Functional Significance of Cannabinoid-Mediated, Depolarization-Induced Suppression of Inhibition (DSI) in the Hippocampus

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The recent discovery of the linkage of the phenomenon known as depolarization-induced suppression of inhibition (DSI), originally reported in hippocampal pyramidal neurons by Alger and colleagues (Pitler and Alger 1992, 1994a,b) to the release of endogenous cannabinoids (endocannabinoids) in hippocampus (Wilson and Nicoll 2001, 2002), has important implications. The reduction in GABAergic transmission, as signaled by the decrease in amplitude of inhibitory postsynaptic currents (IPSCs) that occurs following 1.0-s depolarizations of hippocampal pyramidal cells in slices (Wilson and Nicoll 2001; Wilson et al. 2001) and cultured hippocampal neurons (Ohno-Shosaku et al. 2002), is prevented by the CB1 cannabinoid receptor-antagonist SR141716A. A similar depolarization-dependent cannabinoid suppression of presynaptic release of excitatory transmitter has been demonstrated in the rat cerebellum (Kreitzer and Regehr 2001). In hippocampus, DSI involves activation of CB1 receptors on GABAergic terminals of hippocampal interneurons (Katona et al. 1999; Wilson and Nicoll 2001), which presumably lead to a reduction in voltage-sensitive Ca2+ conductances (Mackie and Hille 1992) and a corresponding decrease in GABA release, signaled by a marked reduction in both evoked (eIPSCs) and spontaneous inhibitory postsynaptic currents (sIPSCs) in hippocampal pyramidal cells (Wilson et al. 2001).

We investigated DSI from two different perspectives: first, as to whether this process could be initiated in vitro by normal patterns of action potentials recorded from animals performing hippocampal-dependent behavioral tasks. This is important because it addresses the functional significance of DSI and associated endocannabinoids that are involved, since it appears that hippocampal neurons need to be significantly depolarized to release endocannabinoids (Lenz and Alger 1999; Wilson and Nicoll 2002). The second purpose was to define precisely the range of frequencies and minimum duration of depolarizing pulses required to elicit DSI in hippocampal pyramidal neurons in vitro. Both objectives were directed at determining whether release of endocannabinoids is possible under what can be considered normal firing conditions for hippocampal neurons in vivo. To address this issue, we utilized trains of depolarizing pulses similar to those that occur during spontaneously increased firing, in various behavioral contexts (Deadwyler et al. 1996; Hampson et al. 1996; Hampson and Deadwyler 2000). This was performed in hippocampal pyramidal cells recorded in vitro in which DSI could in fact be demonstrated. Hence, the following findings: 1) describe the minimal activation conditions required to provoke DSI in hippocampal pyramidal neurons, and therefore, and 2) indicate the circumstances in which endocannabinoid release could contribute to hippocampal operation in vivo.

INTRODUCTION

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METHODS

Hippocampal slices

The preparation of hippocampal brain slices was similar to that described in several previous reports (Alger 1999; Kim et al. 2002; Lenz and Alger 1999; Pitler and Alger 1994; Wilson and Nicoll 2001). One-week-old male Sprague-Dawley rats (Harlan) were CO2 anesthetized and rapidly decapitated. The brains were removed and the hippocampi dissected in cold (4°C) buffered saline medium (see bathing medium, below) and then were sliced at 300 μm thickness along the transverse axis using a Leica vibratome. Slices were incubated in oxygenated buffered saline for 1 h at room temperature before recording. Prior to use, slices were transferred to a custom perfusion chamber (Staff et al. 2000) and viewed through a Zeiss Axioskop2 near-infrared differential interference contrast (DIC) microscope. Slices were constantly perfused with warmed (35°C) bathing medium [140 mM NaCl, 5 mM KCl, 2.5 mM CaCl2, 2 mM MgCl2, 10 mM glucose, 10 mM HEPES, 20 μM 6-cyano-7-nitroquinoline-2,3-dione (CNQX), and 50 μM 2-aminophosphonic acid (APV)]. Carbachol (3.0 μM) was added to the bathing medium after formation of the whole cell patch to increase the frequency of sIPSCs. All experiments were performed on slices within 2 h following transfer to the recording chamber.

