Calcium-Activated Potassium Conductances Contribute to Action Potential Repolarization at the Soma But Not the Dendrites of Hippocampal CA1 Pyramidal Neurons

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Evidence is accumulating that voltage-gated channels are distributed nonuniformly throughout neurons and that this nonuniformity underlies regional differences in excitability within the single neuron. Previous reports have shown that Ca^{2+}, Na^+, A-type K^+, and hyperpolarization-activated, mixed cation conductances have varying distributions in hippocampal CA1 pyramidal neurons, with significantly different densities in the apical dendrites compared with the soma. Another important channel mediates the large-conductance Ca^{2+}-activated K^+ current (I_C), which is responsible in part for repolarization of the action potential (AP) and generation of the afterhyperpolarization that follows the AP recorded at the soma. We have investigated whether this current is activated by APs retrogradely propagating in the dendrites of hippocampal pyramidal neurons using whole-cell dendritic patch-clamp recording techniques.

We found no I_C activation by back-propagating APs in distal dendritic recordings. Dendritic APs activated I_C only in the proximal dendrites, and this activation decayed within the first 100–150 μm of distance from the soma. The decay of I_C in the proximal dendrites occurred despite AP amplitude, plus presumably AP-induced Ca^{2+} influx, that was comparable with that at the soma. Thus we conclude that I_C activation by action potentials is nonuniform in the hippocampal pyramidal neuron, which may represent a further example of regional differences in neuronal excitability that are determined by the nonuniform distribution of voltage-gated channels in dendrites.

Key words: dendrites; hippocampus; calcium-activated potassium conductances; back-propagation; action potentials; patch clamp; calcium

Recent advances in recording techniques have shown that voltage-gated channels are distributed nonuniformly throughout the hippocampal pyramidal neuron; these include Ca^{2+} channels (Elliott et al., 1995; Magee and Johnston, 1995a) and Na^+ channels, which have a relatively constant density throughout the cell but differ in their inactivation properties between the soma and dendrites (Colbert et al., 1997; Mickus et al., 1999). The distributions of the transient A-type K^+ current and the hyperpolarization-activated cation current (I_h) both show dramatic nonuniformity, increasing in density with distance in the apical dendrites (Hoffman et al., 1997; Magee, 1998). The differential distribution of these voltage-gated channels between soma and dendrites yields significant differences in excitability between these two regions. For example, the increased A-current density causes the dendrites to be relatively less excitable than the soma, such that with increasing distance from the soma, dendritic action potential (AP) amplitude progressively decreases, and the threshold for AP initiation increases (Hoffman et al., 1997).

Less is known about the distribution of Ca^{2+}-activated K^+ channels in neurons, in particular the current generated by the large-conductance Ca^{2+}-activated K^+ channel or BK channel. This current (here abbreviated I_C) has been studied with somatic recordings at the hippocampal pyramidal neuron, as well as in

