Subthreshold Inactivation of Na\textsuperscript{+} and K\textsuperscript{+} Channels Supports Activity-Dependent Enhancement of Back-Propagating Action Potentials in Hippocampal CA1

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Pan, Enhui and Costa M. Colbert. Subthreshold inactivation of Na\textsuperscript{+} and K\textsuperscript{+} channels supports activity-dependent enhancement of back-propagating action potentials in hippocampal CA1. J Neurophysiol 85: 1013–1016, 2001. Back-propagating action potentials in CA1 pyramidal neurons may provide the postsynaptic dendritic depolarization necessary for the induction of long-term synaptic plasticity. The amplitudes of back-propagating action potentials are not all or none but are limited in amplitude by dendritic A-type K\textsuperscript{+} channels. Previous studies of back-propagating action potentials have suggested that prior depolarization of the dendritic membrane reduces A-type channel availability through inactivation, resulting in an enhanced, or boosted, dendritic action potential. However, inactivation kinetics in the subthreshold potential range have not been directly measured. Furthermore, the corresponding rates of Na\textsuperscript{+} channel inactivation with depolarization have not been considered. Here we report in cell-attached patches (150–220 μm from the soma, 32°C) that at 20-mV positive to rest, A-type K\textsuperscript{+} channels inactivated with a single exponential time constant of 6 ms, whereas Na\textsuperscript{+} channels inactivated with a time constant of 37 ms. The ratio of available Na\textsuperscript{+} to K\textsuperscript{+} current increased as the duration of the depolarization increased. Thus the subthreshold properties of Na\textsuperscript{+} and A-type K\textsuperscript{+} channels provide a mechanism by which information about the level of synaptic activity may be encoded in the amplitude of back-propagating action potentials.

INTRODUCTION

Back-propagating action potentials in CA1 pyramidal neurons may provide the postsynaptic dendritic depolarization necessary for the induction of associative long-term synaptic plasticity (Hoffman et al. 1997; Magee and Johnston 1997). Unlike somatic or axonal action potentials, back-propagating dendritic action potentials are not all-or-none but are highly variable in amplitude (Colbert et al. 1997; Spruston et al. 1995). This variable amplitude is determined in large part by a high density of dendritic A-type K\textsuperscript{+} (Kv4.2) channels (Colbert and Pan 1999; Hoffman et al. 1997), which are the targets of various neuromodulatory systems (Hoffman and Johnston 1999), and subject to voltage-dependent inactivation (Hoffman and Johnston 1999; Hoffman et al. 1997).

Depolarization of the dendritic membrane by prior synaptic activity can enhance the amplitude of back-propagating dendritic action potentials (Hoffman et al. 1997) (Fig. 1), which increases the likelihood of Ca\textsuperscript{2+} entry through voltage-gated and N-methyl-D-aspartate channels (Hoffman et al. 1997; Magee and Johnston 1997). Thus enhancement of the back-propagating action potential may contribute to the associative nature and the specific timing requirements for the induction of long-term synaptic modification (Bi and Poo 1998; Debanne et al. 1998; Hashemzadeh-Gargari et al. 1991; Levy and Steward 1983; Markram et al. 1997). Because of the important role of A-type K\textsuperscript{+} channels in shaping the dendritic action potential, it has been hypothesized that rapid inactivation of these channels during depolarization underlies the enhancement of dendritic action potential amplitude (Hoffman et al. 1997; Migliore et al. 1999). Certain aspects of this putative mechanism, however, have not been directly investigated. First, the rate of subthreshold Na\textsuperscript{+} channel inactivation has not been determined. Second, A-type K\textsuperscript{+} channels inactivate rapidly in the supra-threshold range (Hoffman et al. 1997), but subthreshold inactivation rates have only been extrapolated. Finally, the various kinetics have not been measured at temperatures above room temperature. Thus to test whether inactivations of Na\textsuperscript{+} and K\textsuperscript{+} channels are consistent with enhancement of back-propagating action potentials, we recorded currents in cell-attached patches from dendrites of CA1 pyramidal neurons. We found that both the rates and degree of inactivation with near-threshold synaptic depolarization led to an increase in the ratio of inward to outward currents consistent with an enhancement of action potential amplitude.

METHODS

Slice methods are essentially those described in Colbert and Pan (1999). Briefly, the experiments used 4- to 6-wk-old male Sprague-Dawley rats. Animals were deeply anesthetized with a combination of ketamine and xylazine and perfused through the heart with cold artificial cerebrospinal fluid (ACSF) containing (in mM) 110 sucrose, 60 NaCl, 3.0 KCl, 1.25 NaH\textsubscript{2}PO\textsubscript{4}, 28 NaHCO\textsubscript{3}, 0.5 CaCl\textsubscript{2}, 7.0 MgCl\textsubscript{2}, and 5 dextrose. Slices 400-μm thick were cut using a Vibratome (Lancer).