Recording methods

Whole cell patch-clamp procedures, similar to that reported previously (Hampson et al. 1993; Mu et al. 1999), were performed using the D/Cl channel blocker to visually localize individual CA1 pyramidal neurons in slices of hippocampus. Briefly, patch electrodes were prepared from 1.5 mm outer diameter, 1.1 mm inner diameter borosilicate glass capillaries to produce 1–2 μm (2–5 MΩ) tip openings. Electrodes were filled by suction and backfilling with a standard intracellular solution [140 mM KCH3SO3 (or 100 mM CsCH3SO3), 40 mM CsCl, 3 mM KCl, 0.2 mM EGTA, 0.02 mM CaCl2, 1 mM MgCl2, 2 mM ATP, 300 μM GTP, 10 mM HEPES buffer (Sigma), and 5 mM QX314]. A critical aspect of pipette solutions that affects buffering, a condition that provides the lowest threshold for provoking calcium increases the depolarization threshold for DSI. Internal calcium concentrations were calculated to be 20 nM, with low calcium buffering, a condition that provides the lowest threshold for provoking DSI (Lenz and Alger 1999).

Sealing the pipette to the neuron and obtaining access to the whole cell followed previously described patch-clamp procedures (Hamill et al. 1981; Mu et al. 1999). Voltage-clamp recordings and command voltage steps were performed with an AxoClamp 2A amplifier and Digidata 1322A controller (Axon Instruments). Whole cell current and voltage records were acquired and stored on magnetic disk using Digidata 1322A controller (Axon Instruments). Whole cell current-voltage steps were performed with an AxoClamp 2A amplifier.

DSI consisted of calculating the area of individual sIPSCs and summing all sIPSCs in the 3-s period prior to the depolarization step, with a similar measure taken over 4–7 s following depolarization at the stage of maximum suppression of sIPSCs (Alger 1999). In most cases, DSI was computed from pre- and post-sIPSC area [%DSI = (pre + post) × 100%] and reported as mean ± SE (Alger 1999; Lenz and Alger 1999). Bar graphs were plotted using mean (±SE) percentage of prepulse sIPSC area [%DSI = 100%] for comparison with current traces. Statistics were calculated from analysis of variance (ANOVA) of within-cell comparisons of the effects of depolarization patterns and drug effects, with individual comparisons calculated as orthogonal linear contrasts either between conditions or against no change in sIPSC area (mean percentage of prepulse sIPSC area = 100%, DSI = 0%).

Drug preparation

WIN 55,212-2 (Research Biochemicals, Natick, MA) was prepared daily from a 10 mM stock solution in ethanol, diluted with extracellular bathing medium, and the ethanol evaporated under a constant stream of nitrogen (Mu et al. 1999). The drug was added to the bathing solution in either 100 or 500 nM concentrations.

