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ION CHANNELS

Voltage-gated ion channels in dendrites of hippocampal pyramidal neurons

Xixi Chen · Daniel Johnston

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Abstract The properties and distribution of voltage-gated ion channels contribute to electrical signaling in neuronal dendrites. The apical dendrites of CA1 pyramidal neurons in hippocampus express a wide variety of sodium, calcium, potassium, and other voltage-gated channels. In this report, we provide some new evidence for the role of the delayed-rectifier K^+ channel in shaping the dendritic action potential at different membrane potentials.

Keywords Dendrites · Hippocampus · Back-propagating action potential · Ion channels · Potassium channels · Patch-clamp

Introduction

Neuronal dendrites coordinate and blend input from thousands of excitatory and inhibitory synapses distributed over their surface. They also dynamically regulate the strengths of those connections. For many years, it was assumed that dendrites were electrically passive and that the integration of synaptic input could be described adequately by a combination of linear cable theory and a morphometric description of dendritic geometry [see 18]. With the development of techniques for recording directly from dendrites using patch-clamp electrodes and high-speed fluorescence imaging, however, it is now clear that dendrites express a wide variety of voltage-gated ion channels [10, 15, 21] and that the processes of dendritic integration and synaptic plasticity are far more complicated than originally believed.

Action potentials were first recorded in dendrites of hippocampal pyramidal neurons by Wong et al. [23] using sharp microelectrodes. Regehr et al. [17] and Jaffe et al. [8] described changes in Ca^{2+} and Na^+ concentrations in CA1 dendrites after electrical stimulation using fluorescence imaging and the intracellular injection of Ca^{2+} and Na^+ indicator dyes. Such changes in ion concentration were thought to reflect the active propagation of action potentials and thus the presence of Ca^{2+} and Na^+ channels in dendrites. Spruston et al. [19] were the first to use whole-cell patch recordings from CA1 dendrites to show that action potentials back-propagate from the soma into the dendrites in a similar fashion to that described by Stuart et al. [20] in layer 5 neurons in the neocortex. Magee and Johnston [12, 13], using cell-attached patch recordings, measured the properties and distribution of single Na^+ channels and a variety of Ca^{2+} channels along the dendrites of CA1 neurons. These and other studies (reviewed in [6]) helped provide a new framework for studies aimed at exploring the functional properties of dendrites.

K^+ channels are perhaps the most molecularly and genetically diverse of all the voltage-gated ion channels expressed in the nervous system [9]. In hippocampal CA1 neurons, Hoffman et al. [7] described a surprising distribution of a fast, transient K^+ channel in the dendrites of CA1 neurons. While sustained or delayed-rectifier-type K^+ channels appeared to be uniformly distributed, at least at the functional level (cf. [16]), the density of a transient, A-type K^+ channel increased with distance from the soma. A structurally similar channel, the h-channel, was later shown by Magge [14] and Lorincz et al. [11] to also be expressed at a high density in the distal dendrites of these neurons. The molecular mechanisms for the dendritic

X. Chen (✉) · D. Johnston
Center for Learning and Memory,
University of Texas at Austin,
Austin, TX 78712, USA
e-mail: xixi@mail.clm.utexas.edu