many other neurons (for review, see Sah, 1996; Vergara et al., 1998). The BK channels that underlie I_C generate a high single-channel conductance for K^+, are both voltage- and Ca^{2+}-sensitive, rapidly open with $[Ca^{2+}]_i$ rises in the range of 1–10 μM, and are blocked by charybdotoxin (CTX) and submillimolar concentrations of tetraethylammonium (TEA) (Barrett et al., 1982; Blatz and Magleby, 1984). I_C activation occurs with Ca^{2+} influx induced by APs and contributes to the rapid repolarization of the AP and subsequent fast afterhyperpolarization (AHP) (Lancaster and Adams, 1986; Storm, 1987). This current is distinct from that generated by small-conductance Ca^{2+}-activated K^+ channels (SK), which are Ca^{2+}- but not voltage-sensitive, activate slowly in response to submicromolar rises in $[Ca^{2+}]_i$, show varying sensitivity to apamin, and generate the slow AHP after a train of APs (Lancaster and Zucker, 1994; Sah, 1996; Vergara et al., 1998).
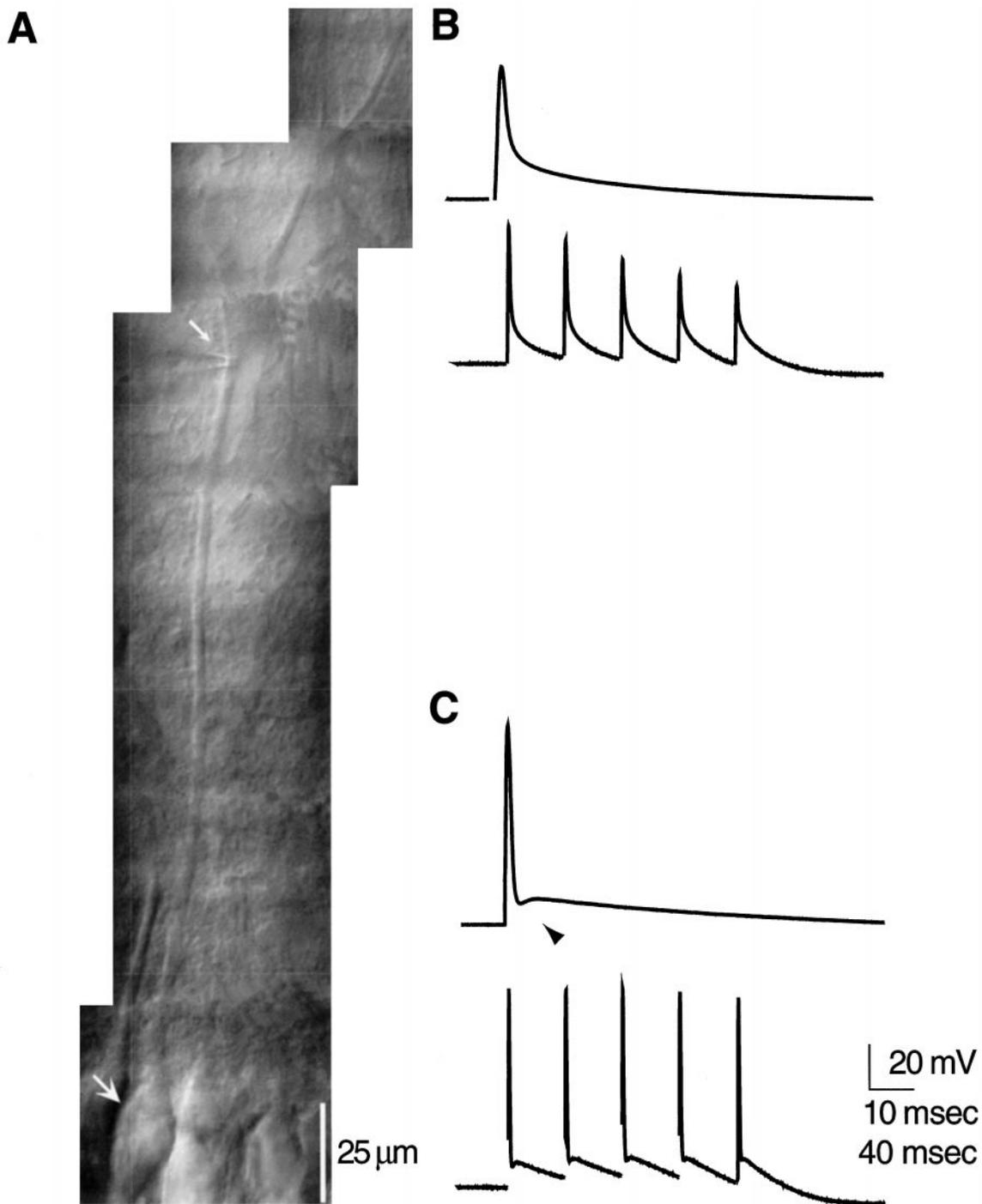
Although the role of I_C in repolarizing the AP is well documented for APs initiated at the soma of hippocampal pyramidal neurons, it is unknown whether this current is present in the dendrites and whether it contributes to the repolarization of the dendritic AP. Since the initial descriptions of retrograde AP propagation in the dendrites (or "back-propagation"), it has been appreciated that dendritic APs differ from somatic APs in that the amplitude of dendritic APs is variable, diminishing with distance from the soma, they lack an afterhyperpolarizing phase, and their repolarization is slower (Andreasen and Lambert, 1995; Spruston et al., 1995; Colbert et al., 1997). We therefore questioned whether I_C is activated by APs back-propagating in the dendrites as it is by APs at the soma. Because we were interested in assessing this functional role of I_C, we used whole-cell patch-clamp recordings in the dendrites of hippocampal CA1 pyramidal

Figure 1. Back-propagating APs recorded in the apical dendrites lack a fast AHP. *A*, Hippocampal CA1 pyramidal neurons were visualized using IR-DIC optics. The *large arrow* points to cell soma; the *small arrow* points to the apical dendrite ~150 μm from the soma, where a patch pipette can be faintly seen. *B*, *Top*, Back-propagating dendritic action potentials (*trace* shown) differ from somatic action potentials in their smaller amplitude, slower repolarization, and lack of a fast AHP. *Bottom*, When APs were repetitively stimulated, dendritic APs decremented in amplitude over the course of the train. *C*, *Top*, Somatic action potentials showed much faster repolarization and a fast AHP (*arrowhead*) after the AP. *Bottom*, When repetitively stimulated, somatic APs had a nearly constant amplitude. Synaptic blocks were omitted in this experiment.

neurons to measure I_C activation by back-propagating action potentials.

MATERIALS AND METHODS

Hippocampal slices were prepared from 6- to 10-week-old male Sprague Dawley rats. Animals were anesthetized with a lethal dose of a combination of ketamine, xylazine, and acepromazine injected intraperitoneally. After being deeply anesthetized, the animal was perfused intracar-

dially with ice-cold modified artificial CSF containing (in mM): 110 choline chloride, 2.5 KCl, 1.25 NaH$_2$PO4, 0.5 CaCl$_2$, 7 MgCl$_2$, 7 dextrose, 3 pyruvic acid, and 1.3 ascorbic acid. The brain was then removed, and 400-μm-thick slices were prepared using a vibratome (Lancer). Hippocampal slices were stored until use in a holding chamber containing external recording solution at room temperature.

Recordings from hippocampal pyramidal neurons were obtained from slices maintained in a submerged chamber at 30–32°C. The external