RESULTS

Characterization of DSI

DSI was produced in the same manner as described previously (Alger 1999; Lenz and Alger 1999; Pitler and Alger 1994b; Wilson and Nicoll 2001). Using two measures of γ-aminobutyric acid (GABA) release, eIPSCs, or sIPSCs, DSI could be elicited in the majority of neurons tested (n = 150). Figure 1A shows the decrease in sIPSCs resulting from a 1.0-s depolarizing pulse. The duration and magnitude of DSI suppression of sIPSCs by this standard depolarizing parameter was appreciable (mean decrease in amplitude from the predepolarization baseline = 52.2 ± 0.6%). Figure 1A also illustrates that sIPSCs were bicusculic sensitive and suppressed by application of the cannabinoid CB1 receptor agonist WIN55,212-2 (500 nM) but recovered in the presence of the CB1 receptor antagonist SR141716A (100 nM). Also, DSI was completely blocked by pretreating slices with SR141716A as evidenced by absence of decrease in sIPSC area from prebaseline (1.1 ± 2.3%: Fig. 1B), indicating reliance on release of endogenous cannabinoids (Kim et al. 2002; Ohno-Shosaku et al. 2002; Wilson and Nicoll 2001; Wilson et al. 2001). Depolarizing pulses of 100-ms duration elicited significant DSI but at an increased latency to onset (2.8 ± 0.06 s) compared with 1.0-s pulses (Wilson et al. 2001). The traces and graph in Fig. 1B show the effects of several agents used to test DSI produced by 1-s depolarizations (DSI = 52.3 ± 2.8%, F1,283 = 257.3, P < 0.001). DSI was not altered by pretreating slices with the phosphenate inhibitor calcineurin A (DSI = 33.7 ± 3.1%, F1,283 = 108.6, P < 0.001) nor the adenylate cyclase activator forskolin (DSI = 31.3 ± 2.4%, F1,283 = 96.2, P < 0.001), but was blocked by the N-type Ca2+ channel blocker ω-conotoxin [DSI = 2.8 ± 1.6%, F1,283 = 0.87, not significant (NS)]. Thus, by a variety of pharmacological criteria, DSI observed under our recording conditions was similar to that described in several other studies (cf. Alger 1999; Wilson and Nicoll 2002).

Involvement of K-currents in DSI

We next examined the whole cell currents associated with depolarizing pulses that elicited DSI, in comparison to simu-
lated action potential and other depolarizing currents that occur in vivo. The outward potassium current associated with spontaneous action potentials evoked under voltage clamp was found to be 800 pA with a duration of 3–5 ms. Voltage parameters that produced action potential-like currents were then systematically examined when voltage-sensitive sodium channels were blocked as in most demonstrations of DSI (Lenz and Alger 1999; Wilson et al. 2001). Injected depolarizing command pulses of <1.0 ms duration were not sufficient to elicit outward potassium current similar to that exhibited by spontaneous action potentials. Depolarizing pulses between 1 and 10 ms produced outward currents identified as a fast inactivating potassium A current of the Kv1.4 type (Dolly and Parcej 1996; Grosse et al. 2000; Sheng et al. 1992). If the depolarizing pulse was ≥10 ms, a subsequent late onset K-current (Dolly and Parcej 1996; Storm 1990) was apparent, which lasted for the duration of the injected voltage with no sign of inactivation (Fig. 2, Control). The outward potassium currents evoked by these depolarizing pulses were found to be altered by either 4-aminopyridine (4-AP) (A-current) or tetraethylammonium (TEA; K-current, Fig. 2, TEA) as documented in prior studies (Hille 1998; Mu et al. 1999; Rudy 1988; Serodio and Rudy 1998; Storm 1990).

