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Lack of Depolarization-Induced Suppression of Inhibition (DSI) in Layer 2/3 Interneurons That Receive Cannabinoid-Sensitive Inhibitory Inputs

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Submitted 23 July 2007; accepted in final form 17 September 2007

Lemtiri-Chlieh F, Levine ES. Lack of depolarization-induced suppression of inhibition (DSI) in layer 2/3 interneurons that receive cannabinoid-sensitive inhibitory inputs. J Neurophysiol 98: 2517–2524, 2007. First published September 19, 2007; doi:10.1152/jn.00817.2007. In layer 2/3 of neocortex, brief trains of action potentials in pyramidal neurons (PNs) induce the mobilization of endogenous cannabinoids (eCBs), resulting in a depression of GABA release from the terminals of inhibitory interneurons (INs). This depolarization-induced suppression of inhibition (DSI) is mediated by activation of the type 1 cannabinoid receptor (CB1) on presynaptic terminals of a subset of INs. However, it is not clear whether CB1 receptors are also expressed at synapses between INs, and whether INs can release eCBs in response to depolarization. In the present studies, brain slices containing somatosensory cortex were prepared from 14–21-day-old CD-1 mice. Whole cell recordings were obtained from layer 2/3 PNs and from INs classified as regular spiking nonpyramidal, irregular spiking, or fast spiking. For all three classes of INs, the cannabinoid agonist WIN55,212-2 suppressed inhibitory synaptic activity, similar to the effect seen in PNs. In addition, trains of action potentials in PNs resulted in significant DSI. In INs, however, DSI was not seen in any cell type, even with prolonged high-frequency spike trains that produced calcium increases comparable to that seen with DSI induction in PNs. In addition, blocking eCB reuptake with AM404, which enhanced DSI in PNs, failed to unmask any DSI in INs. Thus the lack of DSI in INs does not appear to be due to an insufficient increase in intracellular calcium or enhanced reuptake. These results suggest that layer 2/3 INs receive CB1-expressing inhibitory inputs, but that eCBs are not released by these INs.

INTRODUCTION

Recent research has revealed that endogenous cannabinoids (eCBs) are synthesized and released on demand in many areas of the CNS (reviewed in Alger 2002). Postsynaptic depolarization and subsequent calcium influx triggers the release of eCBs that can act as fast retrograde signaling molecules that activate presynaptic type 1 cannabinoid (CB1) receptors. Activation of these receptors can transiently inhibit the release of GABA or glutamate in processes termed depolarization-induced suppression of inhibition (DSI) or excitation (DSE). DSI and DSE are found in many different regions of the brain, including, but not limited to, cerebellum (Kreitzer and Regehr 2001; Llano et al. 1991), hippocampus (Ohno-Shosaku et al. 2001; Pitler and Alger 1992; Wilson and Nicoll 2001), and neocortex (Fortin and Levine 2007; Trettel and Levine 2003). In the neocortex, brief trains of action potentials (APs) in pyramidal neurons triggers DSI of a subset of inhibitory synapses, but it is not known whether inhibitory synapses onto interneurons also express CB1 receptors and whether interneurons themselves can release eCBs onto afferent terminals.

The neocortex is comprised of six discrete layers that contain two major groups of neurons: pyramidal neurons (PNs), which constitute 70–80% of the neuronal population, and local circuit neurons, or interneurons (INs), which constitute the remaining 20–30% of the total population of neurons. PNs are glutamatergic neurons that have relatively stereotyped anatomical, biochemical, and electrophysiological properties, and these cells provide the sole output of the cortex to other brain areas. Local circuit interneurons have varied morphologies, express different calcium-binding proteins (calretinin, calbindin and/or parvalbumin), and although typically GABAergic, they can also express a number of different neuropeptide co-transmitters, e.g., somatostatin, vasoactive intestinal peptide, neuropeptide Y, and cholecystokinin (De Felipe 2002; Markram et al. 2004). These INs play an important role in regulating and synchronizing the activity of PNs. In particular, a well-defined subset of GABAergic basket cells that co-express the neuropeptide cholecystokinin has been shown to express the CB1 receptor (Bodor et al. 2005; Galarreta et al. 2004; Marsicano and Lutz 1999; Tsou et al. 1999).