A soma

B soma

C
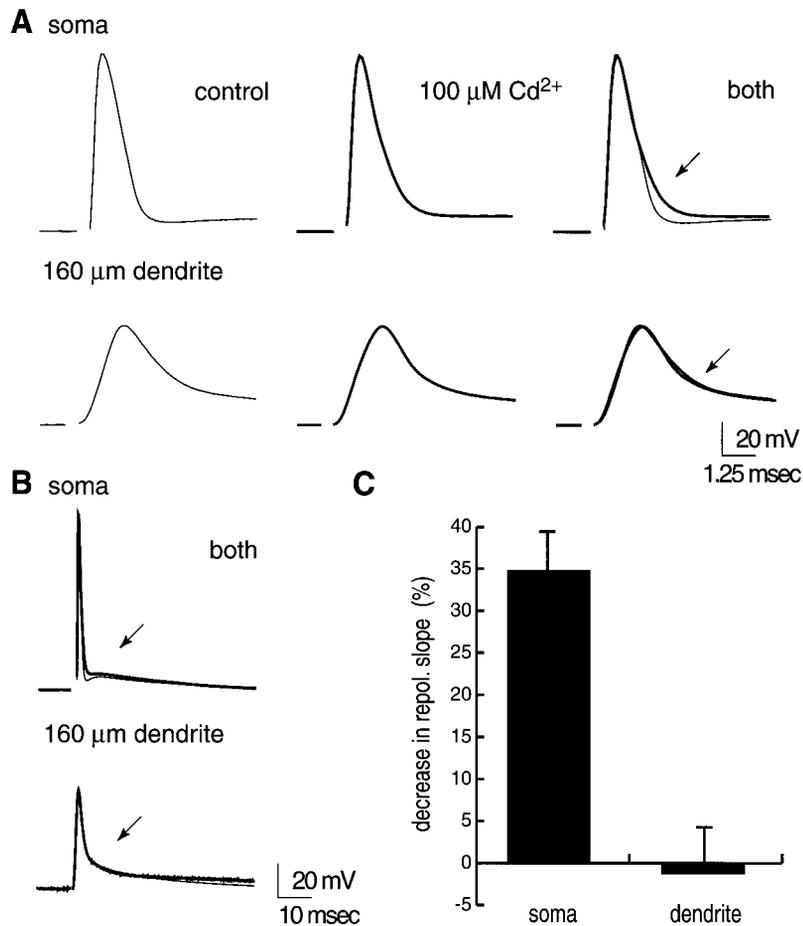
Figure 2. Repolarization of action potentials in the distal dendrites is not affected by Ca^{2+} channel blockade. *A, Top*, Action potentials recorded at the soma are shown under control and 100 μM Cd^{2+} conditions. *Right*, Superimposed APs show that Ca^{2+} channel blockade significantly slowed AP repolarization and blocked the fast AHP (*arrow*). *Bottom*, Repolarization of dendritic APs was not significantly affected by Ca^{2+} channel blockade. *B*, The same responses presented in *A* (*right*) are shown at a longer time scale to show block of the fast AHP (*arrow*) at the soma by 100 μM Cd^{2+}. *C*, Summary of data shows the average decrease in AP repolarization (*repol.*) slope between control and Ca^{2+} channel blockade conditions (grouped data from 100–200 μM Cd^{2+} and from 0 Ca^{2+} and 6 mM Mg^{2+} solutions; $n = 8$ for soma, $n = 9$ for dendrite).

recording solution contained (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH$_2$PO$_4$, 2 CaCl$_2$, 2 MgCl$_2$, and 10 dextrose, bubbled continuously with 95% O$_2$/5% CO$_2$; pH was adjusted to 7.4 with NaOH. The internal recording pipette solution contained (in mM): 120 KMeSO$_4$, 20 KCl, 10 HEPES, 2 MgCl$_2$, 4 Na$_2$-ATP, 0.3 Tris; pH was adjusted to 7.3 with KOH.

Whole-cell patch-clamp recordings were made from hippocampal CA1 pyramidal neuron somata and dendrites that were visualized using infrared differential interference contrast microscopy (IR-DIC; Zeiss Axioskop). Pipettes were fabricated with a Flaming–Brown puller (Sutter Instruments) using borosilicate glass (Fisher Scientific, Houston, TX) and had DC resistances of ~10 MΩ. Whole-cell recordings were obtained with a bridge-mode amplifier (Dagan BVC-700), digitized at 10 kHz (IOTech), and stored with custom data acquisition software. Whole-cell series resistance was 10–25 MΩ for somatic recordings and 15–50 MΩ for dendritic recordings. Recordings without stable series resistance were discarded. Antidromic action potentials were elicited by constant-current pulses (WPI) delivered by a tungsten electrode (AM Systems) placed in the alveus. Recordings are shown with stimulus artifacts truncated for clarity. In some cases, action potentials were elicited by brief depolarizing current pulses delivered through the somatic recording pipette. All drugs were bath-applied. CNQX (10 μM; Research Biochemicals, Natick, MA) and bicuculline methiodide (25 μM; Sigma, St. Louis, MO) were routinely added to the external solution to block postsynaptic potentials. Charybdotoxin (Alomone Laboratories) was prepared and applied in a solution of 0.01% bovine serum albumin (Sigma) to prevent nonspecific binding of the toxin to tubing. Application of bovine serum albumin–containing solution alone had no apparent effect on action potential firing (data not shown).

Changes in somatic action potential repolarization were quantified by measuring the linear slope of repolarization (volts per second) at two time points on the repolarizing phase of the AP: one at 80% of peak AP amplitude (measured peak to baseline) and the other at 20% of peak amplitude. This method allowed measurement of the rate of AP repolarization over a reasonably linear segment of the waveform, as was used by Storm (1987). Dendritic APs were measured similarly except that the

second point was taken at 30% of peak amplitude; because dendritic APs have a slower, more exponential late phase in repolarization, measuring between the 80 and 30% points captured a more linear segment of repolarization. In experiments (see Fig. 6) in which the change in repolarization was compared in the soma and at varying distances along the dendrites, the repolarization slope in both soma and dendrites was measured at the 80–30% points for consistency. Changes in repolarization are shown as the percent decrease in repolarization; that is, slower repolarization is represented by a positive number. Data are reported as mean ± SEM.