The other variable relevant to producing DSI is the level of depolarization. Command voltage steps that produce less depolarization (i.e., steps to 0 mV) typically did not elicit DSI (Lenz and Alger 1999). As such, there are few membrane transients that meet such a requirement in vivo— with the exception of the action potential itself. Injected voltages were therefore tailored to evoke currents that were the following: 1) similar in duration and amplitude to those produced by spontaneous action potentials recorded under voltage clamp in similar bath and pipette conditions with the exception of QX-314; 2) similar in voltage dependence and pharmacological sensitivity with respect to outward potassium currents that occur during spontaneous or evoked action potentials in vitro. Using these guidelines, we subsequently showed that if the injected pulses were prolonged, they not only produced the expected slow onset and noninactivating K-current, but DSI as well (Fig. 2, Control). Blockade of the late onset K-current by TEA eliminated DSI even though A-current remained (Fig. 2, TEA).
This indicates that the time course, magnitude, and threshold for producing DSI were related to the duration of depolarizing K-current and hence the charge transfer necessary for activation of voltage-dependent Ca\(^{2+}\)/H\(_{11001}\) channels (Lenz and Alger 1999). A minimum 70–0 mV depolarizing pulse of 50-ms duration produced only 5% DSI. Figure 3 shows a comparison of DSI as a percentage of sIPSP area elicited by injected depolarizing pulses ranging from 25 ms (DSI = 1.1 ± 1.4%, \(F_{1,283} = 0.11, \text{NS}\)) to 1.0 s (DSI = 52.3 ± 2.8%, \(F_{1,283} = 257.3, P < 0.001\)). It is clear that as the duration of the depolarizing pulse decreased from 1.0 s, the percentage of maximum DSI decreased as well; however, it is also apparent from Fig. 3 that this relationship was not entirely linear across the range of pulses tested. For depolarizing pulses of 25–200 ms (DSI = 37.4 ± 3.6%, \(F_{1,283} = 132.4, P < 0.001\)) decrease in sIPSC frequency (increase in DSI) was close to linear and steep (slope = 0.23% per ms), but as depolarizing pulses exceeded 250 ms (Fig. 3), the slope of this function became much more shallow (slope = 0.02% per ms).

Simulated in vivo neuron firing and DSI

Under most conditions in vivo, pyramidal cell action potentials are not accompanied by large calcium-mediated depolarizing shifts and are of a duration (1–10 ms), even in dendrites, that would be subthreshold for inducing DSI (Staff et al. 2000). It is possible, however, that frequency-summation of action potentials in vivo might produce sufficient depolarization per unit time to elicit DSI. Therefore we tested pulse frequencies and patterns that mimicked increases in hippocampal pyramidal cell firing in vivo for their ability to elicit DSI. Two major conditions that produce increased firing in vivo are as follows: animals traversing place fields in an open arena (Frank et al. 2000; Hampson et al. 1996; Muller et al. 1987) and performance of hippocampal-dependent learning and memory tasks (Deadwyler et al. 1996; Hampson et al. 2002; Eichenbaum et al. 1989; Wood et al. 1999). Figure 4 shows two examples of

FIG. 3. Effect of duration of depolarizing pulses on DSI. Top: traces showing reduction in DSI as a function of decrease in duration of depolarizing pulse (all pulses used a command voltage step from −70 to 0 ms). Bottom: graph depicts magnitude of DSI as mean (±SE) ratio of sIPSC area before and after the depolarizing pulse, at different durations of depolarizing pulses. \(n = 8–12\) cells per condition.

FIG. 4. Portrayal of in vivo hippocampal firing and its effects on sIPSCs. All pulses are 10-ms duration depolarizing voltage steps from −70 to 0 mV. Top: pattern of firing generated as animal traverses place field. Dots illustrate where cell fired on traversal (solid part of path) through place field (ellipse) and not in other places in the arena (dashed line). Depolarizing pulses were delivered to hippocampal neurons in vitro in this same temporal firing sequence, illustrated as a series of depolarizing pulses at right (Stimulation Patterns). I = voltage-clamped currents; V = command voltage steps. Middle: peri-event rasters (in which each row is a single trial and each dot is an action potential) and summed histogram illustrate pattern of firing generated as animal pressed sample lever during performance of delayed-nonmatch-to-sample task (DNMS). The same temporal sequence of pulses delivered to a hippocampal neuron in vitro is illustrated at right. Bottom: lack of DSI produced by DNMS stimulation pattern is illustrated. Five total sweeps were superimposed to show no reduction in sIPSCs for 5 repeats of DNMS pulse pattern.
spike trains recorded while animals either 1) traversed a place field specific for that cell, or 2) responded to behaviorally significant stimuli in a delayed-nonmatch-to-sample (DNMS) memory task. The frequencies ranged from 0.5 to 10.1 Hz for place field firing, and 0.5 to 17.8 Hz for the DNMS task. When injected into cells in hippocampal slices, neither firing pattern was sufficient to produce DSI, even if several trains were delivered within short intervals (1.0-min separations). The bottom of Fig. 4 shows superimposed traces of sIPSCs during five repeated presentations of the DNMS firing pattern (Fig. 4, middle right), with no indication of DSI (2.7 ± 0.9% DSI, \( F_{(1,283)} = 0.84, \) NS).