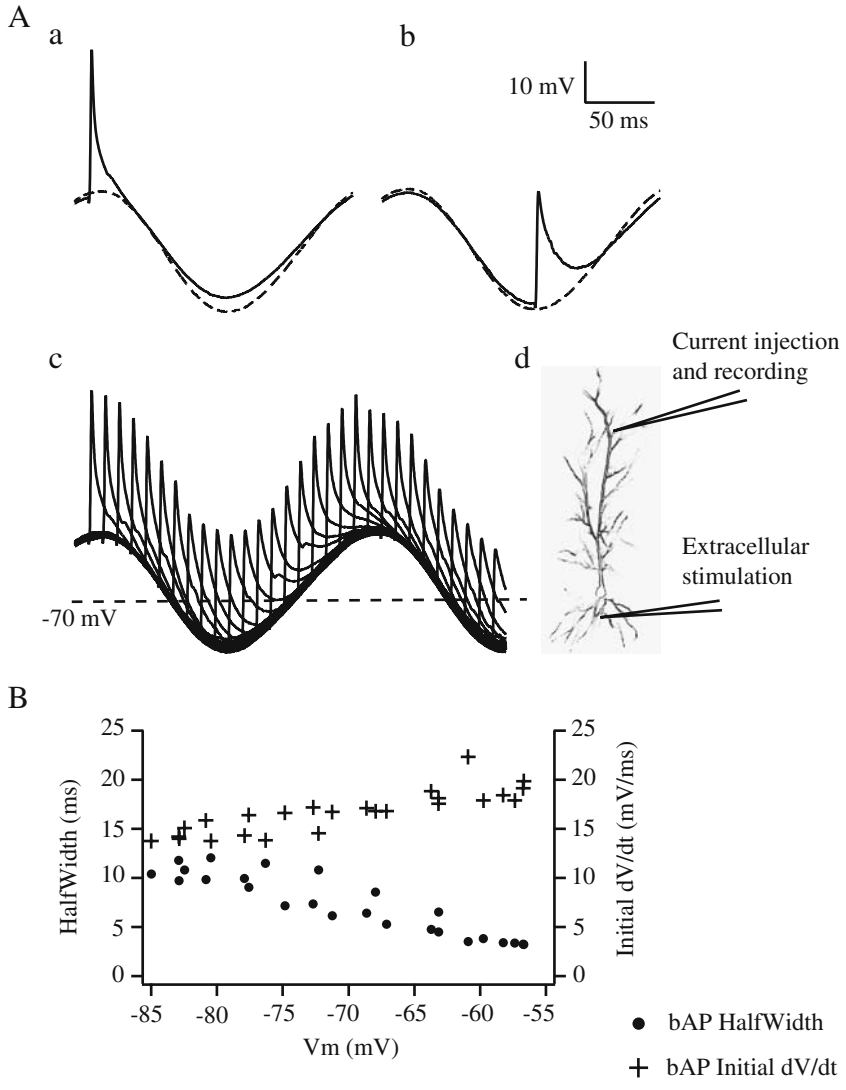

Fig. 1 Theta oscillation affects bAP waveform. Recordings were performed in CA1 dendrites of CD-1 mouse, at a distance of 240 μm from soma. **a** Examples of single bAPs at the peak (*a*) or trough (*b*) of membrane potential oscillation. *Dotted lines* represent only sine-wave current injection, without bAP. *c* Overlay of 35 bAPs like those in (*a*) and (*b*), coinciding with different phases of membrane potential oscillation. *d* Recording configuration, showing a picture of reconstructed CA1 pyramidal neuron and positions of electrodes. **b** bAP half-width and initial d*V*/d*t* were plotted against membrane potentials. Measurements were from the traces in (**a**)

expression of these channels are still poorly understood, although Ca^{2+}–calmodulin-dependent protein kinase II enhances expression of at least one type of dendritic K^+ channel [22].

Chen and Johnston [3] used single-channel recordings from dendrites to identify four biophysically distinct types of depolarization-activated and one inward rectifier K^+ channel [4] in CA1 neurons. The depolarization-activated K^+ channels described in those studies consisted of both a fast and a slowly inactivating channel, a delayed-rectifier channel, and a noninactivating, muscarinic acetylcholine sensitive channel. The hyperpolarization-activated channels were shown to be activated by G-protein coupled receptors and are called GIRK channels.

Being a prominent K^+ conductance preferentially located in the dendrites, the fast, transient A-type K^+ channels contribute to the decrement of the amplitudes of action potentials as they back-propagate into the dendrites. Other than the A-type K^+ channel, the most frequently recorded K^+ channel in CA1 dendrites was a delayed-rectifier-type channel. The present report provides evidence for a role of the delayed-rectifier K^+ channel in regulating the waveforms of back-propagating action potentials (bAPs) in the dendrites of CA1 neurons.

Materials and methods

Slice preparation

Acute hippocampal slices (350 or 400 μm in thickness) were prepared from 6- to 9-week-old male Sprague–Dawley rats or CD-1 mice. Animals were anesthetized with a lethal dose of a combination of ketamine and xylazine, perfused through the heart with ice-cold (4°C) cutting solution (described below), and decapitated. The brain was then removed, dissected, and sliced using a Vibratome. The slices were transferred to a submerged holding chamber containing oxygenated external solution (described below). Pyramidal neurons of the hippocampal CA1 region were visualized with an Olympus 60× water immersion objective and a Zeiss Axioskop with infrared DIC.

Solutions

Solution used for perfusion and slicing contained (mM): 220 sucrose, 2.5 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 0.5 $CaCl_2$, 7 $MgCl_2$, and 7 dextrose. During experiments, the slices were continuously superfused with oxygenated external solution containing (mM): 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 2 $CaCl_2$, 1 $MgCl_2$, and 25 dextrose. For whole-cell recordings, the pipette solution contained (mM): 120 K-gluconate, 20 KCl, 10 HEPES, 0.2 EGTA, 4 NaCl, 4 MgATP, 0.3 Tris–GTP, and 7 phosphocreatine (pH 7.4 with KOH). For cell-attached recordings, the pipette solution contained (mM): 125 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 1 μM TTX (pH 7.4 with NaOH).