Although eCBs and CB1 receptor activation play a role in regulating afferent inhibition onto PNs, it is not clear whether they serve a similar function at interneuron-interneuron synapses. In the hippocampus, it has been shown that INs in the s. radiatum and the s. oriens do receive cannabinoid-sensitive inputs (Hoffman et al. 2003) but fail to show DSI in response to postsynaptic depolarization (Hoffman et al. 2003; Patenaude et al. 2005), although a recent report suggests that a subset of these INs may show DSI (Ali 2007). In cortical layer 5 low-threshold-spiking (LTS) cells, however, prolonged depolarization induces an autocrine hyperpolarization mediated by activation of somatodendritic CB1 receptors, suggesting that these INs can release eCBs, although effects on synaptic inputs were not examined (Bacci et al. 2004). In a recent paper (Beierlein and Regehr 2006), it was demonstrated that interneurons in the cerebellum, specifically stellate cells and basket cells, can mobilize eCBs to modulate excitatory synaptic inputs. These cells showed DSE and synaptically evoked suppression of excitation, which required postsynaptic elevations of free intracellular Ca2+, was blocked by CB1 receptor antagonists, and was lacking in CB1-deficient mice. Thus at least in some situations, local circuit neurons can mobilize eCBs to regulate afferent inputs. In this paper, we set out to compare the cannabinoid sensitivity of inhibitory inputs to the
different cell types in layer 2/3 and to determine whether interneurons in layer 2/3 can mobilize endocannabinoids and induce DSI.

**METHODS**

**Slice preparation**

All experiments were performed on postnatal day 14–21 Swiss CD-1 mice (Charles River, Wilmington, MA) using protocols that were approved by University of Connecticut Health Center Animal Care Committee. Briefly, animals were killed by rapid decapitation without anesthesia, and whole brains were removed and immersed in ice-cold “cutting and incubating solution” composed of (in mM) 125 NaCl, 2.5 KCl, 1.25 NaH2PO4, 25 NaHCO3, 0.5 CaCl2, 4 MgCl2, 4 MgSO4, 4 lactic acid, 2 pyruvic acid, 20 glucose, 0.4 ascorbic acid, and 0.25 kynurenic acid equilibrated with 95% O2-5% CO2 (pH 7.3, 310 mEq/L). Slices were then incubated at room temperature. After 50–60 min, slices were transferred with a Dosaka EM DTK-1000 vibratome (Kyoto, Japan) were cut with a Dosaka EM DTK-1000 vibratome (Kyoto, Japan) and incubated at room temperature. After 50–60 min, slices were transferred to a recording chamber (room temperature) fixed to the stage of the microscope and AP firing patterns. Patch electrodes were pulled from borosilicate glass capillaries using a Flaming/Brown P-97 micropipette puller (Sutter Instruments, Novato, CA). For whole cell recordings of spontaneous inhibitory postsynaptic potentials (sIPSPs) in current-clamp mode, the pipette solution contained (in mM) 65 K-glucuronate, 85 KCl, 10 MgCl2, 0.1 CaCl2, 4 Na2-ATP, 0.3 Na-GTP, and 10 N-2-pyridylbenzo-1,2,4-triazole-3-carboxamide (AM251; Tocris Bioscience, Ellisville, MO) were dissolved in 100% DMSO at 5 × 103 M and 3-(2-carboxypropyl)phosphonic acid (CPP; 3 × 10-3 M) and 3-(2-carboxypropyl)phosphonic acid (CPP; 3 × 10-3 M) were added to the bath solution to block voltage-dependent Na+ channels. Ionotropic glutamate receptor antagonists DNBQX (10 μM) and CPP (3 μM) were used to isolate excitatory activity. The pipette solution contained (in mM) 20 KCl, 100 K-glucuronate, 10 HEPS, 0.1 CaCl2, 4 Na2-ATP, 0.3 Na-GTP, and 10 Tris-phosphocreatine, pH 7.3 (290 ± 5 mmol/kg). INs were chosen based on morphology and the presence of a sag potential when hyperpolarized with a −250-pA current injection. For these voltage-clamp recordings, series resistance (Rs) was compensated to >60% at 10–100 μs lag. Neurons were rejected from analyses if Rs was >25 MΩ at the time of break-in or >10.5 MΩ after compensation, if Rs changed by >15% during the course of an experiment, or if Rs fell <100 MΩ.

