Dendritic Mechanisms of Phase Precession in Hippocampal CA1 Pyramidal Neurons

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Received 10 January 2001; accepted in final form 12 February 2001

Magee, Jeffrey C. Dendritic mechanisms of phase precession in hippocampal CA1 pyramidal neurons. *J Neurophysiol* 86: 528–532, 2001. Dual whole-cell patch clamp recordings from the soma and dendrites of CA1 pyramidal neurons located in hippocampal slices of adult rats were used to examine the potential mechanisms of phase precession. To mimic phasic synaptic input, 5-Hz sine wave current injections were simultaneously delivered both to the soma and apical dendrites (dendritic current was 180° out-of-phase with soma). Increasing the amplitude of the dendritic current injection caused somatic action potential initiation to advance in time (move forward up to 180°). The exact pattern of phase advancement is dependent on the dendritic location of input, with more distal input causing a more gradual temporal shift in spike initiation and a smaller increase in spike number. Patterned stimulation of Schaffer collateral/perforant path synaptic input can produce phase precession that is very similar to that observed with sine wave current injections. Finally, the exact amount of synaptic input required to produce phase advancement was found to be regulated by dendritic voltage-gated ion channels. Together, these data demonstrate that the summation of primarily proximal inhibition with an increasing amount of out-of-phase, primarily distal excitation can result in a form of phase advancement similar to that seen during theta activity in the intact hippocampus.

INTRODUCTION

During exploratory locomotion and REM sleep in rats, the hippocampus exhibits rhythmic oscillatory field potentials at frequencies ranging from 4 to 10 Hz (theta frequency) (Kamondi et al. 1998; Lisman 1999; Vanderwolf 1969; Winson 1974). The synchronization of hippocampal neurons during theta activity could serve as a reference to the information encoded by hippocampal place cells and, therefore, could be fundamental for some operations of the hippocampus (Buzsaki and Chrobak 1995; Jensen and Lisman 1996; Kamondi et al. 1998; Lisman 1999; Skaggs et al. 1996; Tsodyks et al. 1996). Hippocampal place cells are neurons that increase their firing rates when the animal is in a specific part of the environment (the place field) (O'Keefe and Dostrovsky 1971), with sequentially occurring spikes gradually shifting to earlier phases of the theta cycle as the rat approaches and passes through the cell's place field (this is known as phase precession or phase advance) (O'Keefe and Recce 1993; Skaggs et al. 1996). In this way, the phase relationship of the spike to the theta cycle is a good predictor of the rat's position in space (Jensen and Lisman 2000; Tsodyks et al. 1996).

It has been proposed that the distal dendritic regions of CA1 pyramidal neurons may receive rhythmic synaptic excitation during theta oscillations whereas the somatic regions may receive synchronous hyperpolarizing input from interneuron networks (Chapman and Lacaille 1999a,b; Cobb et al. 1995; Kamondi et al. 1998; Kocsis et al. 1999; Leung 1986; Winson 1974). In this scheme, action potential firing induced by excitatory input is phased by rhythmic inhibitory input. As might be expected with increased excitatory input, as the rat moves toward the center of the place field, somatic inhibition is progressively overcome and the neuron fires action potentials earlier in the theta phase. Computer simulations have shown that such events can result in phase advancement of neuronal output in models (Kamondi et al. 1998). The present study further examines the potential mechanisms of phase precession by using simultaneous whole-cell patch clamp recordings both from the soma and from the dendrites of CA1 pyramidal neurons located in hippocampal slices of adult rats.

METHODS

Preparation

Hippocampal slices (400 μm) were prepared from 6–12-wk-old Sprague-Dawley rats using standard procedures that were previously described (Magee 1998). Individual neurons were visualized with a Zeiss Axioskop fit with differential interference contrast optics that use infrared illumination. All neurons exhibited resting membrane potentials between -60 and -75 mV. Area CA3 was surgically removed from each slice just prior to use.