Data acquisition and analysis

Recording electrodes were pulled from thick-wall borosilicate glass pipettes, with resistances of 5–7 MΩ for recordings from rat dendrites, or 8–11 MΩ for recordings from mouse dendrites. Whole-cell, current-clamp recordings were performed using a BVC-700A amplifier (Dagan) at near physiological temperatures of 32–34°C. Records were analog filtered at 10 KHz. Cell-attached, voltage-clamp recordings were performed using an Axopatch-1D amplifier (Axon Instruments) at room temperatures of 22–24°C. Records were analog filtered at 2 KHz. Signals were digitized using an ITC-18 computer interface (Instrutech) at >3× analog filter frequency. After cell-attached recordings, the membrane patch was ruptured and the resting membrane potential of the cell was recorded immediately after break-in. This potential was then used to calculate the absolute voltage of the commands. Data acquisition was controlled by custom software written in Igor (WaveMetrics), which was also used for data analysis.

Results

The waveforms of bAPs depend on membrane potential oscillations

Membrane potential of CA1 pyramidal neurons oscillates at a rhythm of 5–10 Hz (theta oscillation, see [2]) in vivo under certain conditions. To study the effect of theta oscillation on bAPs, we simulated such oscillation by sine-wave (5 Hz) current injections during whole-cell recordings in the dendrites. At the same time, we stimulated the axons of CA1 neurons to initiate bAPs that coincide with different phases during the oscillation (Fig. 1a). This timing between a bAP and the phase of the sine wave affected the waveform of the bAP. For example, when the bAP occurred at the peak of the oscillation (Fig. 1aa), the amplitude was larger than that of a bAP at the trough of the oscillation (Fig. 1ab). Figure 1ac is an overlay of 35 bAPs like those in Fig. 1aa, ab. With depolarization, the initial dV/dt of bAPs increased and the half-amplitude duration (half-width) of the bAPs decreased (Fig. 1b), indicating faster repolarization.

Delayed-rectifier K^+ channels in the dendrites are activated during bAP repolarization

The faster repolarization at depolarized membrane potentials suggests that more channels carrying outward current may be activated at these potentials. From previous studies [3], we know that the delayed-rectifier K^+ channels activate at depolarized membrane potentials. With the experiments illustrated in Fig. 2, we tested whether the delayed-rectifier K^+ channels can be activated by bAP. The recording configuration in these experiments was similar to what is shown in Fig. 1ad. We did cell-attached recordings of K^+ channels in the dendrites (200 μm from soma). With voltage-clamp recordings, we first identified one delayed-rectifier K^+ channel in this dendritic patch [3]. We then stimulated the axons of the neuron for bAP initiation. Under these conditions, the current signals from the cell-attached patch (Fig. 2a) were comprised of a stimulus artifact, followed by a capacitive transient from the bAP [12]. When the patch was held at resting membrane potential (−70 mV), there was no channel activation after the capacitive transient. However, when we held the patch at −50 or −30 mV, there was channel activation after the capacitive transient (Fig. 2a, left, arrows). When we subtracted the stimulus artifact and the bAP capacitive transient from the traces, we obtained single-