Clampfit (Molecular Devices, Sunnyvale, CA) was used to detect and analyze mIPSCs and sIPSPs. We quantified synaptic activity by integrating the area under individual sIPSPs, an index that takes into account event frequency, amplitude and duration. DSI was calculated as the percent suppression in area measured from 5 to 15 s after depolarization. Group data are presented as means ± SE. Tests of statistical significance were based on Student’s paired t-test unless otherwise noted.

For Ca2+ imaging experiments, the cell-impermeant form of Oregon Green 488 BAPTA-1 (100 μM, Molecular Probes, Carlsbad, CA) was dissolved in the internal pipette solution. Recording of Ca2+ fluorescence was carried out 15–20 min after breaking into whole cell mode to allow equilibration of the dye. Changes in Ca2+ fluorescence were detected by a cooled charge-coupled device camera (IMAGO-QE, TILL Photonics, Munich, Germany) at a sampling rate of 20 Hz. Intracellular changes in postsynaptic [Ca2+]i, are expressed in terms of ΔF/FP0, where FR0 is the average background-subtracted fluorescence intensity when the cell was at rest and ΔF is the change in fluorescence intensity immediately after the depolarizing stimuli protocol. Fluorescence intensity was measured in an ≈3 × 5 μm region within the soma. A similar size region of interest was used to detect changes in background fluorescence.

**RESULTS**

**Characterization of layer 2/3 interneurons**

PNs and INs in neocortical layer 2/3 were identified morphologically and classified by AP firing patterns in response to 500-ms current injection pulses ranging from −250 to +250 pA. As shown in Fig. 1, PNs typically displayed a regular firing pattern with amplitude and frequency adaptation, and INs were classified as FS, IS, or RSNP. RSNP cells (Fig. 1D) were the most common and displayed a regular firing pattern with some degree of amplitude and frequency adaptation but were able to fire at higher frequencies than PNs. They also showed a noticeable transient deflection (or sag) when hyperpolarized, which was also seen in other IN types and to a lesser extent in PNs. IS cells (Fig. 1B) fired irregularly in response to depolarizing current pulses and their APs exhibited a noticeable fast afterhyperpolarization (AHP). FS interneurons had the largest and fastest AHPs and discharged at a much higher rate than any
other interneuron type; the example FS cell shown in Fig. 1C is firing at \( \sim 160 \) Hz. The physiological properties of PNs and the different classes of INs are shown in Table 1. In general, INs had a more depolarized resting membrane potential, shorter spike duration, and more pronounced afterhyperpolarization compared with PNs.

We attempted to correlate IN morphology with the electrophysiological classification by imaging recorded cells with the fluorescent dyes Alexa Red or Lucifer yellow. An example montage of photomicrographs for each cell type is shown in Fig. 1. The typical PN shown in Fig. 1A has a long apical dendrite, splitting \( \sim 100 \) \( \mu \)m from the soma as it approaches the superficial cortical layer 1. The axon can also be seen heading toward deeper cortical layers. IS cells, which were found mostly near the border with layer 1, typically had elongated somas and bipolar processes (Fig. 1B). Both FS and RSNP cells were typically multipolar. Some FS cells, like the one shown in Fig. 1C, had a chandelier-like morphology with two long dendrites emerging from the soma and pointing, almost parallel, toward layer 1 while the axon emerges from the opposite direction and split close to the soma. The example shown for the RSNP cell (Fig. 1D) is a typical multipolar cell with processes splitting either immediately or within 10–20 \( \mu \)m from the soma.

Layer 2/3 interneurons receive cannabinoid-sensitive inhibitory inputs

We next investigated whether layer 2/3 INs receive inhibitory inputs that can be suppressed by cannabinoids. In these experiments, we utilized bath application of the muscarinic agonist CCh, which causes a marked increase in the frequency of sIPSPs recorded from cortical PNs. This CCh-induced activity in PNs is potently suppressed by CB1R activation (Trettel et al. 2004). We obtained similar results with PNs in the present study as shown in the top trace in Fig. 2. Interest-