Recordings and solutions

Whole-cell patch-clamp recordings were made using two Dagan BVC-700 amplifiers (Minneapolis, MN) in active "bridge" mode. Data were acquired (10–20 kHz, filtered at 1 kHz) using an Instrutech ITC-16 interface (Great Neck, NY) and Pulse Control software (Richard Bookman, University of Miami) written for Igor Pro (Wavemetrics, Lake Oswego, OR). External solutions contained (in mM) 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 2.0 $CaCl_2$, 1.0 $MgCl_2$, and 25 dextrose. Solutions were bubbled with 95% O_2-5% CO_2 at ~36°C (pH 7.4) for all recordings. Whole-cell recording pipettes (somatic, 2–4 MΩ; dendritic, 5–7 MΩ) were pulled from borosilicate glass. The internal pipette solutions contained (in mM) 120 $KMeSO_4$, 20 KCl, 10 HEPES, 0.1 EGTA, 4.0 Mg_2 ATP, 0.3 Tris guanosine 5′-triphosphate, 14 phosphocreatine, and 4 NaCl (pH 7.25 with

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KOH). Series resistance for somatic recordings was 6–20 MΩ whereas that for dendritic recordings was 15–40 MΩ. Voltages were not corrected for theoretical liquid junction potentials (6–7 mV). For all dendritic sine waves, a positive DC current injection, which was ~75% of sine wave amplitude, was also injected into the dendrite. This functioned to decrease the hyperpolarization induced by the negative portion of the sine wave, thus making the sine wave stimulation more accurately mimic excitatory input (see Fig. 1*B*). To stimulate excitatory synaptic input, tungsten bipolar electrodes (final tip size <1 μm) were placed at the border of the stratum radiatum and the stratum lacunosum moleculare within ~20 μm of the dendrite under study. During these recordings, 10 μm bicuculline methiodide was added to the external solution. ZD7288 was bath applied at a concentration (30 μM) that should block ~75% of the available hyperpolarization-activated (I_H) channels (Magee 1999b).

Analysis

Phase advance was calculated as the average time between the action potential peak (of the first spike if more than one was initiated) and the depolarizing peak of the somatic sine wave given alone. The average phase advance for each cycle in the 2-s stimulation period was calculated. The number of spikes evoked per condition was the average number of action potentials in each cycle of the 2-s stimulation period. Plots either of spike advance or number versus current were arbitrarily fitted by a Boltzmann equation in which $I_{1/2}$ equaled the amount of current required for a half-maximal effect.

RESULTS

To mimic repetitive proximal inhibition, initially hyperpolarizing 5-Hz sine wave current injections (100–200 pA) were made in the soma of CA1 pyramidal neurons (Fig. 1*A*). The associated depolarizing phase of these current injections led to action potential initiation at the peak of the depolarizing wave. To test the ability of dendritic depolarizations to shift the temporal location of the action potential (with respect to the somatic sine wave), 5-Hz depolarizing current sine waves were simultaneously injected into the apical dendritic arborization via a whole-cell pipette located at least 250 μm from the soma. These depolarizing currents were 180° out of phase with the somatic sine waves, to mimic the cyclic arrival of excitatory input to the distal regions of the stratum radiatum. Figures 1 and 2 show that increasing the amplitude of the dendritic 5-Hz sine wave current causes somatic action potential initiation to advance up to 155 ± 17 ms in time ($n = 6$). The number of action potentials initiated also increased with the increase in dendritic current injection (from 1 to 3.3 ± 0.6, $n = 6$). In all cells, maximal spike advancement was seen for dendritic current injections of 600–700 pA, with additional current injections (800–900 pA) being capable of only slightly shifting the temporal location of the spike any further (Fig. 2, *A* and *C*). These data indicate that increasing levels of dendritic depolarization can cause significant phase advancement of action potential output as well as a three- to fourfold increase in the number of spikes.

