Direct Depolarization and Antidromic Action Potentials Transiently Suppress Dendritic IPSPs in Hippocampal CA1 Pyramidal Cells

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Morishita, Wade and Bradley E. Alger. Direct depolarization and antidromic action potentials transiently suppress dendritic IPSPs in hippocampal CA1 pyramidal cells. J Neurophysiol 85: 480–484, 2001. Whole-cell current-clamp recordings were made from distal dendrites of rat hippocampal CA1 pyramidal cells. Following depolarization of the dendritic membrane by direct injection of current pulses or by back-propagating action potentials elicited by antidromic stimulation, evoked γ-aminobutyric acid-A (GABA A) receptor-mediated inhibitory postsynaptic potentials (IPSPs) were transiently suppressed. This suppression had properties similar to depolarization-induced suppression of inhibition (DSI): it was enhanced by carbachol, blocked by dendritic hyperpolarization sufficient to prevent action potential invasion, and reduced by 4-aminopyridine (4-AP) application. Thus DSI or a DSI-like process can be recorded in CA1 distal dendrites. Moreover, localized application of TTX to stratum pyramidale blocked somatic action potentials and somatic IPSPs, but not dendritic IPSPs or DSI induced by direct dendritic depolarization, suggesting DSI is expressed in part in the dendrites. These data extend the potential physiological roles of DSI.

INTRODUCTION

The retrograde signaling process called “depolarization-induced suppression of inhibition” (DSI) can regulate inhibitory postsynaptic potentials (IPSPs) in CA1 pyramidal cells and cerebellar Purkinje cells (Alger and Piter 1995). DSI is induced by a rise in [Ca 2+ ] in pyramidal cells, but is expressed as a decrease in the release of GABA from interneurons. Experimentally, step depolarizations of the soma have generally been used to induce DSI. However, pyramidal cell depolarization is mainly initiated normally by synaptically induced depolarization of the dendrites, and so it will also be important to know if dendritic depolarization is an effective stimulus for DSI induction. Moreover, dendritic inhibitory synapses exist, and regulation of dendritic inhibition could be an important function of DSI.

CA1 DSI is blocked by voltage-gated Ca channel antagonists (Lenz et al. 1998), and L-type and N-type Ca channels are distributed heavily on somata and proximal apical dendrites of CA1 pyramidal cells (Westenbroek et al. 1990, 1995), and dendritic Ca 2+ influx has been measured (Jaffe et al. 1992, 1994; Spruston et al. 1995; Tsukamoto and Ross 1996). Thus we hypothesized that direct depolarization of the dendrites might be an especially effective way of initiating DSI, and that DSI might have pronounced effects on dendritic IPSPs. Because of their potential importance for understanding the physiological roles of DSI, we have begun testing these hypotheses by recording from distal dendrites of CA1 pyramidal cells.

METHODS

Hippocampal slice preparation

Following deep halothane anesthesia and cardiac perfusion with ice-cold saline, brains were removed from male Sprague-Dawley rats (4- to 8-wk-old); the hippocampi were removed and transversely sectioned at 400-μm intervals. Recordings were made from slices in a chamber on a fixed stage of a Nikon E600FN microscope and perfused (1–1.5 ml/min) with oxygenated saline at 20–24°C.

Solutions

The extracellular saline comprised the following (in mM): 120 NaCl, 3 KCl, 1 NaH 2 PO 4 , 25 NaHCO 3 , 2.5 CaCl 2 , 2 MgSO 4 , and 20 d-glucose (pH 7.4 when bubbled with 95% O 2 /5% CO 2 ). 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 20 μM, RBI), MK-801 (150 μM), TTX (1 μM), and biocytin (0.4%, Molecular Probes) were bath applied. Patch pipettes were filled with the following (in mM): 150 KCl, 10 HEPES, 0.2 or 0.4 bis-(oaminophenoxy)-N,N,N’,N’-tetraacetic acid (BAPTA, 2 MgCl 2 ), 2 MgATP, and 10 sodium phosphocreatine (pH adjusted to 7.2 with KOH). In some experiments, biocytin (0.4%, Molecular Probes) was applied during the patch solution, and these slices were fixed and stained. For the experiments of Fig. 4, a whole-cell pipette was filled with 0.1 mM TTX and placed extracellularly in stratum pyramidale near the recorded cell. TTX was pressure-ejected via a Picospitzer (General Valve) using pulses of 20 psi lasting 1 s.

Whole-cell recording and data analysis

Series resistances were between 60 and 110 MΩ and were compensated by bridge balance (Axoclamp 2B amplifier, Axon Instruments). Only cells with stable resting membrane potentials (> −65 mV) and series resistances were accepted. Antidromic action potentials were elicited with a bipolar concentric stimulating electrode (Rhodes Electronics) positioned near the alveus toward the subiculum. IPSPs were evoked at 0.33 Hz with a stimulating electrode in stratum oriens. Voltage signals were filtered at 2 kHz with an eight-pole

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Bessel filter (Frequency Devices) and digitized at 10 kHz (Digidata 1200). Data were stored on a PC and VHS video tape.

