The Journal of Neuroscience, October 1, 1999, *19*(19):8319–8326

Dendritic Ca^{2+}-Activated K^+ Conductances Regulate Electrical Signal Propagation in an Invertebrate Neuron

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Activity-dependent changes in the short-term electrical properties of neurites were investigated in the anterior pagoda (AP) cell of leech. Imaging studies revealed that backpropagating Na^+ spikes and synaptically evoked EPSPs caused Ca^{2+} entry through low-voltage-activated Ca^{2+} channels that are distributed throughout the neurites. Voltage-clamp recordings from the soma revealed a TEA-sensitive outward current that was reduced when Ca^{2+} entry was blocked with Co^{2+} or when the intracellular concentration of free Ca^{2+} was reduced by a high-affinity Ca^{2+} buffer. Ca^{2+} released in the neurite from a caged Ca^{2+} compound caused a hyperpolarization of the membrane potential. These data imply that the AP cell expresses Ca^{2+}-activated K^+ conductances, and that these conductances are present in the neurites. When the Ca^{2+}-activated K^+ current was reduced through the block of Ca^{2+} entry, backpropagating Na^+ spikes and synaptically evoked EPSPs increased in amplitude. Hence, the activity-dependent changes in the intracellular $[Ca^{2+}]$ together with the Ca^{2+}-activated K^+ conductances participate in the regulation of dendritic signal propagation.

Key words: calcium; dendrite; calcium-activated potassium conductance; backpropagating spikes; caged calcium; leech

Calcium ions may enter the cytosol through voltage-gated channels (for review, see McCleskey, 1994; Dolphin, 1996), ligand-gated channels (MacDermott et al., 1986; Schneggenburger et al., 1993), or release from intracellular Ca^{2+} stores (for review, see Berridge, 1998; Svoboda and Mainen, 1999). As a result of calcium diffusion and binding processes, and the restricted geometry of dendrites, fast, large, and local increases in the intracellular $[Ca^{2+}]$ can occur in dendrites (for review, see Augustine and Neher, 1992; Regehr and Tank, 1994; Eilers and Konnerth, 1997). The coupling of the intracellular free $[Ca^{2+}]$ with the membrane conductance depends on the activation of Ca^{2+}-activated K^+ channels (for review, see Blatz and Magleby, 1987; Latorre et al., 1989; Sah, 1996). If Ca^{2+}-activated K^+ channels are expressed in dendrites, their activation by increases in the intracellular $[Ca^{2+}]$ may have profound effects on dendritic processing, such as compartmentalization and negative feedback. Furthermore, if Ca^{2+} ions enter through voltage- or ligand-gated channels, the Ca^{2+} current contributes to (1) a depolarization of the membrane potential and (2) the activation of Ca^{2+}-activated K^+ channels. Which of these actions on the membrane potential is the principal role of the Ca^{2+} current is not obvious and may be system-specific, thereby supporting different functions. Evidence for Ca^{2+}-activated K^+ channels in dendrites has been found in rat Purkinje neurons (Khodakhan and Ogden, 1993), in rat hippocampal pyramidal neurons (Andreasen and Lambert, 1995; Sah and Bekkers, 1996), and in rat neocortical pyramidal neurons (Schwindt and Crill, 1997b).

To investigate the combined effect of the intracellular $[Ca^{2+}]$ dynamics and the Ca^{2+}-activated K^+ conductance on dendritic processing, we chose the leech anterior pagoda (AP) neuron (Stewart et al., 1989; Gu, 1991; Wolszon et al., 1995; Melinek and Muller, 1996; Osborn and Zipser, 1996; Aisemberg et al., 1997; Wessel et al., 1999) (Fig. 1*A*) as a model, because (1) the AP neuron has extensive neurites that receive glutamatergic synaptic inputs (Wessel et al., 1999), (2) its spike initiation zone is far from the soma (Melinek and Muller, 1996), thus allowing us to record backpropagating spikes from the soma, (3) evidence for Ca^{2+} currents has been reported (Stewart et al., 1989), and calcium transients have been recorded from the soma (Ross et al., 1987), and (4) there is preliminary evidence for Ca^{2+}-activated K^+ channels in somatic membrane patches of the AP neuron (Pellegrini et al., 1989) as well as in the somata of other leech neurons (Jansen and Nicholls, 1973). Here we ask: (1) what is the spatial distribution of voltage-gated Ca^{2+} channels in the neurites? (2) are these channels activated by spikes and synaptic inputs? (3) do the neurites express Ca^{2+}-activated K^+ conductances?, and (4) if so, what is the combined effect of the intracellular $[Ca^{2+}]$ dynamics and the Ca^{2+}-activated K^+ conductance on electrical signal propagation?

MATERIALS AND METHODS

Preparation, dissection, and solutions. Leeches, *Hirudo medicinalis*, were obtained from a commercial supplier (Leeches USA, Westbury, NY) and maintained in artificial pond water at 15°C. Animals were anesthetized in ice-cold saline, and individual ganglia were dissected using surgical methods similar to those described previously (Muller et al., 1981; Wessel et al., 1999). Ganglia were pinned ventral side up in Sylgard (Dow Corning, Midland, MI)-lined Petri dishes (bath volume, 10 ml), and the connective tissue sheath over the neuronal somata was removed with fine scissors. For the imaging experiments, somata in the medial ventral packet were removed with fine scissors to reduce light scatter from the ganglia and improve the image quality of the AP cell neurite. For the hemisectioning experiments, the ganglia were cut at the midline with a scalpel blade, and the AP cell was hyperpolarized below −60 mV with current injection for 30 min. Ganglia were superfused (3 ml/min) with normal leech saline containing (in mM): NaCl, 115; KCl, 4; $CaCl_2$, 1.8; $MgCl_2$, 1.5; glucose, 10; Tris-maleate, 4.6; Tris-base, 5.4, pH 7.4 adjusted with NaOH. Equimolar amounts of Co^{2+} replaced Ca^{2+} in 0 Ca^{2+} salines. In solutions with 10 mM TEA, the Na^+ concentration was

Received May 21, 1999; revised July 14, 1999; accepted July 22, 1999.

This work was supported by the National Science Foundation. We thank James Eisenhart for assistance in obtaining Figure 1*A* and Rafael Yuste for discussions.