| Table 1. Physiological properties of layer 2/3 pyramidal neurons and interneurons |
|---------------------------------|-------|-------|-------|-------|
| Resting potential, mV          | -68.8 \( \pm \) 1.1 | -61.9 \( \pm \) 0.8 | -59.0 \( \pm \) 2.0 | -55.0 \( \pm \) 1.5 |
| Input resistance, MΩ            | 272 \( \pm \) 18     | 230 \( \pm \) 28     | 348 \( \pm \) 35     | 349 \( \pm \) 39     |
| Spike amplitude, mV             | 90.5 \( \pm \) 1.2    | 79.4 \( \pm \) 1.7    | 85.5 \( \pm \) 1.9    | 70.7 \( \pm \) 9.8   |
| Spike duration, ms              | 1.4 \( \pm \) 0.06    | 0.6 \( \pm \) 0.04    | 0.9 \( \pm \) 0.06    | 0.8 \( \pm \) 0.04   |
| AHP, mV                         | -3.7 \( \pm \) 0.6    | -19.2 \( \pm \) 1.3   | -10.2 \( \pm \) 0.9   | -12 \( \pm \) 0.8    |
| Freq. accommodation, %          | 68 \( \pm \) 2.7      | 11 \( \pm \) 2.4      | 55 \( \pm \) 5.8      | ND                  |
| Sag potential, mV               | 3.0 \( \pm \) 0.4     | 3.8 \( \pm \) 0.3     | 11.5 \( \pm \) 1.2    | 10.7 \( \pm \) 2.1   |

Values are given as mean \( \pm \) SE. PN, pyramidal neuron; FS, fast spiking; RSNP, regular spiking non-pyramidal; IS, irregular spiking; AHP, afterhyperpolarization; ND, not determined (due to the irregular nature of spiking in IS cells). Physiological parameters were measured as described in Methods. Numbers of neurons are in parentheses.
nabinoid agonist WIN55,212-2 (WIN; 6 A)
tors are present at IN-IN synapses, we bath applied the can-
tary inputs similar to PNs. To determine whether CB1 recep-
gests that these INs may receive cannabinoid-sensitive inhibi-
tions.
sIPSPs, which are depolarizing under our experimental condi-
effects on PNs as well as indirect effects due to the increase in
The depolarizing effects of CCh likely result from direct
antagonist (either 6 A
CB1 receptor, because pretreating slices with a CB1 receptor
sIPSPs within minutes of WIN application that was at least
fully inhibited the effect of WIN. Thus all three classes of
INs receive inhibitory inputs that are activated by CCh and
inhibited by CB1 receptor activation.

Two lines of evidence support the idea that the effect of
WIN was due to activation of presynaptic CB1 receptors. First,
although WIN inhibited sIPSPs, it did not reverse the effect of
CCh on the resting membrane potential of the postsynaptic cell
consistent with a presynaptic effect of WIN rather than an
effect on the postsynaptic cell. In addition, we recorded action
potential-independent mIPSCs in the presence of 1 mM tetro-
dotoxin. As shown in Fig. 3C, we found that WIN significantly
decreased the frequency of mIPSCs (baseline = 2.7 ± 0.4 Hz,
WIN = 1.7 ± 0.4 Hz, P < 0.05, n = 8, range: 1.15–4.53 Hz),
consistent with a presynaptic effect of WIN. There was also
a small but significant reduction in mIPSC amplitude. This
decline in mean amplitude appeared to be due to the fact that

![Figure 2](image.png)

**FIG. 2.** Effects of the muscarinic agonist carbachol (CCh) on spontaneous inhibitory postsynaptic potentials (sIPSPs). Sweeps represents typical examples of the effect of bath-applied CCh (10 µM) on sIPSPs recorded from either a layer 2/3 PN, an IS interneuron, a RSNP cell, or a FS interneuron. sIPSPs are recorded as upward deflections. Note the marked depolarization of the resting membrane potential in the IS cell. Scale bar for all traces is 5 mV, 30 s.