IPSP suppression was quantified by %Reduction = \(1 - \frac{\text{IPSP}_t}{\text{IPSP}_c}\) \times 100\%, where \(\text{IPSP}_c\) is the mean amplitude of seven consecutive IPSPs recorded during the pre-DSI period (the time immediately before the antidromic train or postsynaptic depolarization), and \(\text{IPSP}_t\) is the mean of five consecutive IPSPs evoked during the test period (time following the train or depolarization). The first IPSP was generally not used to calculate \(\text{IPSP}_t\) (Pitler and Alger 1994). Input resistances were obtained from the least-squares linear regression fit of the plots of voltage response versus current steps from \(-100\) to \(+100\) pA. Data were analyzed with PClamp7 (Axon Instruments) and SigmaPlot 4 (SPSS Inc.) software. Student’s t-tests, paired and unpaired as appropriate (\(P < 0.05\)), were used to determine statistical significance of the data. All values given in text and figures are mean ± SE.

RESULTS

Slices were visualized with infrared videomicroscopy (Stuart et al. 1993), and whole-cell current-clamp recordings were made from the distal portion of 22 apical dendrites 250–400 \(\mu\)m from the CA1 pyramidal cell layer. Dendritic recordings were verified by: 1) appearance under videomicroscopy, 2) declining peaks of back-propagating antidromic action potentials (9 of 9 dendrites) (Andreasen and Lambert 1995; Spruston et al. 1995), and 3) location of biocytin-labeled cell somata in the CA1 stratum pyramidale (\(n = 5\) of 5 cells) (Fig. 1, A and B) following filling of the cell through the dendritic electrode.

Figure 1, C1 and C2, show typical dendritic and somatic data. Note the marked inward rectification with strong hyperpolarizations of the dendrite.

Input resistances were \(83.1 \pm 6.06 \, \Omega\) \((n = 7)\) in the dendrites and \(115.4 \pm 9.62 \, \Omega\) \((n = 8, \text{significant, } P \leq 0.02)\) in the somata (different cells). The inward rectification ratio was calculated from the steady-state response (usually taken at 800 ms after the hyperpolarizing step onset) divided by the peak response (usually \(\sim 100\) ms after step onset). The mean rectification ratio in the dendrites was \(0.34 \pm 0.04, n = 7\), whereas in the somata it was \(0.50 \pm 0.03, n = 7 (P \leq 0.01)\), providing further evidence of the dendritic recording site (cf. Andreasen and Lambert 1995; Magee 1998). Both dendritically and somatically recorded IPSPs have a linear conductance (Fig. 1C2), although the dendritic current-voltage plot is shifted slightly to hyperpolarized membrane potentials. The peak amplitudes of the IPSPs in dendrites and somata were similar \((P = 0.08)\) when measured at a membrane potential.
near $-70 \text{ mV}$ ($-7.8 \pm 0.28 \text{ mV}$, $n = 8$, dendrites; $-6.55 \pm 0.62 \text{ mV}$, $n = 8$, somata).

We first asked if depolarizing the dendritic membrane to elicit a train of action potentials would induce DSI of the evoked IPSP. In fact, IPSPs evoked after the 0.65-nA, 500-ms-long depolarizing current step were consistently smaller than IPSPs in control. The suppression was reversible; however, it was modest in magnitude, 15 $\pm 4\%$ peak, $n = 5$ (Fig. 1, D1 and D2).

CA1 DSI is markedly enhanced by bath application of a cholinergic agonist, such as carbachol, acting at a muscarinic receptor (Martin and Alger 1999), and muscarinic agonists can enhance $\text{Ca}^{2+}$ influx into dendrites. Application of 5 $\mu\text{M}$ carbachol caused a large $\text{Ca}^{2+}$ spike to appear following the dendritic depolarization (Fig. 1D1), and there was a significant increase in IPSP suppression (to 36 $\pm 5\%$, $P < 0.05$) (Fig. 1, D1 and D2).

Action potentials can back-propagate into CA1 pyramidal cells and increase intracellular $[\text{Ca}^{2+}]$ (Andreasen and Lambert 1995; Jaffe et al. 1992, 1994; Spruston et al. 1995; Tsubokawa and Ross 1996). A 20-Hz train of back-propagating action potentials lasting 1 s was followed by a transient reduction of evoked IPSPs (6 of 8 dendrites) by 25.9 $\pm 4.11\%$ (Fig. 2, A2 and A4). Extending the antidromic stimulus train in the same dendrites to 2 s of 20-Hz stimuli significantly increased the suppression to 38 $\pm 6\%$ (5 of 5 cells tested) (Fig. 2, A3 and A4; $P < 0.05$).