To examine further the frequency dependence of the depolarizing pulses required to elicit DSI, several additional types of pulse trains were also tested (Fig. 5, A and B). These trains employed depolarizing pulses of differing durations and frequencies, some of which were not typical of normal pyramidal cell activation, but might occur in vivo under specific circumstances (LeBeau and Alger 1998). Frequencies between 5 and 400 Hz in patterns consisting of 1–400 pulse trains with pulse durations ranging between 5 and 20 ms were all ineffective in eliciting significant DSI (Fig. 5B). No significant DSI was detected when irregular bursts of five 10-ms pulses were delivered at an average interval of 50 ms (DSI = 0.2 ± 1.4%, \( F_{(1,283)} = 0.03, \) NS). The maximum effect produced was by trains with pulse durations of 2 ms at frequencies ≤400 Hz (DSI = 3.5 ± 2.1%, \( F_{(1,283)} = 1.15, \) NS, Fig. 5B).

Even trains of 6–10 pulses at 100 Hz, repeated in 6-Hz bursts, which mimicked \( \theta \)-bursts and effectively produced long-term potentiation (LTP) in hippocampal neurons (Rose and Dunwiddie 1986), proved ineffective for inducing DSI (DSI = 6.3 ± 2.2%, \( F_{(1,283)} = 3.38, \) NS). Since depolarizations to 0 mV for 70–100 ms (Fig. 3), the threshold for DSI in vitro, may not be normal occurrences in healthy adult pyramidal neurons (Jefferys 1994; LeBeau and Alger 1998; Leinekugel et al. 2002), it is possible that frequency and duration of cellular depolarization must interact to produce DSI.

To examine the temporal specificity of DSI, a paired-pulse paradigm was used in which two pulses of 35-ms duration were initially separated by >100 ms, a protocol insufficient to elicit DSI. The inter-pulse interval between these pulses was then systematically shortened until DSI was produced, without changing level of depolarization. Figure 6, A and B, shows that the threshold for significant DSI was achieved at an interval of 30 ms and that DSI systematically increased in magnitude when the two pulses were moved closer together until at 10-ms separation, DSI was similar to that effected by a single 70-ms duration pulse (DSI = 18.8 ± 2.4%, \( F_{(1,283)} = 22.4, P < 0.01, \) Fig. 6B). This indicated that at least one critical parameter for producing DSI was the interval (30–35 ms) between depolarizing pulses. DSI could not be elicited, however, by simply delivering the same total amount of depolarization per unit time over the interval. Higher frequency shorter duration...
pulses (i.e., 7 pulses of 10-ms duration), delivered in the same 100 ms interval of time (e.g., 70 Hz) as the two 35-ms duration pulses (Fig. 6A), did not produce DSI (DSI < 3.0%, F(1,283) < 2.3, NS). This was further verified by systematically decreasing the duration of the first 35-ms pulse while the interval between the two pulses was held constant (20 ms). If the initial pulse duration was decreased to ≤ 25 ms, DSI was effectively eliminated, even though the interpulse interval (20 ms) that was effective if both pulses were ≥ 35 ms was maintained (DSI < 2.5%, F(1,283) < 1.8, NS, see Fig. 6B).