![Figure 3](image.png)

**FIG. 3.** Effects of the cannabinoid agonist WIN 55,212-2 (WIN) on inhibitory synaptic activity. A: examples traces for each cell type showing sIPSP activity before, during, and 10 min after WIN exposure (6 µM). All recordings were done in the presence of 10 µM CCh. Scale bar is 5 mV, 5 s. B: group data for each cell type for the effect of WIN on CCh-induced sIPSPs (○) or in the added presence of the type 1 cannabinoid receptor (CB1) antagonist AM251 or SR141716A (6 µM; □). Synaptic activity was integrated over a 1-min period occurring 5 min after onset of WIN application compared with a 1-min baseline period just prior to WIN application. C: group data for the effect of WIN on the frequency and amplitude of miniature inhibitory postsynaptic currents recorded from interneurons in the presence of 1 mM tetrodotoxin. The number above each bar denotes the number of cells tested. *, P < 0.05. Inset: example of the cumulative amplitude distribution of mIPSCs before and during WIN exposure. Note the decreased occurrence of large-amplitude events in the presence of WIN.

The CCh-induced increase in sIPSPs recorded in INs sug-
sists that these INs may receive cannabinoid-sensitive inhibi-
tory inputs similar to PNs. To determine whether CB1 recep-
tors are present at IN-IN synapses, we bath applied the can-
nabinoid agonist WIN55,212-2 (WIN; 6 µM) during CCh
exposure. Typical voltage traces for each cell type before,
during, and after WIN exposure are shown in Fig. 3A. All three
classes of INs, as well as PNs, showed a marked suppression of
sIPSPs within minutes of WIN application that was at least
partially reversible after washout. Group data for all cell types
are shown in Fig. 3B. The effect of WIN was mediated by the
CB1 receptor, because pretreating slices with a CB1 receptor
antagonist (either 6 µM AM251 or 6 µM SR141716A) signifi-
ically inhibited the effect of WIN. Thus all three classes of
INs receive inhibitory inputs that are activated by CCh and
inhibited by CB1 receptor activation.

The CCh-induced increase in sIPSPs recorded in INs sug-

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the decreased frequency of mIPSCs was not uniform across the amplitude distribution but instead was consistently skewed toward larger amplitude events. An example of the amplitude distribution from a single cell before and during WIN exposure is shown in Fig. 3C, inset.

Lack of interneuron DSI

We next investigated whether INs can synthesize and release eCBs by examining whether DSI occurs at IN-IN synapses. First, we confirmed the presence of DSI at inhibitory synapses on PNs, as previously reported. Figure 4A shows a typical example of DSI in a layer 2/3 PN after a 20-AP/40-Hz train, and the blockade of DSI by a CB1 receptor antagonist. Overall, as shown in Fig. 4B, sIPSP area in PNs was transiently suppressed by an average of 40.7 ± 6.9% (n = 9, P < 0.05). DSI was blocked by pretreatment with the CB1 receptor antagonist AM251 or SR141716A (6 μM; 12.0 ± 12.9%, n = 5).

We next attempted to induce DSI in all three classes of layer 2/3 INs. Surprisingly, in light of the potent effects of WIN on sIPSPs, we failed to observe any significant DSI at IN-IN synapses, even with induction protocols ≥800 APs and frequencies as high as 100 Hz. Figure 5A shows examples of traces obtained from each class of IN (IS: top; RSNP: middle; and FS: bottom). Each trace illustrates a 25-s pre-DSI period (baseline), followed by an AP train of 400 APs at 80 Hz (omitted for clarity), and the period immediately after the AP train. The group data shown in Fig. 5B indicates the amount of suppression from each class of IN using a 400-AP/80-Hz train. Note that for the FS cells, the small but consistent suppression that was observed under baseline conditions (12.6 ± 8.0%, n = 9) was unchanged in the presence of the CB1 receptor antagonist AM251 (11.4 ± 3.2%, n = 5), indicating that this transient suppression was not CB1 receptor-mediated. Thus in layer 2/3, only PNs appeared to be capable of mobilizing cannabinoids and inducing DSI in response to AP trains.

It has been shown by our laboratory and others that DSI depends on an increase in intracellular calcium as DSI is blocked by introduction of the calcium chelator bis-((o-aminophenoxy)-N,N,N',N'-tetraacetic acid (BAPTA) to the postsynaptic cell (Lenz and Alger 1999; Trettel and Levine 2003; Wilson and Nicoll 2001). It is possible that the lack of interneuron DSI in the present study is due to an insufficient rise in intracellular calcium. In fact, interneurons have been shown to have a high [Ca2+]i buffering capacity compared with PNs (Baimbridge et al. 1992). We therefore monitored the change in intracellular calcium in PNs and INs in response to DSI