RESULTS

Dendritic action potentials lack a fast afterhyperpolarization

Whole-cell patch-clamp recordings were obtained from visualized CA1 hippocampal pyramidal neurons either at the soma or in the apical dendrites at distances up to 200 μm from the soma (Fig. 1*A*). Antidromic activation of single APs or trains of APs by alvear stimulation resulted in an AP that was recorded at the soma [but presumably initiated at the axon initial segment or first node of Ranvier (see Colbert and Johnston, 1996)] and then propagated retrogradely (back-propagated) into the apical dendrites (Spruston et al., 1995). Action potentials recorded in the dendrites differed from their somatic counterparts in several ways (Fig. 1*B,C*). First, the amplitude of single dendritic APs declined with distance from the soma beyond ~70 μm, such that at the mean distal dendritic recording location (174 ± 5 μm; $n = 9$), their amplitude was 57.5 ± 2.7 mV, compared with 98.4 ± 1.1 mV ($n = 8$) at the soma. Second, during a train of APs, the amplitude of dendritic APs declined continually during the course of the
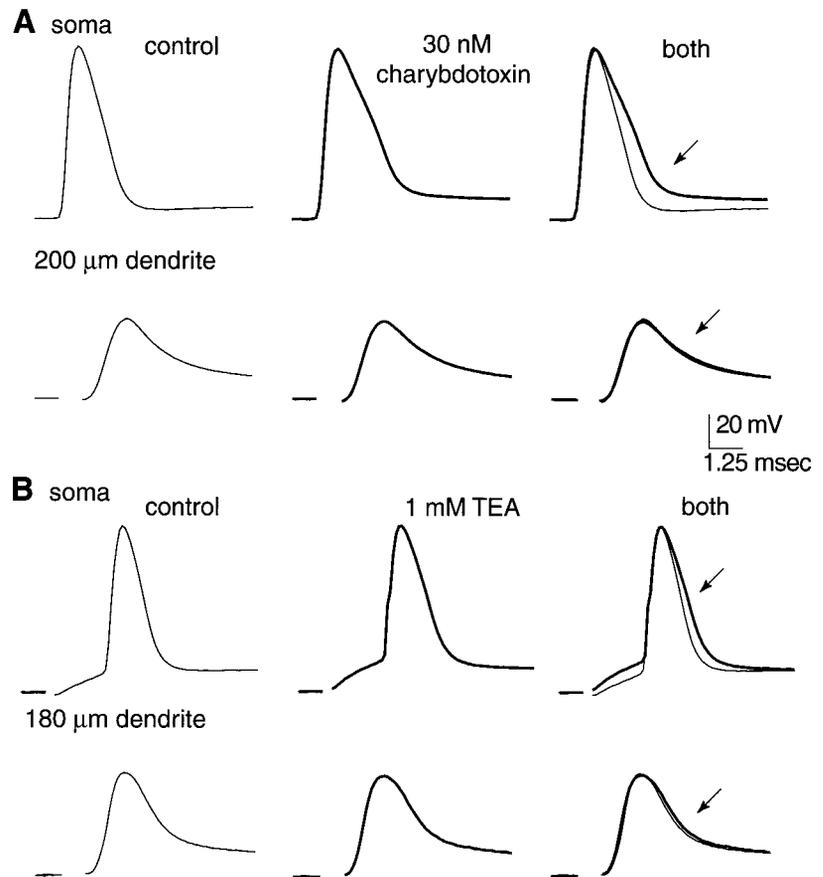
Figure 3. Blockade of Ca^{2+}-activated K^+ currents does not affect repolarization of dendritic action potentials. *A, Top*, Action potentials recorded at the soma showed slowing of repolarization and blockade of the fast AHP (*arrow*) by CTX, an inhibitor of large-conductance Ca^{2+}-activated K^+ conductances. *Bottom*, Repolarization of dendritic action potentials was not affected by CTX. *B*, TEA, in low concentrations, blocks I_C to the exclusion of most other K^+ channels. Application of TEA (1 mM) slowed AP repolarization at the soma (*top*), while having a minimal effect on dendritic APs (*bottom*).

train, whereas the amplitude of somatic APs remained constant. Finally, single dendritic APs had a significantly slower repolarization than did somatic APs and lacked the fast afterhyperpolarizing phase present in somatic APs. These observations were consistent with previous reports on dendritic AP characteristics (Andreasen and Lambert, 1995; Colbert et al., 1997).

Repolarization of action potentials in distal dendrites is not affected by Ca²⁺ channel blockade

Although dendritic action potentials clearly lacked a fast AHP, it was not clear whether Ca^{2+}-activated K^+ currents contributed to dendritic AP repolarization without producing an overt AHP. To study this question, we elicited single antidromic APs in control solution and in solution with 100–200 μM Cd^{2+} added. With action potentials recorded at the soma, the blockade of Ca^{2+} channels with Cd^{2+} caused slowing of repolarization and blockade of the fast AHP (Fig. 2*A,B*). Similar results were also obtained with solutions containing 0 Ca^{2+} and 6 mM Mg^{2+} (data not shown). When this effect was quantified by measuring the decrease in the rate of repolarization (as described in Materials and Methods), the blockade of Ca^{2+} channels caused a 34.7 ± 4.7% (*n* = 8) decrease in repolarization rate. This result was consistent with the previous work of Storm (1987), who found that a decrease of 38 ± 6.3% in repolarization rate appeared to represent the effect of maximal blockade of I_C on AP repolarization at the soma.