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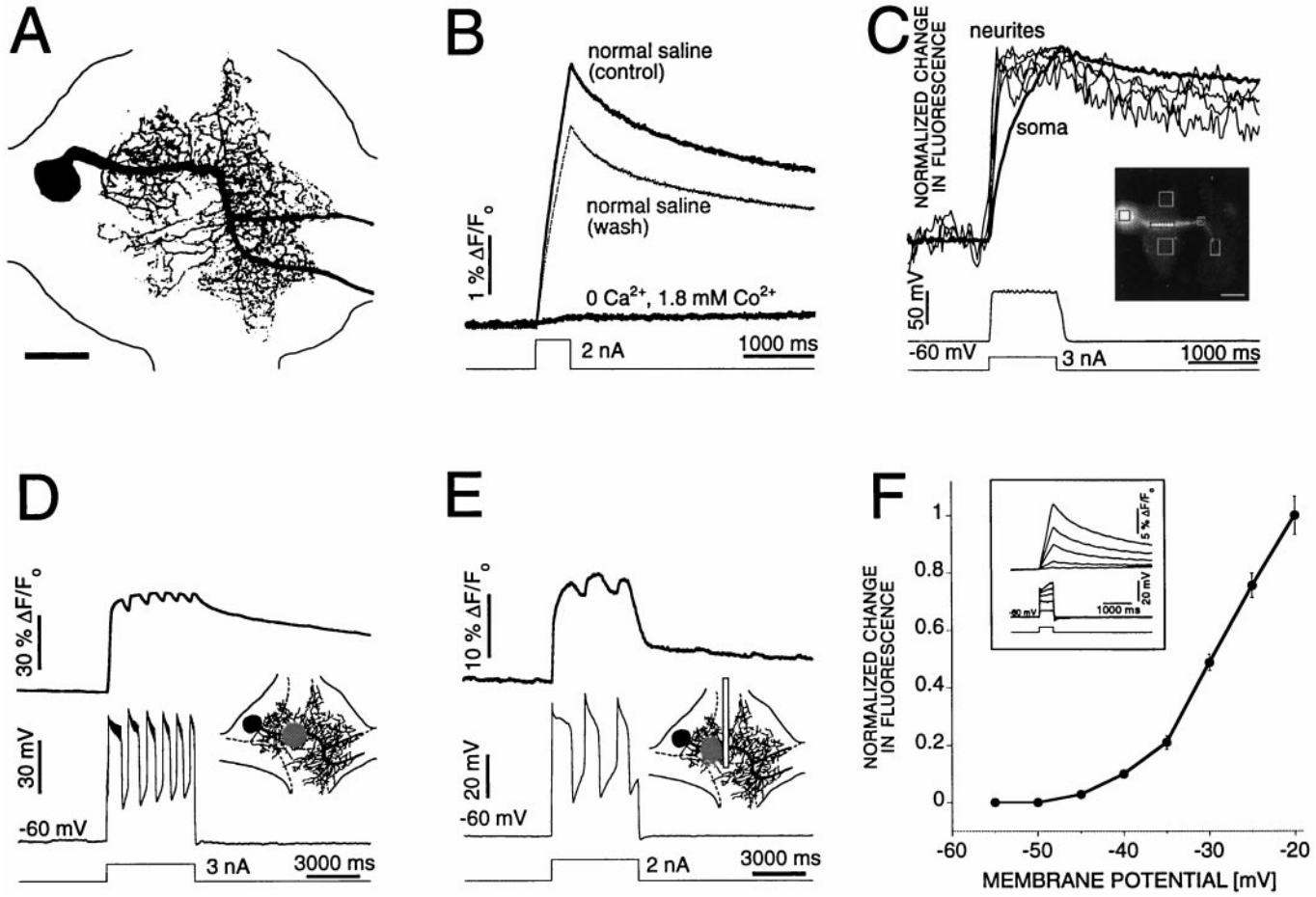

Figure 1. Voltage-gated Ca^{2+} channels are present in the AP cell neurites. *A*, Morphology of the AP cell. Light-microscopic image of the AP cell filled with fluorescein after brightness and contrast adjustment and thresholding. The outline of the ganglion is indicated by the line drawing. Anterior is up. Scale bar, 100 μm. *B,* Increases in the intracellular [Ca^{2+}] in the soma evoked by depolarization to −25 mV via somatic current injection (+2 nA) from a holding potential of −45 mV in normal leech saline and in 0 Ca^{2+}, 1.8 mM Co^{2+} saline. The ganglion was cut at the midline to avoid the occurrence of Na^+ spikes; average of two trials. *C,* Increases in the intracellular [Ca^{2+}] at various neurite locations and in the soma evoked by a depolarization (+3 nA) from a holding potential of −60 mV in 10 mM TEA saline in the intact ganglion. All traces are normalized to their maximum value to facilitate the comparison of the time course. *Inset,* Fluorescent image of the AP cell filled with Calcium Green 1. The *open boxes* indicate the positions for the traces in the main figure. Scale bar, 100 μm. *D,* Repetitive Ca^{2+} spikes with Na^+ spikes riding on the plateaus evoked by a depolarization via current injection (+3 nA) from a holding potential of −60 mV in the intact ganglion in saline containing 10 mM TEA. The fluorescent signal was monitored from the major neurite close to the midline. *Inset,* Schematic of the AP cell and outline of the ganglion. The location of the optical recording site is indicated by the *gray spot* near the midline. *E*, Repetitive Ca^{2+} spikes evoked by a depolarization via current injection (+2 nA) from a holding potential of −60 mV in the truncated ganglion in saline containing 10 mM TEA. The fluorescent signal was monitored from the major neurite close to the midline. *Inset,* Schematic of the AP cell and outline of the ganglion. The location of the optical recording site is indicated by the gray spot near the midline, and the location of the cut is indicated by the *white bar* near the midline. *F,* Voltage dependence of increases in intracellular [Ca^{2+}]. Average peak change in fluorescence in the soma in response to a depolarization from a holding potential of −60 mV to various test membrane potentials via 500-msec-long somatic current injections in normal saline (mean ± SEM; $n = 5$ cells). The ganglion was cut at the midline to avoid Na^+ spikes. Optical data from each experiment were normalized to their value at −30 mV, and test membrane potentials caused by current injection were binned (5 mV bin width). *Inset,* Increases in intracellular [Ca^{2+}] in the soma evoked by a depolarization from a holding potential of −60 mV to various test potentials via somatic current injection in normal leech saline for one representative cell. The *bottom trace* indicates the timing of the current injection.

reduced by equal amount to maintain osmolarity. Experiments were performed at room temperature (20–23°C).