Although the level of advancement of the first spike was relatively constant throughout the 2-s current injection period, the number of spikes generated during the larger-amplitude current injections (>500 pA) decreased with time (from 4.9 ± 0.4 spikes in the first cycle to 3.3 ± 0.6 spikes in the last cycle, $n = 6$) (Fig. 1). This decrease in firing rate is likely the result of the well-known spike accommodation properties of CA1 pyramidal neurons (Storm 1990) and would function to improve the advancement of peak firing rate. Dendritic spike amplitude also decreased during the 2-s current injection period at a rate that was dependent on the frequency of action potential output (Fig. 1*C*). This is likely to be the result of slow inactivation of dendritic Na^+ channels and has also been well described (Colbert et al. 1997; Magee 1999a).

The pattern of phase advancement appears to be dependent on the dendritic location of the input. Figure 2 shows that although the total amount of phase advancement (145 ± 11 ms, $n = 4$) is maintained for more proximal input (soma to <200 μm distal), the initial phase of the advancement is much more abrupt when compared with distal input (Fig. 2*B*). The injection of small amounts of proximal dendritic current (<200 pA) causes an initial jump in the temporal location of the spikes that is not present with distal input. Additional current injection (>200 pA) results in a further, more gradual advance that is similar to that seen for distal input. Another location-dependent difference was that the increase in the number of spikes fired was elevated (6.7 ± 0.8, $n = 4$) for proximal input (Fig. 2*D*). Therefore, distal input causes a more gradual temporal shift in spike initiation (shifts <70–80 ms), with a smaller increase in

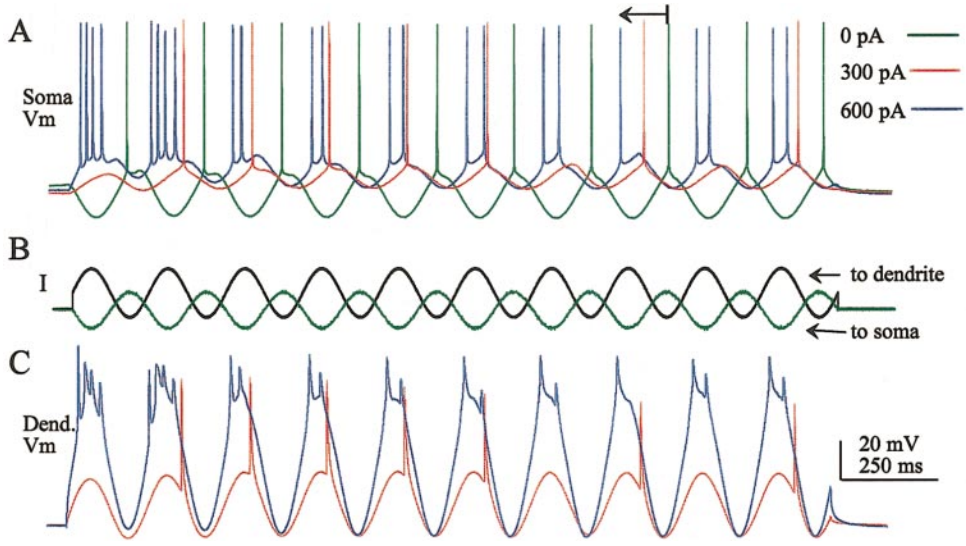

FIG. 1. Phase precession in CA1 pyramidal neurons. *A, green trace*: somatic membrane potential in response to a 5-Hz sine wave current injection to the soma alone (200 pA). *Red trace*: somatic membrane potential in response to a somatic sine plus 180° out-of-phase sine wave current injection to dendrite (~300 μm; 300 pA). *Blue trace*: somatic sine plus a larger-amplitude 180° out-of-phase sine wave current injection to dendrite (600 pA). Dendritic current amplitudes are shown to the right of the traces. *B*: current waveforms for somatic (*green trace*) and dendritic (*black trace*) injections. Both current waveforms were simultaneously injected during the *red trace* in *A*. *C*: dendritic membrane potential during current injections for the like-colored traces in *A*. *A*, arrow: phase advancement of spike initiation for increasing-amplitude dendritic currents.

