons to suppress IPSCs (Glitsch et al., 1996). Therefore, the identity of the retrograde messenger for cerebellar DSI remains an open question.

Here we report that the magnitude of cerebellar DSI is unaffected by antagonists of mGluRs or GABA<sub>B</sub> receptors. However, we find that activation of cannabinoid CB1 receptors is required for suppression, because antagonists of the CB1 receptor completely eliminate cerebellar DSI, and agonists of the CB1 receptor occlude DSI. Cerebellar DSI therefore shares a common retrograde signal with both hippocampal DSI and cerebellar depolarization-induced suppression of excitation (DSE) and im-

This article is published in The Journal of Neuroscience, Rapid Communications Section, which publishes brief, peer-reviewed papers online, not in print. Rapid Communications are posted online approximately one month earlier than they would appear if printed. They are listed in the Table of Contents of the next open issue of JNeurosci. Cite this article as: JNeurosci, 2001, 21:RC174 (1–5). The publication date is the date of posting online at www.jneurosci.org.

http://www.jneurosci.org/cgi/content/full/5720
complicates endogenous cannabinoids as widespread retrograde signaling molecules.

MATERIALS AND METHODS

Sagittal slices (250 μM thick) were cut from the cerebellar vermis of 14- to 21-d-old Sprague Dawley rats. Slices were superfused with an external saline solution containing (in mM): 125 NaCl, 2.5 KCl, 2 CaCl₂, 1 MgCl₂, 26 NaHCO₃, 1.25 NaH₂PO₄, and 25 glucose (bubbled with 95% O₂-5% CO₂). 1,2,3,4-Tetrahydro-6-nitro-2,3-dioxo-benzof[1]quinoline-7-sulfonamide (10 μM) was added to the external solution to suppress synaptic currents mediated by AMPA receptors. (oxy)-α-amino-α-(1S,2S)-2-carboxycyclopropyl-9H-xanthine-9-propanoic acid (LY341495), (2S)-3-[(1S)-1-(3,4-dihydroxyphenyl)ethyl]amino-2-hydroxypropyl[(phenylmethyl)phosphonate (CGP55845), and N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM251) were purchased from Tocris Cookson (Ballwin, MO). R(+)-2,3-dihydro-5-methyl-3-[(morpholin-4-yl)methyl]pyrrolo[1,2-de]-1,4-benzoazainyl]-1H-naphthalenyl methane (WIN55,212-2) was purchased from Sigma (St. Louis, MO).

Whole-cell recordings of Purkinje cells were obtained as described previously (Llano et al., 1991c). Glass electrodes (2–4 MΩ) were filled with an internal solution containing (in mM): 150 CsCl, 1 EGTA, 10 HEPES, 0.1 CaCl₂, 4.6 MgCl₂, 2 Mg-ATP, and 0.3 Na-GTP, adjusted to pH 7.3 with CsOH. Internal solutions also contained 5 mM QX-314 (H9262) and 10–60 mV. The 80 sec trials used to assay DSI were separated by 60 sec. Access resistance and leak currents were monitored, and experiments were rejected if these parameters changed significantly during recording.

DSI was quantified by calculating synaptic charge (Pitler and Alger, 1994). Slow shifts in baseline attributable to calcium-activated currents after depolarization were first subtracted from the recordings by determining the maximum data value for each 100 msec window throughout a trace. An interpolated function fit through these points was then subtracted from the trace. This procedure did not change the amplitude or time course of synaptic currents. After the subtraction, integration of the currents yielded total synaptic charge. However, synaptic charge was not analyzed during the depolarization or in the 3 sec after depolarization because of shunting from large calcium-activated chloride conductances (Llano et al., 1991a). Analysis was performed using custom routines written in Igor Pro (WaveMetrics Inc., Lake Oswego, OR).

EPSCs were filtered at 1 kHz with a four-pole Bessel filter. All signals were digitized at 2 kHz with a 16 bit analog-to-digital converter (InstruTech, Great Neck, NY), with Pulse Control software (Herrington and Bookman, 1995).

RESULTS

We recorded from voltage-clamped Purkinje cells in sagittal cerebellar brain slices and monitored sIPSCs. Cerebellar DSI is characterized by a reduction in the amplitude and frequency of IPSCs (Llano et al., 1991a), and we therefore measured synaptic charge, which is sensitive to changes in these parameters (Pitler and Alger, 1994).