FIG. 4. Depolarization-induced suppression of inhibition (DSI) is present in layer 2/3 PNs and is blocked by a CB1 receptor antagonist. A, top: DSI in a layer 2/3 PN induced by a train of 20 action potentials (APs) at 40 Hz. In lower trace, pretreatment with the CB1 receptor antagonist AM251 (6 μM) completely prevented the expression of DSI. †, occurrence of the AP train, which is blanked for clarity and shown in inset. Scale bars, 10 mV, 10 s. Scale bars for inset, 50 mV, 200 ms. B, normalized group data illustrating the magnitude and time course of DSI in the absence ( ■, n = 9) or presence (○, n = 5) of a CB1 receptor antagonist (AM251 or SR141716a, 6 μM). AP train was delivered at time 0.

FIG. 5. DSI is not observed in layer 2/3 interneurons. A: example traces illustrating the lack of DSI in each class of layer 2/3 interneuron. AP train consisted of 400 APs at 80 Hz, delivered at †. Scale bars, 5 mV, 10 s. B: group data for DSI in layer 2/3 PNs and interneurons. The small but consistent suppression seen in FS cells was not affected by pretreatment with the CB1 receptor antagonist AM251 (6 μM; ○). The number above each bar denotes the number of cells tested. *, P < 0.05.
induction protocols, using the membrane impermeable fluorescent calcium dye, Oregon Green 488 BAPTA-1 (100 μM). After breaking into the cell and allowing 10–20 min for the dye to equilibrate between the pipette and the cell, we collected fluorescence images before, during, and after various DSI induction protocols (trains of 20, 50, 100, or 200 APs at 40 or 80 Hz). In PNs, the magnitude of the Ca\(^{2+}\) signal doubled in response to a train of 20 APs at 40 Hz, a protocol that was sufficient to induce reliable DSI (Fig. 6A). The different classes of INs all showed a smaller calcium increase in response to that particular protocol, but increasing the frequency and duration of the AP train led to relative increases in the Ca\(^{2+}\) signal comparable to that seen in PNs (Fig. 6, B–D). As shown in Fig. 6F, a minimum of 100 APs was able to achieve a relative increase in the Ca\(^{2+}\) signal in all classes of INs similar to that seen with DSI induction in PNs, but as shown in the preceding text, these protocols were still unable to induce DSI in INs. Thus the lack of DSI at IN-IN synapses does not appear to be due to an insufficient rise in intracellular Ca\(^{2+}\).

Another possible explanation for the lack of interneuron DSI is that released eCBs cannot access the site of presynaptic CB1 receptors due to efficient reuptake processes. To address this question, we attempted to induce DSI in the presence of the eCB re-uptake blocker AM404. We replicated our previous results showing that 25 μM AM404 enhances DSI induced in layer 2/3 PNs (Fig. 7A). In all three classes of INs, however, no significant DSI was obtained even in the presence of AM404. Examples of DSI trials in an FS cell before and during AM404 exposure are shown in Fig. 7B, and group data are shown in Fig. 7C. These results suggest that the failure to observe DSI in INs is not due to rapid reuptake of released eCBs.

**DISCUSSION**

We have previously shown that neocortical PNs receive cannabinoid-sensitive inhibitory inputs, and brief trains of APs reliably induce CB1 receptor-mediated DSI (Fortin et al. 2004; Trettel et al. 2004). In the present study, we examined the role of cannabinoids in modulating inhibitory inputs to three distinct classes of physiologically identified neocortical INs. We found that all three classes of interneurons, FS, IS, and RSNP, received inhibitory inputs that could be activated by muscarinic stimulation, and these inhibitory inputs were suppressed by CB1 receptor activation to an extent similar to that seen in PNs. This cannabinoid-mediated suppression was a presynaptic effect, indicating that CB1 receptors are expressed at inhibitory synapses onto INs as well as PNs. Of the cell types recorded in layer 2/3, it is likely that the IS cells provide some of the cannabinoid-sensitive input to INs. IS cells were the only cells that showed a marked depolarization in the presence of CCh sufficient to increase AP discharge. In addition, Galarreta et al. (2004) demonstrated that layer 2/3 INs (with multipolar and/or

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**FIG. 6.** Activity-induced intracellular calcium rise in PNs and interneurons (INs). A: examples illustrating the rise in intracellular calcium in a single PN for trains of 20, 50, and 100 APs at 40 Hz. B–D: examples showing the rise in calcium for 20, 50, 100, and 200 APs at 80 Hz for each class of interneuron. E: superimposition of individual calcium transient traces obtained with 100 APs/80 Hz in all 3 IN types compared with a typical calcium transient from a PN obtained with 20 APs/40 Hz. F: group data for the Ca\(^{2+}\) increase in response to various AP trains in INs (80 Hz; n = 5–7 for each cell type) and PNs (40 Hz; n = 12).