In contrast, the repolarization of dendritic action potentials obtained >150 μm from the soma was not affected by the blockade of Ca^{2+} channels with Cd^{2+} (Fig. 2*A,B*). This lack of effect was evident despite prolonged solution superfusion times (up to

30 min) and was similarly observed with 0 Ca^{2+} and 6 mM Mg^{2+} solutions. Quantification of the effect of Ca^{2+} channel blockade on distal dendritic AP repolarization showed a −1.3 ± 5.5% (*n* = 9) slowing of action potential repolarization (i.e., a small increase in repolarization rate that was not significantly different from 0). Comparison of the effects of Ca^{2+} channel blockade on AP repolarization at the soma and distal dendrites (Fig. 2*C*) showed this effect was significantly different between the two groups (*p* < 0.0005). This result shows that for the distal dendrites, Ca^{2+}-activated conductances do not contribute to action potential repolarization as they do at the soma. In dendritic recordings obtained in the proximal dendrites at distances <150 μm from the soma, Ca^{2+} channel blockade had an effect on AP repolarization that was intermediate between that at the soma and in the distal dendrites. The results obtained in the proximal dendrites are further discussed below.

Blockade of Ca²⁺-activated K⁺ channels does not affect repolarization of dendritic action potentials

To determine whether I_C contributes to AP repolarization in the dendrites, we applied blockers of this current and elicited backpropagating APs. As shown in Figure 3*A*, bath application of CTX (30 nM), a potent blocker of BK channels at nanomolar concentrations (Miller et al., 1985), caused significant broadening of the somatic action potential. This effect was fairly rapid in onset (4–5 min after initiation of perfusion) but had a slow and incomplete reversal after washing in control saline (data not shown). Application of CTX caused a 37.1 ± 3.7% (*n* = 3) decrease in the repolarization slope of somatic APs, similar in magnitude to the slowing caused by Ca^{2+} channel blockade. The
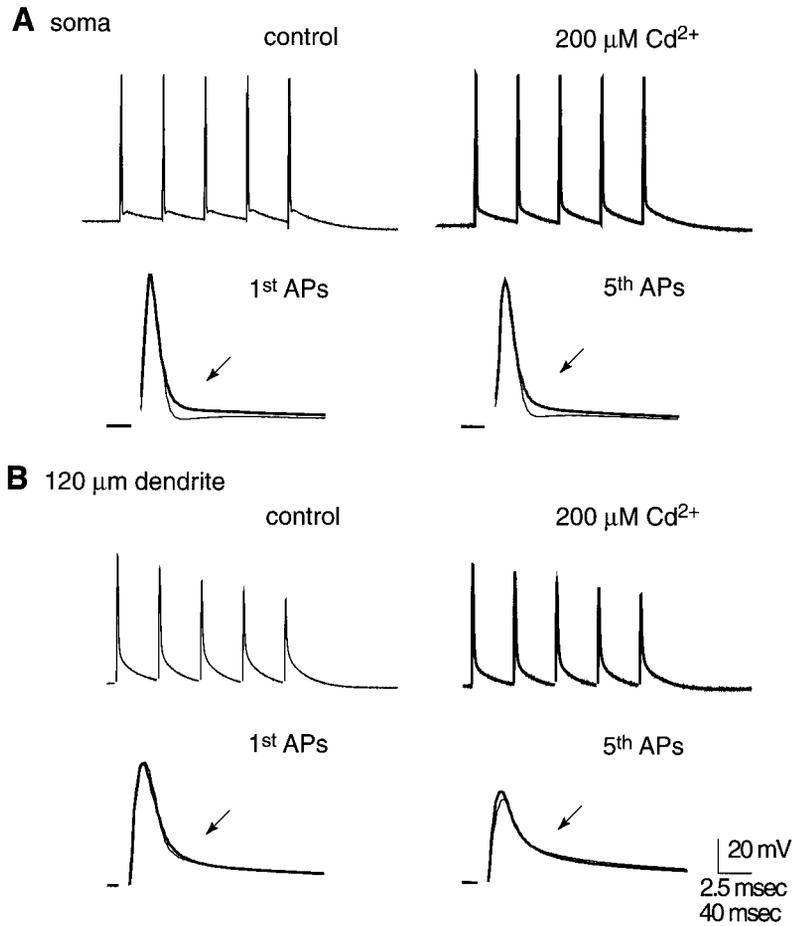
Figure 4. Repetitive firing does not cause activation of dendritic I_C. *A, Top,* Short trains of antidromic action potentials were elicited in control and Cd^{2+} solutions. *Bottom,* Comparison of superimposed APs in control and Cd^{2+} solutions from the beginning (*left*) and end (*right*) of the train shows that Ca^{2+} channel blockade had a similar effect on AP repolarization (*arrow*) at the beginning and end of the train. This suggests that I_C activation was nearly maximal with the first AP. *B,* A similar comparison of dendritic APs shows that I_C was minimally activated at both the beginning (*bottom left*) and end (*bottom right*) of the train of APs.

AP broadening that occurred in CTX differed from that with Ca^{2+} channel blockers in that CTX caused broadening that began at an earlier point on the repolarizing waveform. This difference may stem either from the possible presence of other voltage-gated non-Ca^{2+}–dependent K^+ channels that are sensitive to CTX [such as Kv1.3 (Kaczorowski et al., 1996)] or from the contribution of inward Ca^{2+} currents to the AP waveform, which would be blocked by Cd^{2+} but unaffected by CTX.

In contrast, application of CTX caused no significant change in the AP waveform recorded in the dendrites (Fig. 3*A*), despite perfusion times up to 25–30 min. CTX caused a 1.36 ± 10.1% ($n = 3$) decrease in repolarization rate, which was significantly different from the repolarization slowing at the soma caused by CTX ($p < 0.01$). This result, obtained with a high-affinity blocker of I_C, is a specific demonstration that I_C makes little contribution to the repolarization of dendritic action potentials. Similar effects were obtained with perfusion of TEA at concentrations (0.5–1 mM) that primarily affect I_C (Storm, 1987; Rudy, 1988). As shown in Figure 3*B*, TEA caused somatic AP broadening (29.4 ± 4.7%; $n = 3$) that was similar in appearance to that of CTX. No significant effect was seen when TEA was applied during dendritic recordings (0.22 ± 4.4%; $n = 4$; $p < .005$), further demonstrating that I_C makes little or no contribution to AP repolarization in the distal dendrites.