Electrophysiology. Dual intracellular recordings were made with sharp borosilicate microelectrodes (1 mm outer diameter, 0.75 mm inner diameter; A-M Systems, Carlsborg, WA) pulled on a micropipette puller (P-80; Sutter Instruments, Novato, CA), filled with 3 M potassium acetate, and had resistances of 40–80 MΩ. An Axoclamp 2B (Axon Instruments, Foster City, CA) was used for either current-clamp (bridge mode) or two-electrode voltage-clamp (TEVC mode) measurements. Analog data were low-pass filtered (4 pole Butterworth) at 1 kHz, digitized at 2 kHz, stored, and analyzed on a personal computer equipped with an AT-MIO-16E-1 (National Instruments, Austin, TX) and LabView software (National Instruments, Austin, TX). The AP cell was impaled with two microelectrodes, one for passing current and one for measuring voltage. Data are expressed in the text and figures as mean ± SEM.

Imaging. Individual cells were filled with the calcium-sensitive dye, Calcium Green 1 (Molecular Probes, Eugene, OR), by iontophoresis through intracellular microelectrodes. The electrodes were filled with 7 mM Calcium Green 1 in 300 mM potassium acetate. The soma was impaled, and the dye was injected with hyperpolarizing current (−0.5 nA for 10 min). After the injection, the dye diffused in the cytosol and filled the neurites within 30 min. The image of the filled neurites was then projected with a fluorescent microscope (Zeiss, Oberkochen, Germany), equipped with a 40×, 0.75 NA water immersion objective (Zeiss), onto a photodiode (UV140, EGG Ortec, Ontario, Canada), using a 150 W Xenon arc lamp (Osram) illumination with a stabilized power supply (model 1600; Opti-Quip, Highland Mills, NY), and the following filter combination: excitation, 500 DF22; dichroic, 515 DRLP; and emission, 530 LP (Omega Optical, Brattleboro, VT). The aperture of the illuminating light was reduced to a spot of 20 μm diameter to record from

selected neurites. The photodiode current was converted to voltage (Ithaco 1211 current preamplifier, Ithaca, NY), and the voltage signal was low-pass filtered (10 Hz; Ithaco 4212 electronic filter). For experiments that required simultaneous recording from multiple sites, the image of the filled neurites was projected with a 20×, 0.4 NA dry objective (Nikon) onto a CCD camera (PXL; Photometrics, Tucson, AZ) that was controlled with commercial software (IPLab Spectrum; Scanalytics, Fairfax, VA).

Photolytic release of Ca^{2+}. Individual cells were filled with the caged Ca^{2+} compound, DM-Nitrophen (DMNP-EDTA; Molecular Probes, Eugene, OR) by iontophoresis through 30 MΩ intracellular microelectrodes. The electrodes were filled with 100 mM DM-Nitrophen in H_2O. The soma was impaled, and the DM-Nitrophen was injected by passing current (−1 nA, 10 min). To photolyze the DM-Nitrophen (Lando and Zucker, 1989; Adams and Tsien, 1993; Zucker, 1993) in the neurite, the aperture of the illuminating light from a 150 W Xenon arc lamp, reflected from a dichroic mirror (420DCLP02; Omega Optical), was reduced and focused with a 40×, 0.75 NA water immersion lens (Zeiss) onto the medial packet.

Histology. To observe AP cells in light microscopy, an AP cell was iontophoretically injected with fluorescein dextran (5% w/v in H_2O; 3000 molecular weight; Molecular Probes, Eugene, OR) using sharp electrodes of 10–30 MΩ resistance and pulsed current (−7 to −3 nA; 10 Hz; 30 min). The ganglia were fixed in 2% paraformaldehyde in 0.1 M PBS for 2–12 hr, rinsed in PBS, and mounted in a solution of 20% PBS and 80% glycerin. Digital images were taken at 20× magnification with a confocal microscope (MRC1024; Bio-Rad, Hercules, CA) equipped with a krypton–argon laser using the 488 nm line for excitation and the emission filter 540/30. Images were adjusted with respect to brightness and contrast and thresholded using Adobe Photoshop (Adobe Systems, Mountain View, CA).

RESULTS

Neurites express voltage-gated Ca^{2+} channels

If Ca^{2+} channels were expressed in the AP cell neurite, activation of these channels by Na^+ spikes and EPSPs would cause an increase in the intracellular $[Ca^{2+}]$. To test whether AP cells express voltage-gated Ca^{2+} channels, the calcium sensitive dye, Calcium Green 1, was injected into the AP cell, and its fluorescence was recorded from the soma. Because TTX does not block voltage-gated Na^+ channels in leech (Kleinhaus and Angstadt, 1995), the spike initiation site, located contralateral to the soma, approximately between the midline and the bifurcation of the primary process (Melinek and Muller, 1996) (Fig. 1*A*), was surgically removed with a cut at the midline to avoid the involvement of Na^+ spikes. In current clamp, starting at a membrane potential of −45 mV, a depolarizing current injection into the soma caused a depolarization to approximately −25 mV and an increase in the intracellular $[Ca^{2+}]$ in the soma (Fig. 1*B*). The increase in the intracellular $[Ca^{2+}]$ in the soma was reversibly abolished in 0 Ca^{2+}, 1.8 Co^{2+} saline. Because Co^{2+} is a blocker for voltage-gated Ca^{2+} channels (Hille, 1992), this observation indicates that Ca^{2+} entered the cell through voltage-gated Ca^{2+} channels during the depolarization.

Optical recordings from multiple sites in the neurite of the intact AP neuron revealed simultaneous increases in the intracellular $[Ca^{2+}]$ in response to a current injection that caused a depolarization from −60 mV to a membrane potential of approximately +20 mV in 10 mM TEA saline (Fig. 1*C*). The changes in intracellular $[Ca^{2+}]$ in response to a depolarization were also abolished in 0 Ca^{2+}, 1.8 Co^{2+} saline (data not shown). Because calcium ion diffusion within the cytoplasm is slow ($D \sim 0.6\ \mu m^2$/msec; for review, see Koch, 1999), which implies a diffusion length of $<40\ \mu m$ over the 1000 msec time scale of the experiment, the increase in the intracellular $[Ca^{2+}]$ in response to a depolarization suggests that voltage-gated Ca^{2+} channels are present in the neurites of the AP cell. The increase in the intracellular $[Ca^{2+}]$ was slower in the soma than in the neurites. Most likely the different time course for changes in the intracellular $[Ca^{2+}]$ are associated with differences in surface to volume ratios in the different compartments (Hernandez-Cruz et al., 1990; Schiller et al., 1995), rather than a lack of voltage-gated Ca^{2+} channels in the soma and Ca^{2+} diffusion from the primary neurite.