**FIG. 7.** Effects of endocannabinoid reuptake blockade on DSI. A: example of DSI in a PN in the absence or presence of 25 μM AM404. Scale bars, 4 mV, 10 s. B: lack of DSI in a FS interneuron in the absence or presence of AM404. Scale bars, 4 mV, 10 s for control trace, 2 mV, 10 s for AM404 trace. C: group data illustrating the effect of AM404 on DSI in PNs (n = 2) and INs (n = 8).
bitufted morphologies) that express CB1 receptors exhibit electrophysiological characteristics of IS cells. For those reasons, we believe that it is most likely that the IS cells are responsible for the increase in sIPSPs induced by CCh application.

In marked contrast to PNs, there was no eCB-mediated DSI of inhibitory inputs onto INs, even with prolonged high-frequency spike trains. One of the requirements for activity-induced DSI is a steep increase in free intracellular calcium concentration ([Ca^{2+}]). Estimates of the level of calcium needed to induce DSI range from 4 μM in hippocampal CA1 pyramidal neurons (Wang and Zucker 2001) to 15 μM for the Purkinje cells in the cerebellum (Brenowitz and Regehr 2003), although retrograde inhibition may also be triggered by lower calcium levels (Glitsch et al. 2000). In the present studies, we found that the relative calcium increase in INs is less than seen in PNs for the same activity trains. INs express higher levels of calcium binding proteins such as calbindin D28k, parvalbumin, and calretinin, and therefore they generally have a high [Ca^{2+}] buffering capacity compared with the PNs (Baimbridge et al. 1992). However, the lack of DSI did not appear to result from an insufficient rise in intracellular calcium because prolonged high-frequency spike trains in INs resulted in relative calcium increases comparable to what is seen in PNs during DSI induction. It should be noted, however, that absolute calcium concentrations were not monitored, and baseline calcium levels may differ between INs and PNs. These results are in agreement with a study in the hippocampus, where it was reported that INs in the s. oriens and s. radiatum also receive cannabinoid-sensitive inputs but fail to show DSI (Hoffman et al. 2003). A more recent study, however, suggests that DSI may occur between cholecystokinin-expressing INs in the hippocampus (Ali 2007). In the neocortex, although it has been shown that layer 5 LTS cells can release endocannabinoids to modulate self-excitability (Bacci et al. 2004), it is not clear whether these INs can also produce DSI or DSE.

The lack of DSI at IN-IN synapses suggests that either layer 2/3 INs are not capable of producing endocannabinoids or releasing them at relevant synaptic sites or that insufficient stimulation was delivered to mobilize eCBs from these cells. INs might be lacking some of the components of the cellular enzymatic cascade required for eCB synthesis. It is also possible that there is a lack of co-localization between the site of eCB release by INs and the presynaptic terminals expressing CB1 receptors. A related explanation could be the presence of more efficient endocannabinoid re-uptake at IN-IN synapses compared with IN-PN synapses, although the inability of the reuptake inhibitor AM404 to unmask DSI in interneurons suggests that this is probably not the case. It may be that depolarization alone is not a sufficient trigger for eCB mobilization in these INs, and some combination of neuronal activity plus metabotropic stimulation (other than muscarinic) or NMDA receptor activation is required. However, if INs do not release eCBs, could there be some other functional relevance for CB1 receptor expression at IN-IN synapses? One possibility is that these receptors are activated by spillover of eCBs from neighboring PNs when PNs display high levels of prolonged activity. Under these conditions, activation of CB1 receptors at IN-IN synapses would disinhibit IN activity, and the resulting increase in inhibitory input to PNs could act as a negative feedback mechanism to dampen PN excitability.

**GRANTS**

This research was supported by National Institute of Drug Abuse Grant DA-16791 to E. S. Levine.

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