Repetitive firing does not cause activation of dendritic I_C

Because it was apparent that single back-propagating dendritic APs did not cause activation of I_C, we questioned whether this might be a result of insufficient Ca^{2+} influx during a single dendritic AP. Previous work using imaging of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) had demonstrated that repetitive firing of back-propagating APs led to increases in $[Ca^{2+}]_i$ in CA1 pyramidal neuron dendrites that progressively increased with the number of APs for brief trains of stimuli (Jaffe et al., 1992; Callaway and Ross, 1995). Thus, we investigated whether repetitive firing of back-propagating APs could lead to activation of dendritic I_C.

Trains of five back-propagating APs were elicited by antidromic stimulation at 20 Hz under control and Ca^{2+} channel blockade conditions (Fig. 4). Somewhat surprisingly, there was little change in the somatic AP broadening by Ca^{2+} channel blockers when compared at the beginning and end of the train. In the example shown in Figure 4*A*, application of 200 μM Cd^{2+} caused a fairly constant slowing of repolarization for all APs in the train, with a 39.9% slowing of the first AP and a 41.1% slowing of the last AP in the train. This suggests that activation of I_C at the soma is nearly maximal with a single action potential.

When the effect of Ca^{2+} channel blockade was studied during repetitive firing of dendritic APs, it was found that Ca^{2+} channel blockers continued to have no effect on AP repolarization despite repetitive firing. As shown in Figure 4*B*, comparison of APs elicited at the beginning and end of a 20 Hz train of stimuli shows that Ca^{2+} channel blockade has little or no effect on AP repolarization throughout the train. Thus, despite presumably rising levels of dendritic $[Ca^{2+}]_i$ during repetitive firing, there is no evidence of dendritic I_C activation by back-propagating action potentials.

A soma

B 25 μm dendrite

C 70 μm dendrite

D 120 μm dendrite
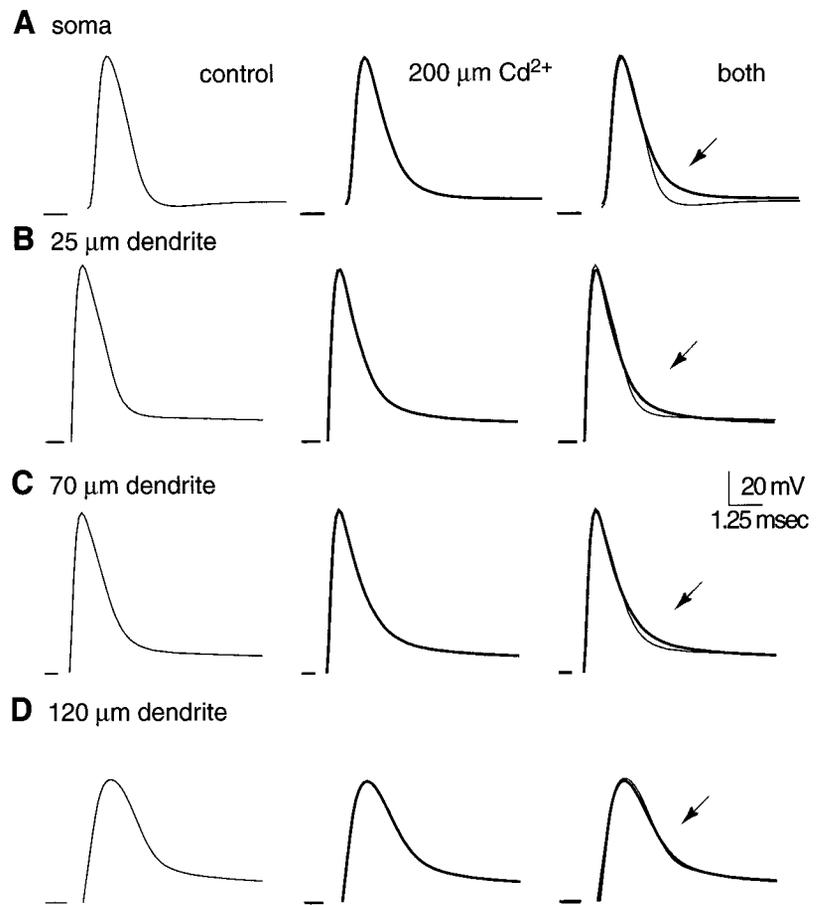
Figure 5. Activation of I_C diminishes in the proximal dendrites. *A*, Action potentials recorded at the soma showed a marked slowing of repolarization in 200 μM Cd^{2+} (*arrow*). *B–D*, Action potentials recorded in the proximal dendrites at progressively greater distances from the soma showed a gradual decrease in the activation of I_C (*arrows*).

Activation of I_C diminishes in the proximal dendrites

One factor that could underlie the absence of I_C activation by action potentials in the distal dendrites might be the diminished amplitude of back-propagating APs with increasing dendritic distance. Because the activation of I_C is both voltage- and Ca^{2+}-dependent, decreased peak AP amplitude would decrease I_C activation both because of the decreased peak depolarization and because of decreased Ca^{2+} influx through intrinsic voltage-gated Ca^{2+} conductances. To investigate this possibility, we studied the relation of I_C activation and AP amplitude in the proximal dendrites where AP amplitude starts to decline from its value at the soma.