When K^+ channels were blocked in saline with 10 mM TEA, regenerative Ca^{2+} spikes with concomitant increases in the intracellular $[Ca^{2+}]$ were observed in response to a prolonged current injection in the intact ganglion, with Na^+ spikes riding on the Ca^{2+} plateau potentials (Fig. 1*D*). Sodium spikes were not necessary to trigger Ca^{2+} spikes, because Ca^{2+} spikes were also generated in the truncated ganglion with the spike initiation for Na^+ spikes surgically removed (Fig. 1*E*). In both cases, the changes in intracellular $[Ca^{2+}]$ were recorded from the primary neurite near the midline. The presence of Ca^{2+} spikes strongly suggests a Ca^{2+} inward current of sufficient amplitude to overcome the outward leak current in 10 mM TEA saline (compare Fig. 3*A*).

To determine the voltage dependence of the intracellular $[Ca^{2+}]$ transients, we measured changes in the somatic intracellular $[Ca^{2+}]$ in response to a depolarization from −60 mV to various test potentials via current injection (current clamp) in the truncated ganglion with the spike initiation for Na^+ spikes surgically removed. Increases in the intracellular $[Ca^{2+}]$ were detectable above membrane potentials of −50 mV and increased in size with further depolarization (Fig. 1*F*). Because the increase in the intracellular $[Ca^{2+}]$ in the soma was reversibly abolished when Ca^{2+} entry through voltage-gated Ca^{2+} channels was blocked in 0 Ca^{2+}, 1.8 Co^{2+} saline (Fig. 1*B*), the data suggest that voltage-gated Ca^{2+} channels are activated at membrane potentials positive to −50 mV.

Activation of Ca^{2+} channels by Na^+ spikes and EPSPs

The fact that voltage-gated Ca^{2+} channels are activated above a membrane potential of −50 mV, close to the resting potential of these neurons, suggests that these channels can be activated by Na^+ spikes and EPSPs. Increases in the intracellular $[Ca^{2+}]$ in response to Na^+ spikes were recorded from the primary neurite near the midline in response to Na^+ spikes (Fig. 2*A*). Similar results were seen in all five AP cells tested. Such changes were abolished when Ca^{2+} entry through voltage-gated Ca^{2+} channels was blocked in 0 Ca^{2+}, 1.8 Co^{2+} saline (data not shown). The intracellular $[Ca^{2+}]$ transients in response to Na^+ spikes decayed exponentially with time constants ranging between 210 and 750 msec (470 ± 90 msec; mean ± SEM; $n = 5$ cells). Because the time constant increases with increasing dye concentration (Helmchen et al., 1996) as a result of $[Ca^{2+}]$ buffering by Calcium Green 1, the measured values are upper bounds of the time constant for intracellular $[Ca^{2+}]$ decay without dye. These data suggest that Na^+ spikes cause an increase in the intracellular $[Ca^{2+}]$ through the activation of voltage-gated Ca^{2+} channels.

EPSPs evoked by a burst of spikes in the presynaptic ipsilateral dorsal and ventral pressure-sensitive cells caused increases in the intracellular $[Ca^{2+}]$ in the primary neurite near the midline (Fig. 2*B*). Similar results were seen in all five AP cells tested in this way. Such increases were abolished when the AP cell was hyperpolarized to a membrane potential of −70 mV (Fig. 2*C*), indicating that at a membrane potential of −45 mV (Fig. 2*B*) Ca^{2+} entered through voltage-gated Ca^{2+} channels rather than via the synaptic current. This interpretation is supported by the fact that