Effects of mGluR and GABAB antagonists on cerebellar DSI

To test the hypothesis that mGluRs and GABAB receptors are involved in DSI, we recorded sIPSCs from Purkinje cells using 80 sec trials (Fig. 1A). During each trial, baseline synaptic charge was determined during the first 20 sec of recording. The Purkinje cell was then depolarized for 1 sec (Fig. 1A, arrow), and the recording continued for 60 sec. DSI is clearly visible in these trials as a decrease in the amplitude of sIPSCs after depolarization (Fig. 1A). To quantify DSI, we measured synaptic charge for each 2 sec epoch during the trials. Figure 1C shows the average synaptic charge for five trials in control conditions. After application of the high-affinity broad-spectrum mGluR receptor antagonist LY341495 (100 μM) (Fitzjohn et al., 1998) and the high-affinity GABAB receptor antagonist CGP55845 (2 μM) (Davies et al., 1993), the magnitude of DSI is unchanged (Fig. 1B,D). In Figure 1E, baseline synaptic charge (open circles; calculated from times shown in Fig. 1C) and synaptic charge during DSI (filled circles; calculated from times shown in Fig. 1C) are plotted for each trial in the experiment. The amount of DSI, calculated as chargeDSI/chargebaseline, remains unchanged for the duration of the experiment, even after application of mGluR and GABAB antagonists (Fig. 1F). This shows the stability of DSI during the course of an experiment and also demonstrates that activation of mGluRs and GABAB receptors is not required for cerebellar DSI. Summary data are shown in Figure 4.

Effects of cannabinoids on cerebellar DSI

To test whether endogenous cannabinoids play a role in cerebellar DSI, we used the same protocol outlined for Figure 1. We recorded sIPSCs from Purkinje cells and elicited DSI at the time marked by the arrow in Figure 2A. In Figure 2C, the average
synaptic charge was calculated for four trials in control conditions. After application of the high-affinity CB1 receptor antagonist AM251 (1 μM) (Gatley et al., 1996), DSI is completely eliminated in all subsequent trials (Fig. 2F) (see Fig. 4C). In Figure 2E, baseline synaptic charge (open circles; calculated from times shown in Fig. 2C) and synaptic charge during DSI (filled circles; calculated from times shown in Fig. 2C) are plotted for each trial in the experiment.

DSI in the cerebellum, but not the hippocampus, is characterized by a reduction in mIPSC frequency (Alger and Pitler, 1995). Therefore, we performed additional experiments (n = 5 cells) in the presence of TTX (1 μM) to test whether the inhibition of mIPSCs during DSI is also mediated by endogenous cannabinoids. Application of TTX reduced synaptic charge by ~80%. After Purkinje cell depolarization, mIPSC frequency is reduced to 69 ± 3% of baseline, and synaptic charge is reduced to 75 ± 3% of baseline. After bath application of AM251, Purkinje cell depolarization did not elicit any reduction in mIPSC frequency (102 ± 1% of baseline) or synaptic charge (104 ± 3% of baseline).

Similar experiments were conducted using WIN55,212-2, an agonist of the CB1 receptor (Figure 3). We first recorded sIPSCs in control conditions and elicited DSI at the time marked by the arrow in Figure 3A. The average synaptic charge for five trials in control conditions is shown in Figure 3C. After application of the cannabinoid receptor agonist WIN55,212-2 (5 μM), IPSCs were reduced and Purkinje cell depolarization did not elicit any additional suppression (Fig. 3B,D). In Figure 3E, baseline synaptic charge (open circles; calculated from times shown in Fig. 3C) and synaptic charge during DSI (filled circles; calculated from times shown in Fig. 3C) are plotted for each trial in the experiment. After application of WIN55,212-2, DSI is completely eliminated (Figs. 3F, 4D).

**DSI is mediated by endogenous cannabinoids**

A summary of the pharmacological manipulations used to identify the retrograde messenger for cerebellar DSI is shown in Figure 4. The amplitude and time course of cerebellar DSI, as measured by normalized synaptic charge (percentage of baseline), is shown in control conditions (n = 18 cells) (Fig. 4A), in the presence of the mGluR and GABAA antagonist LY341495 (100 μM) and CGP55845 (2 μM; n = 4 cells) (Fig. 4B), in the presence of AM251 (1 μM; n = 5 cells) (Fig. 4C), and in the presence of WIN55,212-2 (5 μM; n = 4 cells) (Fig. 4D). After depolarization, synaptic charge, relative to baseline, was 44 ± 3% (Fig. 4A) in control conditions, 47 ± 5% in the presence of LY341495 and CGP55845 (Fig. 4B), 95 ± 5% in the presence of AM251 (Fig. 4C), and 93 ± 6% in the presence of WIN55,212-2 (Fig. 4D). WIN55,212-2 also decreased baseline synaptic charge to 44% of control.
DISCUSSION

We demonstrate that cerebellar DSI is mediated by endogenous cannabinoids that are released by Purkinje cells during depolarization and act presynaptically to inhibit IPSCs for tens of seconds. We show that endogenous cannabinoids may be widespread in the CNS.

REFERENCES