Simulated in vivo firing and membrane depolarization

The finding that DSI was temporally dependent in this manner indicates that the pulse trains shown in Figs. 4 and 5 did not meet minimal criteria for eliciting DSI. To determine whether the depolarizing pulse trains that did not produce DSI in voltage clamp might sum and produce sufficient membrane depolarization if the membrane were not clamped (Pitler and Alger 1994b), the same trains were delivered to cells in current-clamp mode. Cells were held at −74 mV by application of slight hyperpolarizing current and recorded without QX 314 in the pipette solution to allow spontaneous action potentials to occur. Figure 7A (left) shows an action potential elicited by 25 pA (1.6 ms) depolarizing current, and a train of 20 such action potentials (Fig. 7A, right), delivered in a pattern similar to that shown in Fig. 4. The accompanying depolarizing shift in the unclamped membrane was far from sufficient to produce DSI. The bar graph in Fig. 7B shows that neither the simulated baseline firing rate (5 pulses, mean rate 0.5 Hz) nor the increases produced by several different patterned trains of 20 pulses (mean rate 20 Hz) produced significant DSI (DSI < 1.8%, F(1,283) < 0.7, NS). However, a depolarizing current step of 100-ms duration of the same amplitude did induce significant DSI (DSI = 38.7 ± 4.9%, F(1,283) = 71.2, P < 0.001). The graph also shows that addition of WIN 55,212-2 (500 nM) reduced sIPSP amplitudes (DSI = 24.8 ± 4.2%, F(1,283) = 44.6, P < 0.001) and that the reduction was blocked by co-administration of (100 nM) SR141716A (DSI = 2.7 ± 3.1%, F(1,283) = 3.1, NS). Thus in current-clamp mode, DSI could be generated using conventional long-duration depolarizing protocols, but patterns of action potentials that occur in hippocampus in vivo were not sufficient to elicit DSI.

FIG. 6. Temporal dependence of DSI. A: paired-pulse paradigm in which two 35-ms pulses (−70 to 0 mV) do not produce DSI if separated by 60 ms (left), but if reduced to 20-ms separation, produce slight DSI (right). Below, delivery of the same total current per unit time as above left (10-ms pulses at 70 Hz) fails to produce DSI. B: left: graph shows DSI as a function of interpulse interval between the two 35-ms pulses. Also shown are results for equivalent depolarization in the same unit time (7 × 10 = 7 pulses, 10-ms duration, 70 Hz; 4 × 20 = 4 pulses, 20-ms duration, 40 Hz). Right: change in DSI as duration of first pulse in the pair is decreased, but interval is maintained at ≥ 20 ms. DSI is reduced even though the interpulse interval is the same. Parameters of paired-pulse durations tested are shown as combinations of interpulse interval and pulse duration (e.g., 10 + 35, 15 + 35, ...). Mean ± SE across different cells; n = 5–7 cells per bar.
DISCUSSION

The above results support recent investigations of DSI and its relation to endocannabinoid mediation at certain GABAergic synapses in hippocampus and confirm prior reports of the parametric and pharmacological characteristics of DSI (Fig. 1, see Wilson and Nicoll 2002). The investigation revealed quite precisely the limits of both frequency and pulse duration required to elicit DSI in hippocampal neurons (Fig. 3). We confirmed that blockade of voltage-dependent N-type Ca\(^{2+}\) channels was sufficient to eliminate DSI (Fig. 1B) and showed that DSI was systematically increased as the duration of the depolarizing pulse delivered to the cell (Fig. 3) was also increased as previously reported (Lenz and Alger 1999). In addition the threshold values for eliciting DSI described here are in the same range (75- to 100-ms duration) reported by Wilson et al. (2001) with similar calcium buffering to that reported by Lenz and Alger (1999). Hence, DSI may be systematically increased as the duration of the depolarizing pulse increases.