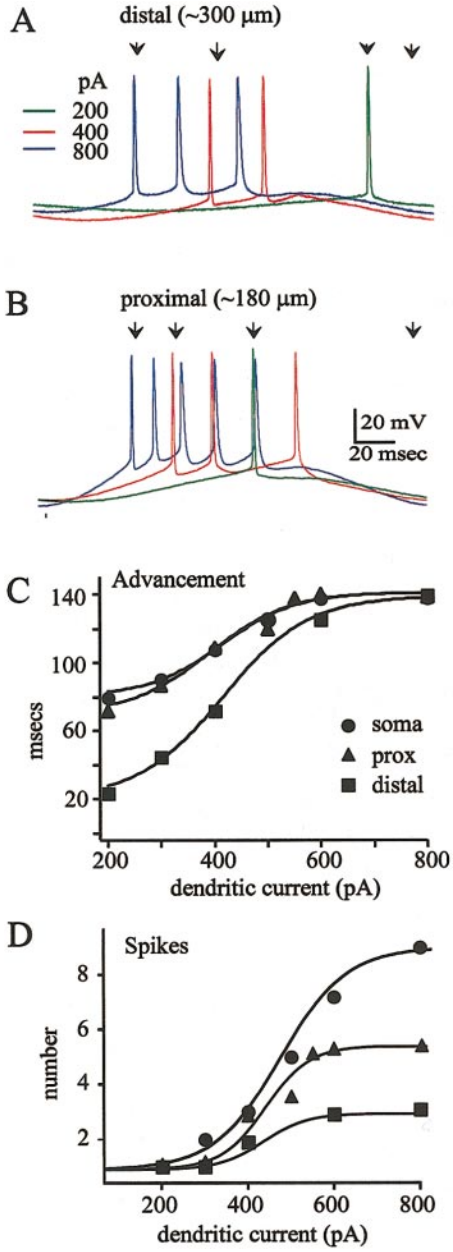

FIG. 2. Increasing dendritic current amplitude causes action potential initiation to progressively advance with respect to the phase of the somatic sine wave. *A*: somatic membrane potential during simultaneous somatic and distal dendritic 5-Hz sine wave current injections of different amplitude. Differently colored traces correspond to different levels of dendritic current injection; the corresponding amplitudes are listed to the left of the traces. The dendritic current injection site was ~300 μm from the soma. *B*: somatic membrane potential as the amplitude of a proximal dendritic (~170 μm) sine wave is increased. *A* and *B*, arrows: location of the first spike initiated for each dendritic current amplitude; rightmost arrows: time of action potential initiation during soma-only current injection. *C*: plot of phase advancement as a function of dendritic current amplitude (●, soma; ■, distal; ▲, proximal). Phase advancement is defined as the time difference between action potentials induced by a sine wave current injection to the soma alone and those induced by combined dendritic and somatic sine waves. *D*: plot of the number of spikes induced by sine wave injections as a function of dendritic current amplitude (●, soma; ■, distal; ▲, proximal). For the soma-only injections in *C* and *D* (●), a waveform that was a combination of the standard somatic waveform and a dendritic current waveform (180° advanced relative to the somatic waveform) was delivered to the soma only.

the number of spikes initiated when compared with more proximal input. The reason for this more gradual shift appears to be that dendritic filtering causes a temporal shift or delay in the peak of the propagated depolarization, which allows for a more even summation with somatic current injection.

To determine if actual synaptic input could produce similar phase advancement, a mixture of distal Schaffer collateral and proximal perforant path synaptic input was evoked by electrical stimulation at the border of the stratum radiatum and stratum moleculare. A patterned synaptic stimulation (5 excitatory postsynaptic potentials at 100 Hz given every 200 ms for 1 s) was simultaneously paired with a 5-Hz hyperpolarizing sine wave current injection to the soma (Fig. 3). Increasing the level of electrical stimulation resulted in a phase precession that is very similar to that observed with the simple dendritic