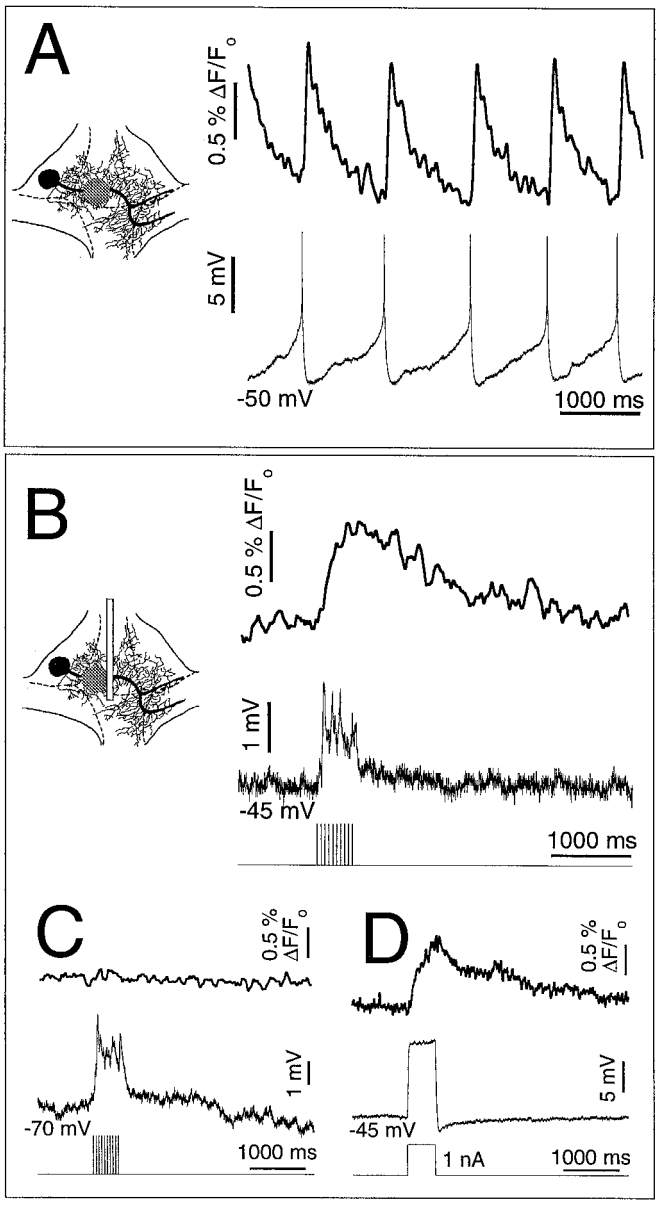

Figure 2. Activation of Ca^{2+} channels by Na^+ spikes and synaptic inputs. *A,* Increases in the intracellular [Ca^{2+}] in the neurite evoked by Na^+ spikes in the intact ganglion in normal saline. *Inset,* Schematic of the AP cell and outline of the ganglion. The location of the optical recording site is indicated by the *gray spot* near the midline. *B,* Increases in the intracellular [Ca^{2+}] in the neurite evoked by EPSPs in normal saline from a holding potential of −45 mV. The ganglion was cut at the midline to avoid Na^+ spikes. The EPSPs were evoked by a burst of presynaptic spikes (indicated by the *bottom trace*) in the ipsilateral dorsal and ventral pressure-sensitive cells. *Inset,* Schematic of the AP cell and outline of the ganglion. The location of the optical recording site is indicated by the *gray spot* near the midline. The location of the cut is indicated by the *white bar* near the midline. *C,* EPSPs did not cause an intracellular [Ca^{2+}] transient in the neurite when the cell was hyperpolarized to −70 mV. *D,* A depolarization from a holding potential of −45 mV generated by a current injection (+1 nA) caused an increase in the intracellular [Ca^{2+}] in the neurite similar to the one generated by synaptic stimulation in *B*.

a small depolarization generated by a somatic current injection caused an increase in the intracellular [Ca^{2+}] (Fig. 2*D*) similar in shape to the one caused by an EPSP (Fig. 2*B*). In addition, Ca^{2+} entry through NMDA-R channels is excluded, because NMDA receptors are absent from these synapses (Wessel et al., 1999). These data suggest that EPSPs cause an increase in the intracellular [Ca^{2+}] through the activation of voltage-gated Ca^{2+} channels.

Neurites express Ca^{2+}-activated K^+ conductances

If Ca^{2+}-activated K^+ conductances are present in the AP cell neurite, these conductances are potentially activated by the increase in the intracellular [Ca^{2+}] caused by Na^+ spikes and EPSPs. To test for Ca^{2+}-activated K^+ conductances, a two-electrode voltage clamp was used to measure the *I–V* characteristics of AP cells above membrane potentials of −50 mV, the range of membrane potentials at which Ca^{2+} entry was detectable (Fig. 1*F*). The voltage clamp is assumed to be effective only in the AP cell soma. When the somatic membrane potential was stepped to test voltages between −50 and −10 mV from a holding potential of −60 mV with the ganglion in normal leech saline, the outward current activated rapidly and, for test voltages more than −30 mV, largely inactivated during the 3 sec voltage steps used in these experiments (Fig. 3*A, inset*). In 0 Ca^{2+}, 1.8 Co^{2+} saline, chosen to block Ca^{2+} entry through voltage-gated Ca^{2+} channels, the outward current above −30 mV was reduced (Fig. 3*A, inset*). The outward current, measured from the value of the current 100 msec after the onset of the voltage step, was significantly reduced for test voltages above −30 mV when the saline was changed from normal saline to 0 Ca^{2+}, 1.8 Co^{2+} saline (Fig. 3*A; n* = 7 cells). The outward current was almost completely blocked in 10 mM TEA saline (Fig. 3A; *n* = 5 cells). The remaining current had a linear *I–V* curve, indicating that this current was caused by a voltage-independent leak conductance.

High-affinity Ca^{2+} buffers reduce the intracellular concentration of free Ca^{2+} (Swandula et al., 1991; Regehr and Tank, 1992; Helmchen et al., 1996). Moderate concentrations of kinetically fast, high-affinity calcium buffers, like Calcium Green 1, are known to reduce Ca^{2+}-activated K^+ currents in other preparations (Roberts, 1993; Sobel and Tank, 1994). In normal saline, the outward current above a test voltage of −30 mV was significantly reduced after a moderate quantity of Calcium Green 1 was injected into the AP cell (Fig. 3*B*). The Co^{2+}, TEA, and Ca^{2+} buffer voltage clamp data together suggest the presence of a Ca^{2+}-activated K^+ current in the AP cell.

Ca^{2+}-activated K^+ conductances are known to cause an afterhyperpolarization (AHP) after a depolarization (Hille, 1992; Berridge, 1998). To test whether AP cells display AHP, current pulses (1 sec, +3 nA) were injected into the soma of cells with the spike initiation surgically removed. All cells tested displayed an AHP. This AHP was reduced from 11.0 ± 1.0 mV in normal saline to 4.7 ± 0.8 mV (*n* = 9 cells) when Ca^{2+} entry through voltage-gated Ca^{2+} channels was blocked in 0 Ca^{2+}, 1.8 Co^{2+} saline (Fig. 3*C*). Additionally, in normal saline, the AHP was reduced from 7.5 ± 1.1 mV in a control to 1.6 ± 0.7 mV (*n* = 9 cells) after the injection of Calcium Green 1 (Fig. 3*D*).

The voltage-clamp data, as well as the observation of a Ca^{2+}-dependent AHP, indicate that Ca^{2+}-activated K^+ conductances are present in the AP cell. To test whether these conductances are expressed in the neurite, rather than in the soma alone, the caged-Ca^{2+} compound DM-Nitrophen was injected into the AP cell. DM-Nitrophen releases Ca^{2+} after photolysis and transiently raises the intracellular [Ca^{2+}] (see Materials and Methods). When Ca^{2+} was released inside the AP cell neurite through a flash of light focused onto the neurite in the medial packet, a significant transient hyperpolarization (ΔV = −1.7 ± 0.4 mV;