Dendritic recordings were obtained in the proximal dendrites at distances between 25 and 150 μm, and the effect of Ca^{2+} channel blockade with 200 μM Cd^{2+} on AP repolarization was measured. Recordings from several distances are shown in Figure 5. Comparing the effects at the soma and at increasing distances in the proximal dendrites, it appears that the effect of Cd^{2+} on AP repolarization is maximal at the soma and then progressively declines with distance in the proximal dendrites. A plot of the decrease in AP repolarization slope in Cd^{2+} versus dendritic distance (Fig. 6*A*) shows that the effect of Ca^{2+} channel blockade decreases continuously with distance, with the decrease in repolarization equaling one-half of the somatic value at a distance of ~70 μm from the soma.

When the amplitude of the back-propagating AP was normalized to its value at the soma and plotted versus dendritic distance (Fig. 6*B*), it can be seen that AP amplitude in the dendrites remains similar to its value in the soma (~100 mV) for the first

~70 μm of the proximal dendrites and then begins to decline such that at 175 μm, AP amplitude is ~0.6 of its value at the soma (or ~60 mV). Comparison of the normalized decrease in AP repolarization with AP amplitude shows that in the first 70 μm of the proximal dendrites, the AP repolarization change progressively diminished while AP amplitude remained nearly constant. For example, at 70 μm, AP amplitude is similar to the somatic value, whereas the change in repolarization is approximately one-half its value at the soma. Thus, the decrease in I_C activation in the dendrites appears unrelated to AP amplitude. It is likely that other factors account for the decline of dendritic I_C activation, such as a decreased density of Ca^{2+}-activated K$^+$ channels in the dendrites or a similar decrease in the density of dendritic voltage-gated Ca^{2+} channels activated by back-propagating APs.

DISCUSSION

In this study we have investigated action potential repolarization in the distal dendrites of CA1 hippocampal pyramidal neurons and have found that, unlike APs at the soma, dendritic APs fail to activate large-conductance Ca^{2+}-activated K$^+$ current. This lack of I_C activation in the distal dendrites was demonstrated by the failure of both Ca^{2+} channel blockade and I_C blockers to affect AP repolarization in the distal dendrites at concentrations that completely blocked I_C at the soma. Repetitive firing of APs likewise failed to activate dendritic I_C. Investigation of AP repolarization in the proximal dendrites showed that I_C activation by single APs progressively diminished within the proximal dendrites and disappeared at a distance of 100–150 μm from the soma. Thus, the activation of I_C by action potentials appears to be
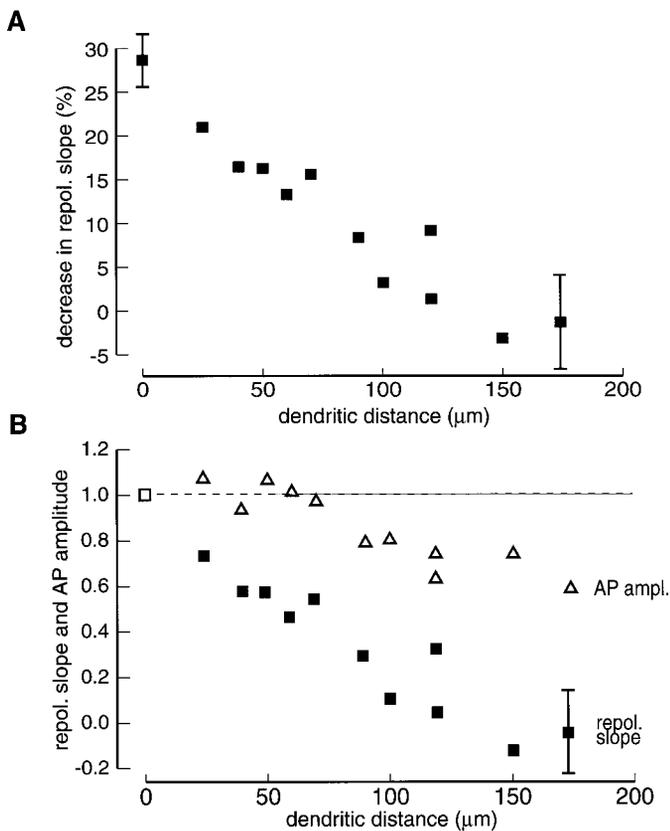
Figure 6. The decrease in dendritic I_C activation does not depend on action potential amplitude. *A*, A plot of the decrease in action potential repolarization rate with Ca^{2+} channel blockade versus distance in the dendrites from the soma. In this case, the repolarization rate at both the soma and dendrites was computed as the slope of AP repolarization measured between 80 and 30% of action potential amplitude. Each point represents a single experiment, with the values at the soma (dendritic distance = 0) and at 174 μm (mean distal dendritic recording location) representing the mean values of experiments at that location ($n = 8$ at the soma; $n = 9$ in the distal dendrites). Note that I_C activation decreased continuously from its value at the soma with increasing dendritic distance. *B*, The decrease in repolarization slope (*squares*) and AP amplitude (*triangles*) normalized to these values at the soma and plotted versus dendritic distance. [The SEM of the mean AP amplitude (*ampl.*) in the distal dendrites was less than the *symbol size*.] In the proximal dendrites up to ~70 μm, AP amplitude was nearly invariant, whereas the activation of I_C declined. Thus, the diminishing activation of I_C in the dendrites was not a function of decreasing AP amplitude.

nonuniform within the CA1 pyramidal neuron, diminishing with distance in the apical dendrites. Because this current is responsible in part for the rapid repolarization of the somatic AP and the fast AHP that follows the AP, its lack of activation in the distal dendrites results in dendritic action potentials that repolarize significantly more slowly than somatic APs and show no fast AHP.