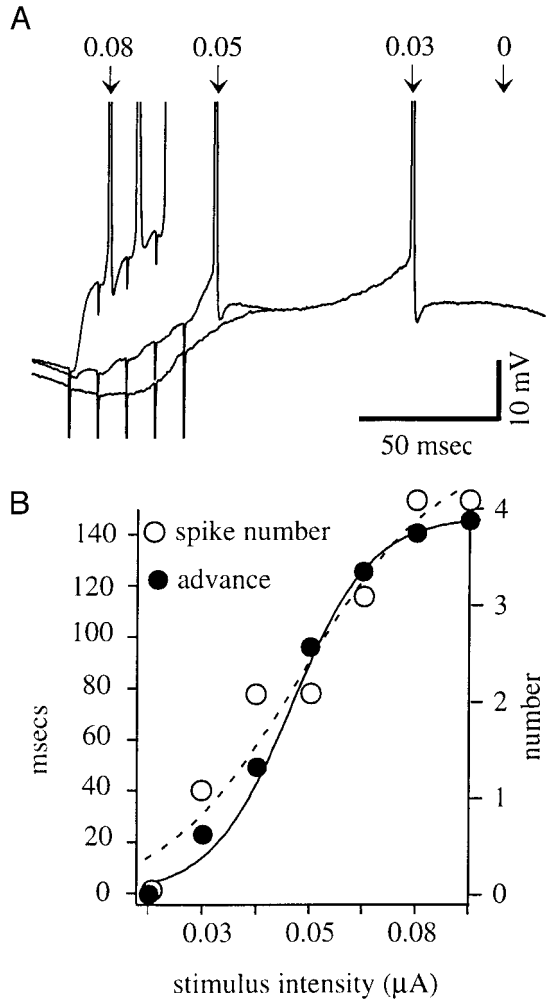

FIG. 3. Increasing synaptic input amplitude causes action potential initiation to progressively advance with respect to the phase of the somatic sine wave. *A*: somatic membrane potential during simultaneous injection of a 5-Hz sine wave to the soma and patterned 100-Hz stimulation of the Schaffer collateral and perforant pathways. *A*, arrows: location of the first spike initiated for the different stimulus amplitudes. Traces are truncated shortly after spike initiation for clarity. *B*: plot of time difference between the action potential induced by sine wave current injection to the soma alone (0 pA) and the action potential induced by combined somatic sine wave and synaptic input as a function of stimulus amplitude (advance). Also plotted is the number of spikes induced by combined somatic sine wave and synaptic input as a function of stimulus amplitude (spike number). 0 pA was somatic sine only. Stimulation currents ranged from 0.03 to 0.1 μA.

sine wave current injections. In this case, the temporal location of action potential initiation gradually advanced to 149 ± 6 msec ($n = 5$) and the number of spikes increased from 1 to 4.1 ± 0.7 ($n = 5$) over the range of stimulation intensities. Therefore, patterned distal synaptic stimulation can produce phase advancement similar to that observed with dual current injections and in vivo.

Finally, the ability of dendritic voltage-gated ion channels to regulate phase precession was examined. The H channel was chosen as a model dendritic channel population because of its primarily dendritic distribution, its slower activation/deactivation kinetics, and its susceptibility to relevant channel modulation (Magee 1998; Pape 1996). Application of a moderate concentration of the H-channel blocker ZD7288 (30 μM) caused a shift to lower current values in the current versus spike advance plot ($I_{1/2} = 345 \pm 9$ vs. 264 ± 8 pA for control and ZD7288, respectively; $n = 4$) while causing only a slight increase in the maximum amount of advancement (155 ± 16 vs. 168 ± 10 ms for control and ZD7288, respectively; $n = 4$) (Fig. 4). Therefore, blocking a moderate proportion of the H-channel population can substantially reduce the amount of dendritic input required to advance spike initiation. This indicates that the exact amount of synaptic input required to produce phase precession in CA1 pyramidal neurons is regulated by dendritic voltage-gated ion channels (H channels in particular).