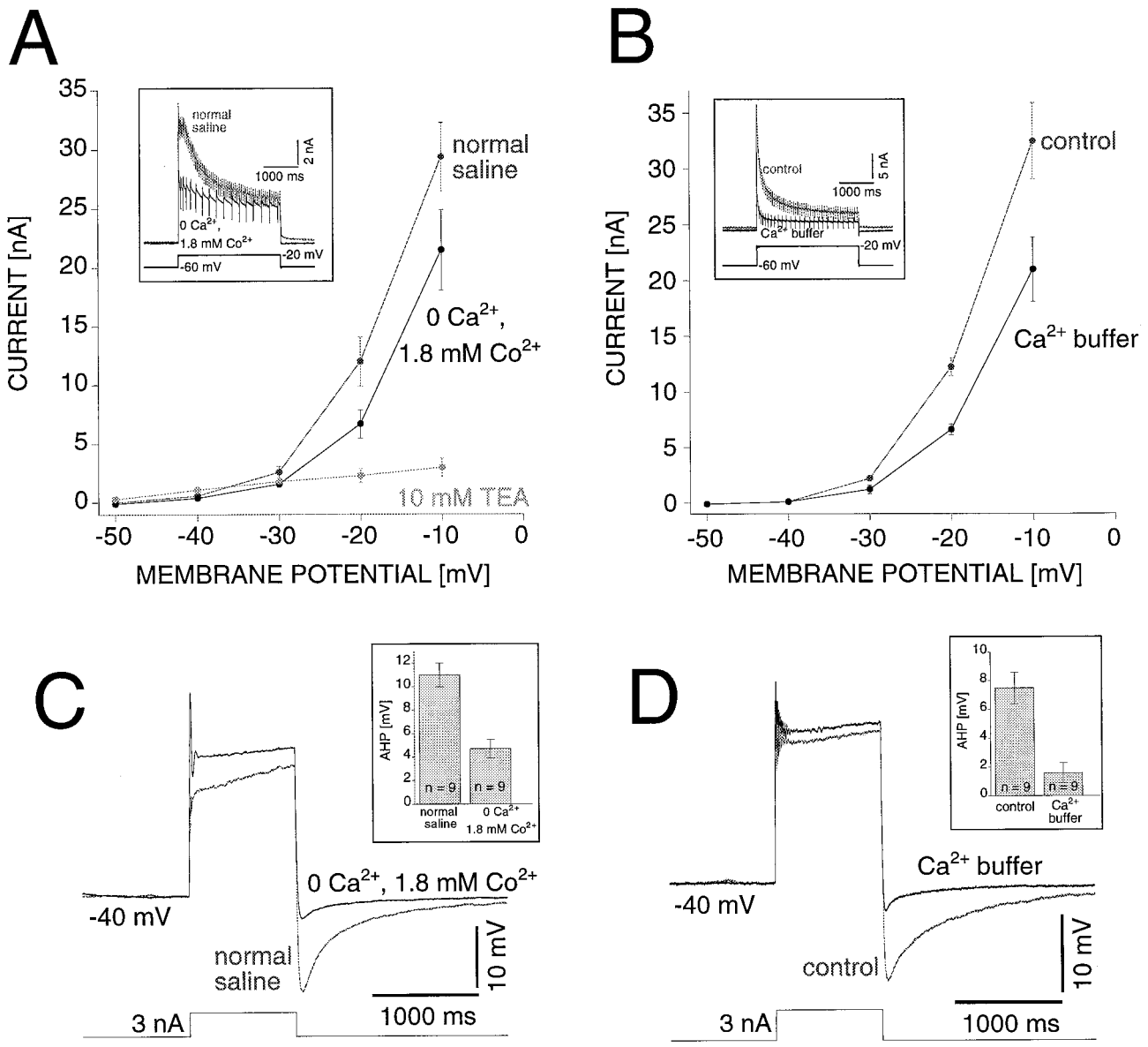

Figure 3. Evidence for Ca^{2+}-activated K^+ conductances. *A,* The current measured at 100 msec after a voltage step from a holding potential of −60 mV to various test membrane potentials between −50 and −10 mV in the intact ganglion in normal leech saline (mean ± SEM; $n = 7$ cells), in 0 Ca^{2+}, 1.8 mM Co^{2+} saline ($n = 7$ cells), and in saline with 10 mM TEA ($n = 5$ cells). *Inset,* The current response to a voltage step from a holding potential of −60 mV to a test membrane potential of −20 mV in normal leech saline and in 0 Ca^{2+}, 1.8 mM Co^{2+} saline. The current spikes are caused by Na^+ action potentials generated in the nonclamped region of the neuron. *B,* The current measured at 100 msec after a voltage step from a holding potential of −60 mV to various test membrane potentials between −50 and −10 mV in the intact ganglion in normal saline before and after filling the cell with a moderate concentration of Calcium Green 1, a high-affinity calcium buffer (mean ± SEM; $n = 4$ cells). *Inset,* The current response to a voltage step from a holding potential of −60 mV to a test membrane potential of −20 mV before and after filling the cell with a moderate concentration of Calcium Green 1. *C,* Voltage response to a 1 sec, +3 nA current pulse in normal saline and in 0 Ca^{2+}, 1.8 mM Co^{2+} saline. The ganglion was cut at the midline to avoid Na^+ spikes. *Inset,* On average the AHP was reduced from 19.3 ± 1.7 mV in normal saline to 4.7 ± 0.8 mV in 0 Ca^{2+}, 1.8 mM Co^{2+} saline ($n = 9$ cells). *D,* Voltage response to a 1 sec, +3 nA current pulse before and after filling the cell with a moderate concentration of Calcium Green 1. The ganglion was cut at the midline to avoid Na^+ spikes. *Inset,* On average the AHP was reduced from 7.5 ± 2.8 mV before to 1.6 ± 0.7 mV after filling the cell with a moderate concentration of Calcium Green 1 ($n = 9$ cells).

$n = 6$ cells) and a reduction in spike rate ($\Delta f = -2.3 \pm 0.6$ Hz; $n = 6$ cells) was observed (Fig. 4). Hyperpolarization or a decreased spike rate was not observed in response to light flashes in the absence of DM-Nitrophen and was not observed after two or three flashes delivered within a period of ~2 min (data not shown), at which point all caged-Ca^{2+} was probably photolysed. These data provide evidence for a calcium-activated outward current in the neurites. The TEA sensitivity of the outward current measured in voltage clamp (Fig. 3*A*) suggests that the outward current is carried by potassium ions.