Attempts were made to elicit DSI with an extensive array of depolarizing pulse parameters and protocols (Figs. 4–6), patterns of stimulation that mimicked the range of firing of hippocampal neurons in behaviorally relevant circumstances (Deadywler et al. 1996; Hampson et al. 1996, 1999, 2002). However, none of the parameters resulted in significant DSI. While logical, this assumption is not necessarily correct as action potential shapes and durations can vary considerably depending on where generated and whether they traverse into dendrites or are localized to somal regions of the cell (Golding et al. 2001; Magee 2001; Watanabe et al. 2002). However, depolarizations resulting from inputs to dendrites are typically associated with fewer action potentials than those elicited by the same depolarizing inputs to the soma (Harris et al. 2001; Leinekugel et al. 2002; Magee 2001) and bursts of action potentials in hippocampal pyramidal cells are more likely to be generated nearer to than further from the soma (Harris et al. 2001). Hence, pulses injected into the soma would appear to be the most potent as well as the most capable of producing DSI (Fig. 3). From this perspective, burst-type firing in pyramidal cells would provide the best possible means of producing DSI (LeBeau and Alger 1998). However, as shown here, the timing and duration of such bursts are extremely critical (Fig. 6). Alger and colleagues have shown definitively that DSI is directly dependent on calcium charge differences in depolarized pyramidal cells (Lenz and Alger 1999). Hence it is possible, as suggested, that DSI and this form of endocannabinoid release is related to processes not routinely encountered in behaviorally or cognitively activated hippocampal neurons (Christie and Vaughan 2001; Seward et al. 1995; Seward and Nowicky 1996).

Because the duration of depolarizing pulses required (Figs. 3 and 6) to produce DSI (Pitler and Alger 1994), certain outward currents can be eliminated as factors responsible for its occurrence. These include potassium A and D currents whose time course of activation is shorter, resulting in nearly complete inactivation (Fig. 2) during sustained depolarizations ≥25 ms (Hille 1998; Jan and Jan 1994; Mu et al. 1999; Storm 1990; Watanabe et al. 2002). This minimum duration necessary to
provoke DSI leaves only “K” and “leak” potassium currents as possible candidates for providing the outward current during depolarizations necessary for DSI. We confirmed this relationship by showing that DSI was attenuated in the presence of TEA which blocks K but not A and D voltage-dependent potassium current (Hille 1998; Storm 1990). Since the reduction in K-current by TEA also blocked DSI (Fig. 4), it is likely that the depolarizing pulse parameters sufficient to elicit DSI involve TEA-sensitive potassium conductances. This dependence between DSI and K-current may be circumstantial due to the fact that both are a direct function of injected depolarizing pulses, but since the specific nature of this interaction with respect to presynaptic “expression” of DSI is undetermined at this point, it remains a possibility.

The fact that DSI could not be evoked within the range of characteristic in vivo firing patterns of hippocampal pyramidal cells (Figs. 4–5) questions the relevance of this type of endocannabinoid release process in the control or modulation of behaviorally relevant hippocampal pyramidal cell activity. This view is reinforced when it is noted that the above experiments were carried out under conditions that have been shown to be optimal for inducing DSI with the lowest possible duration of depolarizing pulses (Pitler and Alger 1994a), including high concentrations of carbachol (3 μM) and calculated concentrations (20 nM) of intracellular free Ca2+ (Lenz and Alger 1999). At the same time, it was demonstrated that the DSI evoked with longer duration pulses was in fact cannabinoid receptor (CB1) sensitive (Fig. 1A) and that reductions in sIPSCs were readily produced with WIN 55,212-2 and reversed by SR141716A applied to these same cells. Because there was no effect of the CB1 receptor antagonist (SR) alone on sIPSCs, constitutive “release” of endocannabinoids was apparently nonexistent in the absence of suprathreshold depolarizing pulses necessary for DSI (Wilson and Nicoll 2002). These results are consistent with our prior reports that the antagonist alone had no effects on cannabinoid-altered memory processes (Hampson and Deadwyler 2000). However since the precise mechanism of endocannabinoid release under these conditions is not known (Davies et al. 2002; Kim et al. 2002; Pitler and Regehr 2002; Piomelli et al. 2000; Wilson and Nicoll 2002), it is not possible at this time to rule out other factors that might induce endocannabinoid action in vivo.