We attributed the suppression of IPSCs following the alveus stimulation to a DSI-like process because: 1) During the IPSC suppression there were no changes in passive cell properties; e.g., Fig. 2A2 (5 of 5 cells). 2) Limiting back-propagating action potential invasion by a strong hyperpolarization of the dendrites abolished the IPSC suppression (Fig. 2, B1 and B2; $n = 3$). Because of the small size of the reversed IPSP (Fig. 2B1) in three cases, we blocked back-propagating spikes by hyperpolarizing the dendrite only during the antidromic train, so that the larger IPSPs were recorded at the resting membrane potential. This also prevented DSI (data not shown). 3) Bath application of 50 $\mu\text{M}$ 4-AP, a treatment that blocks DSI (Alger et al. 1996), significantly reduced the IPSC suppression caused by back-propagating action potentials (from 30.8 to 17.1%, $P < 0.03$, paired $t$-test, $n = 7$).

When carbachol, 5 $\mu\text{M}$, was bath-applied to the same dendritic recording, the 20-Hz/1-s antidromic train caused a significantly greater suppression of the IPSP (control suppression 20 $\pm 2\%$; carbachol suppression 30 $\pm 4\%$, $n = 5$, Fig. 3, A1 and A2; $P < 0.05$), which was reversible (Fig. 3A2). Interestingly, carbachol failed to enhance IPSP suppression by a 20-Hz/2-s train (Fig. 3, B1 and B2). Presumably, DSI was saturated by the 2-s train.

Both dendritic depolarization and antidromic stimulation produced somatic action potentials; it was not clear if DSI depended on somatic action potentials, nor if dendritic IPSPs were reduced (somatic IPSP reduction might be recorded distally). To address these issues, we placed an extracellular pipette containing 0.1 mM TTX into the slice in s. pyr. and

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**FIG. 2.** Antidromic trains of action potentials transiently suppress dendrically recorded IPSPs. A1: arrangement of stimulating and recording electrodes for the following experiments. A2, top left: average of 5 consecutive IPSPs (a) prior to stimulation (Pre-stim) of the alveus. A hyperpolarizing current pulse (0.15 nA, 500 ms) preceded the IPSP to monitor changes in input resistance. To the right are antidromic spikes arising from a 20-Hz/1-s stimulation of the alveus. The 3 consecutive poststimulus (Post-stim) IPSPs are illustrated in the lower left panel (b). To the right are superimposed traces of (a) and (b). A3: as in A2 but a 2 s antidromic train was delivered to the alveus. Average of 6 consecutive IPSPs following the antidromic train (Post-stim, bottom left panel, b). To the right are superimposed traces of (a) and (b). B2: graph of the effect of reducing the number of antidromic spikes by hyperpolarizing the dendritic membrane (+CLAMP) on IPSP suppression.
pressure-applied a small bolus of TTX (see Fig. 4A1). When DSI was induced by antidromic action potentials, TTX reduced the IPSP and blocked both the antidromic spikes and DSI (Fig. 4A2). Note the remaining IPSP evoked by stratum radiatum stimulation must have been produced by dendritic synapses in this case. However, when DSI was induced in the same cells by direct dendritic depolarization, somatic TTX application did not block DSI, even though the somatic spikes were clearly blocked (Fig. 4A3). Because the residual IPSP was dendritic in origin and was reduced by DSI, we infer that DSI is expressed...
at least partly in the dendrites. Figure 4A4 summarizes data from all six dendrites tested as in Fig. 4, A2 and A3.

DISCUSSION

This work suggests that direct dendritic depolarization can induce a DSI-like phenomenon in CA1 pyramidal cells. The present results extend our previous observations (Pitler and Alger 1994) by showing that antidromic action potentials can induce DSI when they invade the soma-dendritic regions, and thus demonstrating another physiological mode of inducing DSI. However, as nearby cells that are antidromically activated probably also undergo DSI (Fig. 4A2), the influence of DSI induced in these cells evidently does not spread effectively to the recorded cell when it is prevented from firing. Thus CA1 DSI appears to be a local form of communication, unlike cerebellar DSI (Vincent and Marty 1993). This demonstration, which has not previously been reported, would have significant functional implications. A caveat is that we do not have direct evidence that all pyramidal cells were equally well activated by the antidromic stimulation.

Our results show for the first time that DSI can be recorded in distal dendrites and can be expressed on dendritic IPSPs. Nevertheless, the relatively small magnitude of DSI induced by direct dendritic depolarization in the absence of carbachol did not support our prediction that DSI would be especially prominent when induced in this way. This is partly attributable to our recent finding that the “slow” component of the dendritic GABA<sub>A</sub> IPSP (Pearce 1993) is not affected by DSI (L. A. Martin, D.-S. Wei, and B. E. Alger, unpublished observations); i.e., the greater the proportion of IPSP that is not susceptible to DSI, the smaller is the percentage reduction in the total IPSP i.e., the greater the proportion of IPSP that is not susceptible to DSI. In any case, dendritic IPSPs were significantly reduced by DSI, and thus DSI may influence dendritic electro-responsiveness. Further experiments will be needed to test this hypothesis about the functional roles of DSI.

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