Backpropagating spikes and EPSPs are attenuated by a Ca^{2+}-activated K^+ conductance

We have shown that Na^+ spikes and EPSPs cause a transient increase in the intracellular [Ca^{2+}] in the neurites (Fig. 2*A,B*) and that Ca^{2+}-activated K^+ conductances are present in the

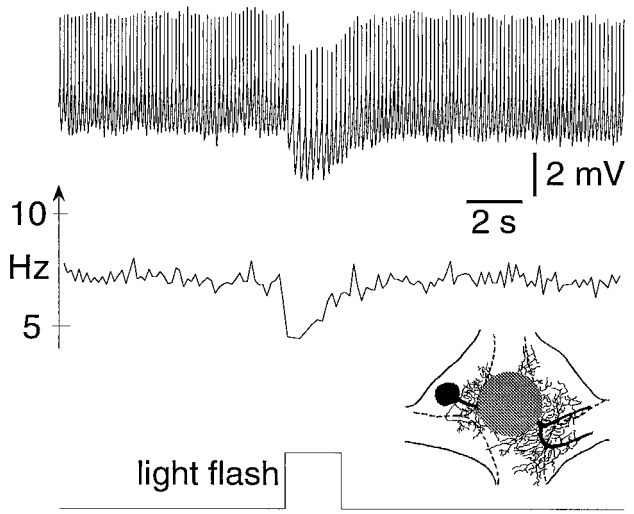

Figure 4. Evidence for dendritic Ca^{2+}-activated K^+ conductances. *Top trace,* The electrical response to calcium released intracellularly in the neurite by photolysis of DM-Nitrophen. *Bottom trace,* The open time of a shutter that allowed ultraviolet light to illuminate the neuropil in the medial packet and release the calcium bound to DM-Nitrophen. Depolarizing current was injected into the soma to hold the baseline firing rate at ~7 Hz (*center trace*). When Ca^{2+} was released inside the AP cell neurite through a flash of light on the neurite in the medial packet, a transient hyperpolarization and a reduction in spike rate was observed. *Inset,* Schematic of the AP cell and outline of the ganglion. The location of the illumination is indicated by the *gray spot* covering the medial packet.

neurites (Figs. 3, 4). How does this intracellular $[Ca^{2+}]$ transient together with the Ca^{2+}-activated K^+ conductances affect the propagation of Na^+ spikes and EPSPs in the neurite? To answer this question, the effect of blocking Ca^{2+} entry on signal propagation was studied.

Spikes are generated in the neurite contralateral to the soma near the bifurcation of the primary process (Melinek and Muller, 1996) and propagate along the axons into the periphery as well as into the neurites and along the primary neurite toward the soma, the site of electrical recording in these experiments. When Ca^{2+} entry was blocked, the spike amplitude and shape, recorded in the soma, changed (Fig. 5*A*). On average, the spike amplitude increased from 19 ± 2 mV in normal saline to 31 ± 3 mV in 0 Ca^{2+}, 1.8 mM Co^{2+} saline, and the spike half-width increased from 8 ± 1 msec in normal saline to 14 ± 1 msec in 0 Ca^{2+}, 1.8 mM Co^{2+} saline (Fig. 5*A*, *inset; n* = 5 cells).

To mimic synaptic inputs in normal and in 0 Ca^{2+}, 1.8 Co^{2+} saline, puffs of glutamate were applied. Pressure injection of glutamate into the neuropil between the medial and contralateral lateral packet (Fig. 5*B*, *bottom inset*) evoked EPSPs in normal saline, which increased in amplitude by 65 ± 8% (Fig. 5*B*, *top inset; n* = 5 cells) when Ca^{2+} entry was blocked in 0 Ca^{2+}, 1.8 Co^{2+} saline (Fig. 5*B*). These data indicate that the calcium entry activates Ca^{2+}-activated K^+ conductances, which attenuate backpropagating spikes and EPSPs.

DISCUSSION

The major findings of the present experiments are: (1) AP cell neurites express voltage-gated Ca^{2+} channels and Ca^{2+}-activated K^+ channels, (2) voltage-gated Ca^{2+} channels are activated by backpropagating spikes as well as EPSPs, and (3) the increase in the intracellular $[Ca^{2+}]$ together with the activation