The external ACSF contained (in mM) 125 NaCl, 2.5 KCl, 1.25 NaH\textsubscript{2}PO\textsubscript{4}, 25 NaHCO\textsubscript{3}, 2.0 CaCl\textsubscript{2}, 1.0 MgCl\textsubscript{2}, and 25 dextrose. Slices were maintained submerged in normal ACSF bubbled continuously with 95% O\textsubscript{2}-5% CO\textsubscript{2}. The pipette solution used for whole cell
recordings contained (in mM) 140 K-Gluconate, 10 HEPES, 1 EGTA, 4.0 NaCl, 4.0 Mg\textsubscript{2}ATP, 0.3 Mg\textsubscript{2}GTP, and 14 phosphocreatine; pH was 7.25. The pipette solution for cell-attached patch recordings of Na\textsubscript{1} currents contained (in mM) 110 NaCl, 20 tetraethylammonium (TEA) chloride, 10 HEPES, 2 CaCl\textsubscript{2}, 3 KCl, 1 MgCl\textsubscript{2}, and 10–12 4-aminopyridine (4-AP). pH was adjusted to 7.4 with NaOH. For cell-attached recordings of Na\textsubscript{1} and A-type K\textsubscript{1} currents, 4-AP was replaced by NaCl (5 mM). For cell-attached recordings of A-type currents alone, 4-AP was replaced by NaCl (5 mM) and tetrodotoxin (TTX, 1 m\textsubscript{M}) was added.

Dendrites of CA1 pyramidal neurons were visualized using infra-red-illuminated, differential interference contrast optics (BX50WI, Olympus) and a newvicon camera (DAGE-MTI) according to standard techniques (Stuart et al. 1993). Whole cell patch-clamp recordings in the apical dendrites were made using an intracellular amplifier (BVC-700, Dagan). Cell-attached patch recordings were made using a patch-clamp amplifier (Axopatch 200, Axon Instruments). Pipettes (5–7 M\text{\Omega} for whole cell, 7–10 M\text{\Omega} for cell-attached) were made from EN-1 glass (Garner) and pulled using a P-97 Flaming-Brown pipette puller (Sutter Instruments). All recordings were made at 32 ± 0.5°C, mean ± SD. Membrane potentials were determined by rupturing patches at the end of the recording sessions and were about −70 mV, consistent with other studies of CA1 dendrites (Hoffman et al. 1997; Magee 1998). However, data were most often collected until loss of the patch, precluding an estimate of membrane potential. Thus command potentials are reported here as depolarization from rest. Conditioning pulse durations were interleaved to minimize any effects of systematic changes in potential during an experiment. Ensemble waveforms were constructed from 15–25 individual sweeps. Whole cell recordings were low-pass filtered at 3 kHz (6 dB/octave) and digitized at 10 kHz. Cell-attached patch recordings were filtered at 2 kHz (8-pole Bessel filter) and sampled at 10 kHz. Data were digitized at 16-bit resolution (ITC18, Instutech) and stored by computer (Intel) for off-line analysis. Excitatory postsynaptic potentials (EPSPs) and antidromic action potentials were evoked by constant current pulses [0.1 ms, 30–100 \mu A (Neurolog, Digitimer)] through tungsten electrodes (AM Systems) placed in stratum radiatum and the alveus, respectively. Exponential curve fits were made with DISCRETE (Provencher 1976). Summary data are reported as means ± SE.

FIG. 1. Prior depolarization boosts back-propagating action potential amplitude. Waveforms are membrane potential (V\textsub{m}) recorded in the apical dendrite 180 μm from the soma. All waveforms are at the same scale. Action potentials were evoked antidromically by a stimulating electrode in the alveus. A: back-propagating action potentials alone (Anti) or paired with a train of excitatory postsynaptic potentials (EPSPs, Paired + Anti). B: superimposed consecutive sweeps (0.02 Hz), each with a single antidromic action potential as in Paired Anti in A. In each successive sweep, the latency of the antidromic action potential has been increased by 20 ms. C: rates of rise (dV/dt) of paired action potentials in B. Note that each waveform is nominally centered under the corresponding action potentials in B. Note also that each waveform is nominally centered under the corresponding action potential in B, but the scale bars are different. Note that the amplitude and peak rate of rise of the action potential increases with duration of prior depolarization.

recording time. Each sweep contained 1,638,400 sample points at 10 kHz. Data were digitized at 16-bit resolution (ITC18) and stored by computer (Intel) for off-line analysis. Excitatory postsynaptic potentials (EPSPs) and antidromic action potentials were evoked by constant current pulses [0.1 ms, 30–100 \mu A (Neurolog, Digitimer)] through tungsten electrodes (AM Systems) placed in stratum radiatum and the alveus, respectively. Exponential curve fits were made with DISCRETE (Provencher 1976). Summary data are reported as means ± SE.