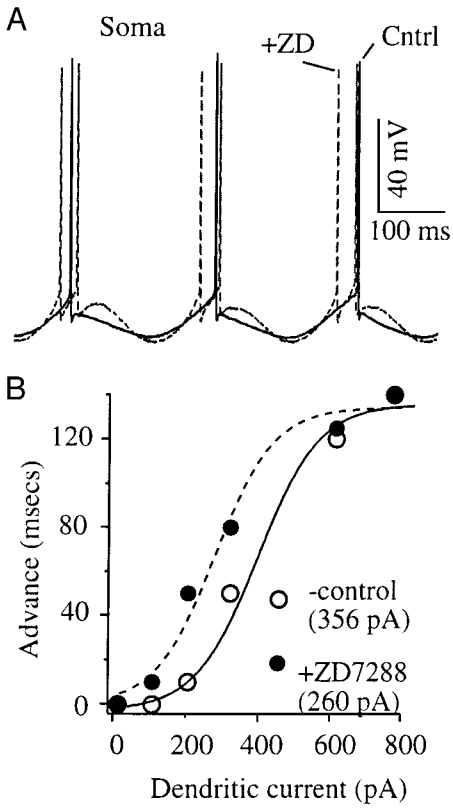

FIG. 4. H-channel blockade reduces the dendritic current required for phase advance. *A*: somatic membrane potential in response to dendritic (300 pA) and somatic (−100 pA) sine wave current injections under control conditions (solid line) and in the presence of 30 μM ZD7288 (dashed line). *B*: plot of spike advance as a function of dendritic current amplitude for control conditions (solid line) and with 30 μM ZD7288 (dashed line).

DISCUSSION

These experiments show that, in CA1 pyramidal neurons receiving out-of-phase sine wave current injections to the soma and apical dendrites, phase precession can occur by increasing the amplitude of a depolarizing dendritic input. Furthermore, patterned distal excitatory synaptic stimuli were able to mimic the spike advancement induced by dendritic sine wave current injections. The phase precession was highly reminiscent of that observed in vivo and in various computational models where progressive spike advancement and an increase in the number of action potentials is usually observed (Fenton and Muller 1998; Kamondi et al. 1998; O'Keefe and Recce 1993; Skaggs et al. 1996; Tsodyks et al. 1996). During theta oscillations, CA1 pyramidal neurons have been reported to receive strong hyperpolarizing inhibitory input to the soma and proximal dendritic regions that is synchronized with a depolarizing excitatory input located in the more distal regions of the apical dendrite (Chapman and Lacaille 1999a,b; Cobb et al. 1995; Kamondi et al. 1998; Kocsis et al. 1999; Leung 1986; Winson 1974). The data presented here suggest that the phase advancement observed in hippocampal CA1 place cells can occur as the level of rhythmic synaptic excitation increases with movement of the rat toward and through the cell's place field (Kamondi et al. 1998).

The particulars of phase precession (the progressiveness of spike advancement and the number of spikes initiated) appear to depend on the spatial pattern of the input. In the present study, increased proximal excitatory input resulted in more abrupt spike advancement as well as a larger increase in the number of action potentials initiated, when compared with distal input. This suggests that a combination of proximal and distal excitatory input (similar to the synaptic stimulation used in Fig. 3) would most closely approximate the intact situation. In this case, kinetically filtered distal synaptic activity would provide a gradual spike advance whereas more proximal input could supply additional, less-filtered current for a more dramatic increase in the number of spikes fired.

Finally, we observed that the exact amount of synaptic excitation required for spike advancement was under the control of dendritic voltage-gated ion channels. Although the contribution of H channels to this phenomenon was demonstrated, other channel types may be involved as well. Dendritic A-type K^+ channels and various Ca^{2+} channel subtypes are examples of other channels that could easily affect the level of synaptic input required for phase precession (Magee 1999a). The activity of all of these channels is highly regulated by common neuromodulators, including acetylcholine (Magee 1999a; Pape 1996). A reduction of I_H activity (via adenylate cyclase inhibition) could be one way in which muscarinic input is able to modify hippocampal theta activity. In summary, out-of-phase proximal and distal theta oscillations could produce phase precession in CA1 pyramidal neurons, with the exact level of synaptic activity required being under the regulation of dendritic voltage-gated ion channels.

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