Because I_C activation is both voltage- and Ca^{2+}-dependent, the progressive loss of I_C activation with distance in the dendrites could have several explanations: decreasing AP amplitude, diminished Ca^{2+} influx triggered by back-propagating APs, or a lack of I_C itself in the dendrites. Dendritic AP amplitude decrements with distance from the soma and could affect I_C activation both by decreased membrane depolarization and by decreased Ca^{2+} influx through intrinsic Ca^{2+} conductances driven by the depolarization. This is unlikely to explain the results for two reasons.

First, I_C activation began to decline in the proximal dendrites where AP amplitude was equal to that of the soma (~100 mV); for example, at 70 μm from the soma, AP amplitude is still ~100 mV, but I_C activation had declined to approximately one-half the somatic value. Second, Ca^{2+}-imaging experiments have shown that increases in $[Ca^{2+}]_i$ induced by back-propagating APs are actually larger in the proximal dendrites than in the soma (reaching a peak at ~100 μm from the soma) and thus would have been expected to cause maximal activation of I_C (Jaffe et al., 1992; Callaway and Ross, 1995).

It is possible that I_C activation depends on Ca^{2+} influx through a specific Ca^{2+} channel subtype that is nonuniformly distributed throughout the pyramidal neuron. Marrion and Tavalin (1998) have reported that BK channels in excised membrane patches from hippocampal pyramidal neuron somata are selectively activated by N-type Ca^{2+} channels and not by L- or P/Q-type channels. N-type channels have been found in significant density at the soma but occur at a much lower density in CA1 pyramidal neuron dendrites (Magee and Johnston, 1995a); thus, it is possible that insufficient Ca^{2+} influx is mediated by these channels to activate dendritic I_C. This assumes that BK channels in the dendrites have the same predilection for Ca^{2+} influx through N-type Ca^{2+} channels as do somatic channels and cannot be activated by Ca^{2+} influx through other channel subtypes.

An additional possible explanation for the lack of activation of dendritic I_C depends on the distribution of the underlying conductance itself. The most straightforward hypothesis is that BK channels are primarily confined to the soma or to the soma and proximal dendrites. This would be in agreement with the findings of Knaus et al. (1996) who described the distribution of *slo* (the BK channel gene) mRNA and protein in the hippocampus as confined primarily to the pyramidal cell layer and to mossy and perforant path fibers. Furthermore, *slo* has been found to undergo alternative splicing to yield numerous isoforms with wide variation in their Ca^{2+} sensitivity (Tseng-Crank et al., 1994), so dendritic BK channels might exist as isoforms that are markedly less sensitive to Ca^{2+} than is their somatic counterpart and thus are not activated by dendritic APs.

Other than the present study, there are little previous data that describe the activation of I_C in dendrites. One study using blind intradendritic recordings of presumed pyramidal dendrites found that AP repolarization was sensitive to prolonged application of Co^{2+} in some recordings, although it was not reported at what dendritic distance this observation occurred (Andreasen and Lambert, 1995). Sah and Bekkers (1996) examined decay time constants of IPSCs to infer that slow AHP currents mediated by SK channels were distributed on the proximal dendrites, but this study did not investigate the currents underlying the fast AHP. Previous cell-attached recordings in CA1 pyramidal neuron dendrites have noted the infrequent appearance of BK-like channel openings, usually under circumstances of poor cell health (D. Hoffman, personal communication). This last finding would support the presence of BK channels in dendrites, although at an unknown density and of unknown biophysical properties.

The lack of dendritic I_C activation has a significant effect on the shape of the back-propagating dendritic action potential, causing it to repolarize more slowly. This change in AP shape likely affects dendritic integrative properties. For example, slower dendritic AP repolarization will cause greater inactivation of both dendritic Na^+ and A-type K^+ currents, which may limit the ability of the dendrites to follow fast repetitive firing at the soma. Broad dendritic APs will also have an increased unblocking effect

on the voltage-dependent conductance of NMDA receptors, which can lead to increased Ca^{2+} influx during paired back-propagating APs and synaptic stimuli (Magee and Johnston, 1997; Koester and Sakmann, 1998; Schiller et al., 1998). Furthermore, the lack of I_C may explain in part the lowered dendritic threshold for Ca^{2+} spiking, because these regenerative responses are mediated by high-threshold Ca^{2+} channels that are usually activated on the repolarizing phase of preceding dendritic Na^+ APs (Andreasen and Lambert, 1995; Magee and Johnston, 1995b). Conversely, the localization of BK channels predominantly to the soma and axon terminals, as shown by anatomic data (Knaus et al., 1996), suggests that I_C is most important for regulating the shape of orthodromically propagating action potentials, especially at the axon terminal, where AP width can have significant effects on neurotransmitter release.

Although the relative lack of I_C activation significantly affects dendritic AP shape, it is clearly not the only factor. Dendritic AP back-propagation is also affected by the progressive increase in A-current with dendritic distance, which decreases AP amplitude and raises the threshold for dendritic AP initiation (Hoffman et al., 1997). Under conditions of I_C blockade or both I_C and A-current blockade (Hoffman et al., 1997), dendritic APs still repolarize significantly more slowly in the dendrites than in the soma. This would suggest a relative lack of some other conductance, most likely a rapidly activating delayed rectifier; one possibility would be K^+ channels containing the α subunit encoded by Kv2.1, which have been found to be a significant component of the delayed-rectifier K^+ current in hippocampal neurons and appear to be confined to the soma and proximal dendrites (Murakoshi and Trimmer, 1999). This possibility, along with the present finding of a relative lack of dendritic I_C activation, joins accumulating evidence that voltage-gated ion channels are distributed nonuniformly within the hippocampal pyramidal neuron and that this regional nonuniformity shapes the signal-processing capabilities of the neuron.

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