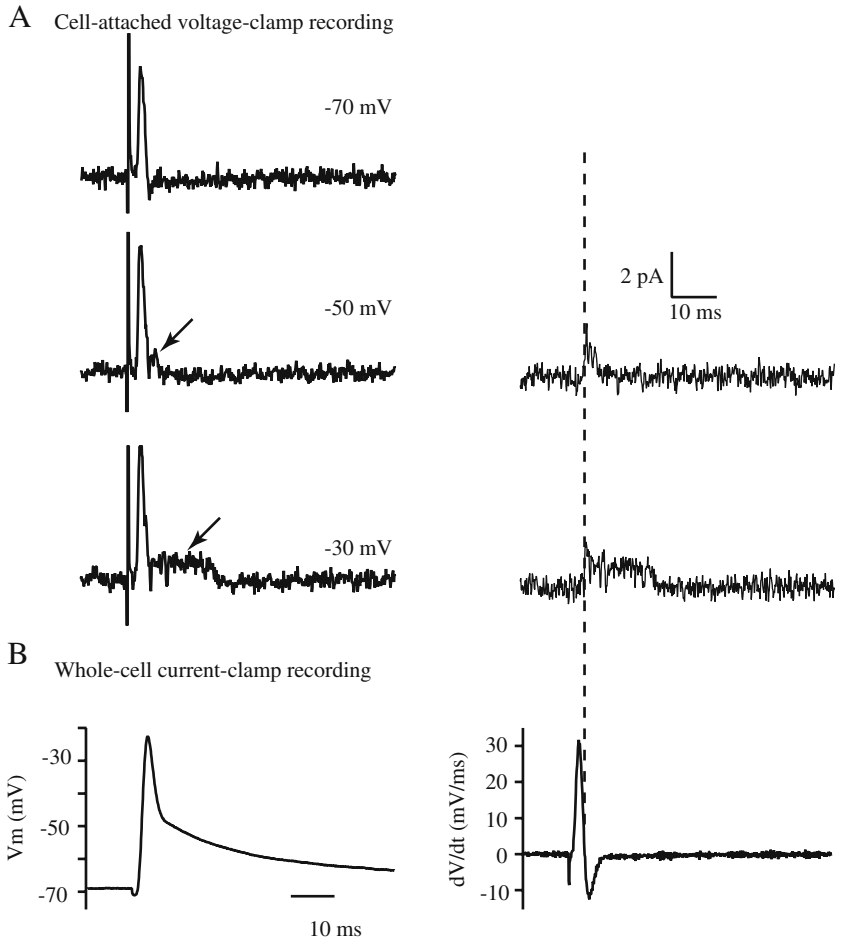

Fig. 2 Activation of single delayed-rectifier K+ channel by bAP. Recordings were performed from CA1 dendrites of Sprague–Dawley rat at room temperature, at a distance of 200 μm from soma. The whole-cell recordings were performed after rupturing the membrane at the end of cell-attached recordings. **a** Cell-attached recordings at noted membrane potentials. bAPs resulted in capacitive current following the stimulus artifacts (*left column*). Subtracting the capacitive transients from these records led to traces showing only channel activities (*right column*). **b** bAP recorded in the whole-cell configuration. Both Vm (*left*) and d*V*/d*t* (*right*) are shown. Channel activities coincided with the repolarization phase of the bAP (*dotted line*)

channel current from activation of the delayed-rectifier K^+ channel (Fig. 2a, right). After the cell-attached recordings, we ruptured the membrane and recorded the bAP in the whole-cell configuration under current-clamp (Fig. 2b, left). The first derivative (d*V*/d*t*) of the whole-cell bAP under current-clamp should be proportional to the capacitive current under voltage-clamp: $dV/dt=(1/C)\times(dQ/dt)$. Therefore, d*V*/d*t* of the whole-cell bAP qualitatively resembles the capacitive transient in cell-attached, voltage-clamp recordings (Fig. 2b, right). The negative values of d*V*/d*t* represent the repolarization phase of the bAP, which corresponded well with the activation of the delayed-rectifier K^+ channel (dotted line).

Discussion

Our results suggest that the delayed-rectifier K^+ channels can contribute to bAP repolarization, depending on the membrane potential (Fig. 2). This hypothesis predicts that bAPs should repolarize faster at more depolarized membrane potentials than at more negative membrane potentials. This is because at depolarized membrane potentials, more delayed-rectifier K^+ channels can be recruited to repolarize the bAP. We found that the waveforms of bAPs were affected by membrane potential oscillation in mouse dendrites (Fig. 1). A similar phenomenon was also observed in rat dendrites [1].

The oscillation of membrane potentials could presumably affect the activation/inactivation states of multiple ion channels. Besides the delayed-rectifier K^+ channel, the A-type K^+ channels and Na^+ channels are the two types of ion channels that greatly contribute to the amplitude and/or waveform of bAPs [5, 7]. The increase of initial d*V*/d*t* with membrane depolarization (Fig. 1b) suggests that more Na^+ channels were activated with membrane depolarization. This greater activation of Na^+ channels, together with

possible inactivation of A-type K^+ channels during the depolarizing phase of the oscillation, may contribute to larger bAP amplitude at the peak of the oscillation (Fig. 1; [1]). If the A-current were the only repolarizing current of the bAP, one would expect the bAP at the peak of the membrane oscillation to become broader because A-current was inactivated by the depolarizing phase of the oscillation (reflected by larger bAP amplitude) and was no longer available to repolarize the bAP.

However, the opposite was observed in the experiments. As shown in Fig. 1, when the bAP coincided with the peak of the voltage fluctuation, the half-width of the bAP was shorter than that at the trough of the voltage fluctuation, indicating faster spike repolarization at higher membrane potentials. The faster repolarization may indeed come from greater recruitment of the delayed-rectifier-type K^+ channels at depolarized membrane potentials (Fig. 2).

In conclusion, advances in patch-clamp techniques have enabled in-depth study of the active properties of neuronal dendrites. In the CA1 pyramidal neurons of hippocampus, Na^+ channels and multiple types of Ca^{2+} channels and K^+ channels were identified with single-channel recording [3, 4, 7, 12]. These channels contribute to electrical signaling of the dendrites in ways that reflect their biophysical properties. The data presented in this study provides evidence for the involvement of the uniformly distributed delayed-rectifier K^+ channels in regulating bAP waveforms during membrane potential oscillations.

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