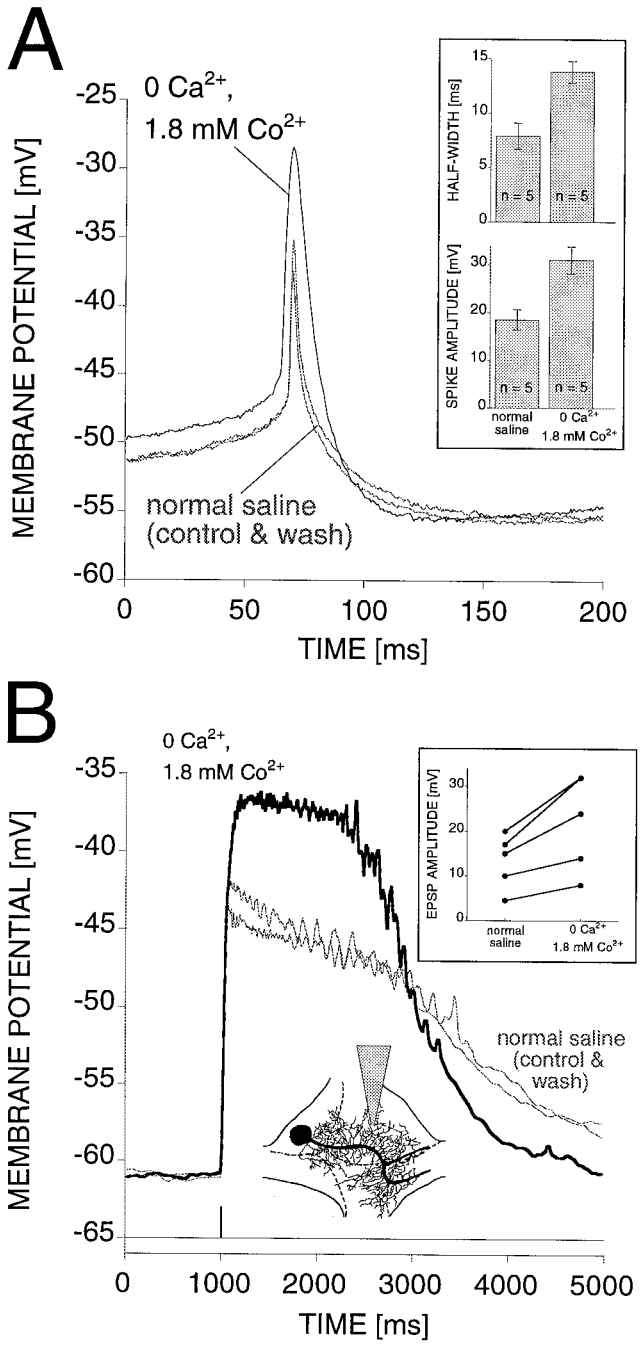

Figure 5. Backpropagating spikes and EPSPs are attenuated by a Ca^{2+}-activated K^+ conductance. *A,* Na^+ spike in normal saline (control and wash) and in 0 Ca^{2+}, 1.8 mM Co^{2+} saline. *Inset,* On average, the spike half-width increased from 8 ± 1 msec in normal saline to 14 ± 1 msec in 0 Ca^{2+}, 1.8 mM Co^{2+} saline, and the spike amplitude increased from 19 ± 2 mV in normal saline to 31 ± 3 mV in 0 Ca^{2+}, 1.8 mM Co^{2+} saline (n = 5 cells). *B,* Responses to glutamate puffs located in the contralateral neurites in normal saline and in 0 Ca^{2+}, 1.8 mM Co^{2+} saline (average of eight trials) for one representative cell. Spikes have been filtered out with a sliding window average (20 msec window width) for clarity. The *bottom trace* indicates the timing of the glutamate puffs. *Bottom inset,* Schematic of the AP cell and outline of the ganglion. The location of the puff pipette is indicated by the *gray triangle* in the contralateral side. *Top inset,* For all five cells tested, the EPSP amplitude increased when the saline was changed from normal saline to 0 Ca^{2+}, 1.8 mM Co^{2+} saline.

of the Ca^{2+}-activated K^+ conductances attenuate backpropagating spikes and EPSPs.

The TEA sensitivity of the Ca^{2+}-activated outward current is consistent with the Ca^{2+}-activated K^+ channel of the BK type, whose activity depends on the intracellular $[Ca^{2+}]$ as well as the membrane potential (for review, see Blatz and Magleby, 1987; Latorre et al., 1989; Sah, 1996). The BK type channel is also known as "maxi-K" channel, and the corresponding macroscopic current is known as I_C. The BK type Ca^{2+}-activated K^+ channel is blocked selectively by nanomolar concentrations of iberiotoxin in mammalian systems (Galvez et al., 1990; Vazquez et al., 1990; Suarez-Kurtz et al., 1991). In contrast, in the leech AP neuron, the Ca^{2+}-activated and TEA-sensitive outward current was not affected by high concentrations of iberiotoxin (300 nM; $n = 5$ cells; data not shown). Such pharmacological differences between mammalian systems and the leech are common (for review, see Kleinhaus and Angstadt, 1995). In particular, charybdotoxin, another blocker of BK type Ca^{2+}-activated K^+ channels, which is less selective than iberiotoxin (for review, see Garcia and Kaczorowski, 1992), has been found previously not to be effective on Ca^{2+}-activated K^+ channels in leech (Johansen et al., 1987; Stewart et al., 1989).

Ca^{2+}-activated K^+ conductances shape action potentials

The role of Ca^{2+}-activated K^+ conductances in shaping somatic action potentials has been demonstrated in the bullfrog sympathetic ganglion B-type cell (Yamada et al., 1998) and in rat hippocampal pyramidal neurons (Storm, 1987). The Ca^{2+}-activated K^+ current (BK type) quickly activates on the entry of calcium through calcium channels during an action potential. It rapidly repolarizes the membrane potential, shutting off in the process. As a result, the Ca^{2+}-activated K^+ current shortens the duration of an action potential (Storm, 1987; Yamada et al., 1998). Similarly, the Ca^{2+}-activated K^+ current shortens the duration of dendritic action potentials recorded from the distal apical dendrite in rat hippocampal pyramidal neurons (Andreasen and Lambert, 1995). In contrast to those studies, in the AP cell the spike amplitude was reduced in addition to the reduced spike width when the Ca^{2+}-activated K^+ current was present (Fig. 5*A*). This difference is possibly caused by the fact that in the AP cell the spike was attenuated in the neurite on its way from the remote spike initiation zone to the somatic recording site, whereas in the vertebrate studies the spike was recorded at the spike initiation zone. Action potentials backpropagate into the dendrite of many neuronal types (for review, see Stuart et al., 1997) and cause an increase in the intracellular $[Ca^{2+}]$ (Jaffe et al., 1992; Callaway and Ross, 1995; Schiller et al., 1995; Spruston et al., 1995; Helmchen et al., 1996). In the light of the evidence for dendritic Ca^{2+}-activated K^+ channels in many of these cell types (Khodakhan and Ogden, 1993; Andreasen and Lambert, 1995; Sah and Bekkers, 1996; Schwindt and Crill, 1997b) it is possible that backpropagating action potentials are attenuated by dendritic Ca^{2+}-activated K^+ channels in mammalian neurons as well.

The effect of the Ca^{2+} current on the membrane potential

When Ca^{2+} ions enter through voltage- or ligand-gated channels, the Ca^{2+} inward current contributes to (1) a depolarization of the membrane potential and (2) the activation of Ca^{2+}-activated K^+ channels. Which of these actions on the membrane potential is the principal role of the Ca^{2+} current is not obvious and may be system-specific, thereby supporting different functions. The observed attenuation of spikes and EPSPs in the AP cell neurite in the presence of Ca^{2+} indicates that the principal role of the Ca^{2+} inward current might be to activate the Ca^{2+}-activated K^+ conductance, rather than to depolarize the AP cell neurite. A similar conclusion came from a study of bullfrog sympathetic ganglion "B"-type cells (Yamada et al., 1998). In these neurons, the contribution of the Ca^{2+} inward current in depolarizing the cell body during an action potential is small, however, the Ca^{2+} inward current activates Ca^{2+}-activated K^+ outward currents, leading to a fast repolarization and spike frequency adaptation. In other systems, however, the principal effect of the Ca^{2+} inward current on the membrane potential might be to depolarize the cell, rather than activating the Ca^{2+}-activated K^+ current. For instance, under normal recording conditions, i.e., without blocking K^+ currents, (1) Purkinje cell dendrites generate Ca^{2+} spikes (Llinas and Sugimori, 1980; Tank et al., 1988), even though Ca^{2+}-activated K^+ channels are present (Khodakhan and Ogden, 1993), (2) rat neocortical pyramidal neuron dendrites generate Ca^{2+} spikes (Kim and Connors, 1993; Schiller et al., 1997; Schwindt and Crill, 1997a; Larkum et al., 1999; Svoboda et al., 1999), even though Ca^{2+}-activated K^+ channels are present (Schwindt and Crill, 1997b), and (3) EPSPs are amplified in rat hippocampal pyramidal neurons by low-voltage-activated Ca^{2+} channels (Gillessen and Alzheimer, 1997), even though Ca^{2+}-activated K^+ channels are present (Andreasen and Lambert, 1995; Sah and Bekkers, 1996).

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