The threshold duration for evoking DSI with a single pulse under recording conditions employed here was 50–75 ms; single pulses of less duration (Fig. 5), no matter the frequency, failed to elicit significant DSI (Fig. 3). At the same time, the process appears to have a dynamic that has not been described in prior reports, namely that mere depolarization per unit time is not adequate to induce endocannabinoid release. This was demonstrated by injecting pulse pairs of the same amplitude in which DSI was dependent on pulse separation (25–30 ms, Fig. 6B). This temporal dependence of DSI could not be mimicked by pulses of shorter duration presented at higher frequencies, nor by decreasing the duration of the first pulse and maintaining the temporal separation between pulses. These results provide clues as to the potential kinetics of intracellular events underlying endocannabinoid release and support the proposal by Lenz and Alger (Lenz and Alger 1999; Lenz et al. 1998) for a two-step process, possibly involving diffusion of intracellular calcium to a prescribed release site. The recent demonstration of enhancement of endocannabinoid-mediated DSI by co-activation of group I metabotropic receptors (Varma et al. 2001) suggests facilitation in this process. However, since Ca2+ appears to be required for release of endocannabinoids in most contexts (Piomelli et al. 2000), it is not clear how reduced sIPSCs produced by activation of group I metabotropic receptors alone (as recently reported by Kim et al. 2002) could explain the relationship between depolarization-induced endocannabinoid release (i.e., DSI), and how this might be facilitated during normal firing of hippocampal neurons in vivo.

The failure to demonstrate DSI with trains of depolarizing pulses that mimicked those generated in animals engaged in hippocampal-dependent tasks does not rule out cannabinoid participation in these processes. However, previous research showing effects of cannabinoids on hippocampal cells indicates that systemically administered cannabinoids suppress firing of hippocampal neurons in behaviorally relevant circumstances (Hampson and Deadwyler 2000, 2003; Heyser et al. 1993). Similar suppressive effects have been reported on hippocampal GABA, mediated potentials in vitro (Hoffman and Lupica 2000) and in vivo (Hajos et al. 2000). While there may be circumstances in which the threshold for producing endocannabinoid-dependent DSI is exceeded in hippocampal neurons (LeBeau and Alger 1998), such as depolarization shifts as a result of asphyxia, excitotoxicity, or epileptogenesis, most of these conditions are considered to be pathologic (Arabadzisz et al. 2002; Gorter et al. 2002; Perez-Velazquez et al. 1997; Shuttleworth and Connor 2001; Tanaka et al. 2002; Yin et al. 2002). The one normal circumstance in which prolonged action potential bursts occur frequently in hippocampal neurons in the rat is during sleep (Nadasdy et al. 1999); however, even these episodes are transient and, outside of providing a synchronizing influence, tend to mimic firing patterns that occur in waking (Louie and Wilson 2001).

Whatever the process responsible for release of endogenous cannabinoids in vivo and its relation to DSI, its significance will not be completely understood until cannabinoi-mediated DSI is demonstrated in vivo (Piomelli et al. 2000). The above paired-pulse analyses revealed the required frequency range and duration of such depolarizing events (Figs. 3 and 6B) and showed that production of the same depolarization per unit time via trains of shorter action potential-like pulses was not sufficient to induce release of endocannabinoids (Fig. 7B). This temporal dependence appears to have a direct relationship to events that provoke calcium entry (see Wilson and Nicoll 2002), but how this form of depolarization is induced, either synaptically or by other cellular events in vivo, has not yet been identified. That endogenous cannabinoids may in fact have an important role in hippocampal function and in the sculpting of critical firing patterns, remains a distinct possibility, especially with respect to the profound and selective effects of systemically administered CB1 receptor agonists on hippocampal-mediated short-term memory processes (Hampson and Deadwyler 2000, 2003). However, since administration of the CB1 receptor antagonist (SR) alone is ineffective in the latter circumstance and does not alter GABA sIPSCs in the absence of DSI (Wilson and Nicoll 2002), the role of endocannabinoid signaling in hippocampal-dependent behavior remains unresolved.

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