FIG. 2. Subthreshold depolarizations rapidly inactivate A-type K\textsubscript{1} currents. A: voltage commands used to determine inactivation. Cell-attached patches were held at rest (about −70 mV), stepped to a conditioning potential 20-mV positive to rest for 0–20 ms then stepped to 60-mV positive to rest to evoke currents. B: ensemble averages of 15 consecutive sweeps in a patch with isolated A-type K\textsubscript{1} current. Times indicated are duration of conditioning step. C: ensemble averages of 20 consecutive sweeps in a patch with Na\textsubscript{1} and A-type K\textsubscript{1} currents. D: summary data for patches as in C. Currents are normalized to the 0-ms conditioning duration. Numbers in parentheses are the number of patches tested at each duration. Note that the K\textsubscript{1} current inactivates considerably over the short duration of 20 ms.
where both currents were present, A-type K⁺ channels inactivated with a single exponential time constant of 6 ms (Fig. 2D). After 20 ms of conditioning, only 29 ± 5% (n = 7) of the current at rest remained available. In the same patches after 20 ms of conditioning, 75 ± 12% (n = 7) of Na⁺ current at rest remained available. In some patches, a potential 10 mV above rest was also tested. The A-type K⁺ current inactivated with a time constant of 5 ms to a value of 44 ± 5% (n = 3) of its resting value.

To further characterize the time course of inactivation of Na⁺ current, we recorded Na⁺ currents in additional patches using longer durations of conditioning depolarization (40–400 ms, Fig. 3A). Voltage commands to 20-mV positive to rest evoked currents as shown by representative ensemble averages (Fig. 3B) and by summary data (Fig. 3C). Na⁺ current inactivated with a rate of 37 ms (single-exponential time constant). At 440 ms, 50 ± 9% (n = 5) of the Na⁺ current at rest remained available.

**DISCUSSION**

In the present study, we have investigated subthreshold inactivation of ion channels underlying the back-propagating action potential. Given the surprising result that the K⁺ channels inactivated so much faster than the Na⁺ channels, the key experiment here was to compare Na⁺ and K⁺ channel inactivation in the same patches directly. Thus any errors in our estimates of membrane potential, temperature, or differences in pipette solutions can be ruled out.

Previously inactivation rates for A-type K⁺ channels were estimated by evoking currents and directly measuring the rate at which the current decreased (Hoffman et al. 1997). Such measurements were necessarily limited to supra-threshold potentials (at least −25 mV), because the currents evoked at subthreshold potentials were too small to measure accurately. From the observation that the rate of inactivation of A-type K⁺ channels was faster with less depolarization, it was concluded that subthreshold inactivation should proceed rapidly. The present results provide direct support for this idea.

The very slow rate of Na⁺ channel inactivation was somewhat of a surprise. In an earlier study of CA1 dendrites using depolarizations to −40 mV to evoke currents, Magee and Johnston (1995) found that Na⁺ current inactivated with two distinct rate constants. The fast exponential rate constant was in the 1- to 4-ms range, the value also reported by Sah et al. (1988). The slower time constant, however, was similar to that seen in the present study (30–50 ms). Thus entry into the inactivated state may take one of two distinct pathways. The modest depolarizations used in the present study (which evoked little current) might favor inactivation by the slower pathway resulting in a large fraction of the resting current being available for up to 100 ms after depolarization begins.

Back-propagating action potentials in the dendrites of CA1 pyramidal neurons have been hypothesized to be a rapid signal to the synapses that the postsynaptic cell has fired (Hoffman et al. 1997; Magee and Johnston 1997). Such a mechanism would seem to mirror long range signaling in the axon. However, the amplitude of dendritic action potentials is highly variable, allowing additional information, such as modulation by neurotransmitter systems (Hoffman and Johnston 1999) or the inactivation studied here, to be integrated into the signal. One
well-established property of long-term synaptic plasticity in many systems is that induction has specific timing requirements (Bi and Poo 1998; Hashemzadeh-Gargari et al. 1991; Levy and Steward 1979, 1983; Markram et al. 1997). In particular, synaptic activity following the strong input (i.e., that responsible for the postsynaptic depolarization) does not induce potentiation. Such restricted timing seems more consistent with the brief, strong depolarization of an action potential than with the more prolonged depolarization associated with strong excitatory synaptic input. Consistent with this idea, both back-propagating dendritic action potentials and activation of NMDA receptors were required for induction in a study of long-term potentiation (Magee and Johnston 1997). If, under physiological conditions, a back-propagating action potential is necessary to relieve the Mg$^{2+}$ block of the NMDA receptor (Nowak et al. 1984), then it is critical to maintain action potential amplitude in the face of dendritic depolarization. Furthermore if a back-propagating action potential with enhanced amplitude is necessary to activate NMDA receptors, then synaptic modification will only progress if an adequate number of synapses are activated in the period before an action potential is initiated.

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