Mechanisms Underlying Burst and Regular Spiking Evoked by Dendritic Depolarization in Layer 5 Cortical Pyramidal Neurons

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Schwindt, Peter and Wayne Crill. Mechanisms underlying burst and regular spiking evoked by dendritic depolarization in layer 5 cortical pyramidal neurons. *J. Neurophysiol.* 81: 1341–1354, 1999. Apical dendrites of layer 5 pyramidal cells in a slice preparation of rat sensorimotor cortex were depolarized focally by long-lasting glutamate iontophoresis while recording intracellularly from their soma. In most cells the firing pattern evoked by the smallest dendritic depolarization that evoked spikes consisted of repetitive bursts of action potentials. During larger dendritic depolarizations initial burst firing was followed by regular spiking. As dendritic depolarization was increased further the duration (but not the firing rate) of the regular spiking increased, and the duration of burst firing decreased. Depolarization of the soma in most of the same cells evoked only regular spiking. When the dendrite was depolarized to a critical level below spike threshold, intrasomatic current pulses or excitatory postsynaptic potentials also triggered bursts instead of single spikes. The bursts were driven by a delayed depolarization (DD) that was triggered in an all-or-none manner along with the first Na$^+$ spike of the burst. Somatic voltage-clamp experiments indicated that the action current underlying the DD was generated in the dendrite and was Ca$^{2+}$-dependent. Thus the burst firing was caused by a Na$^+$ spike-linked dendritic Ca$^{2+}$ spike, a mechanism that was available only when the dendrite was adequately depolarized. Larger dendritic depolarization that evoked late, constant-frequency regular spiking also evoked a long-lasting, Ca$^{2+}$-dependent action potential (a “plateau”). The duration of the plateau but not its amplitude was increased by stronger dendritic depolarization. Burst-generating dendritic Ca$^{2+}$ spikes could not be elicited during this plateau. Thus plateau initiation was responsible for the termination of burst firing and the generation of the constant-frequency regular spiking. We conclude that somatic and dendritic depolarization can elicit quite different firing patterns in the same pyramidal neuron. The burst and regular spiking observed during dendritic depolarization are caused by two types of Ca$^{2+}$-dependent dendritic action potentials. We discuss some functional implications of these observations.

**INTRODUCTION**

The injection of current into a neuron through a recording microelectrode in the soma was used for decades to study the input–output relation of a single neuron (Granit et al. 1963). The injected current is meant to mimic the summated synaptic current that would reach the soma from sites on the dendritic tree. In a neuron with passive dendrites, the synaptic current reaching the soma would be a linear sum of the synaptic current generated at each dendritic synapse weighted by the distance of the synapse from the soma. In this model the input–output relation evoked by tonic synaptic current and current injected into the soma are equivalent. However, if the dendrites contain voltage-gated ion channels adequate depolarization of the dendrite may activate these channels, thereby altering the current flowing to the soma. In this case the input–output relation evoked by somatic depolarization and dendritic depolarization would not be equivalent. More complex effects are possible. For example, in turtle spinal motoneurons a radically different input–output relation is obtained under conditions where plateau potentials are generated (reviewed by Kiehn 1991). The plateau potential recorded in the soma is thought to be caused by a prolonged Ca$^{2+}$ action potential initiated in the dendrites (Booth et al. 1997).

Although plateau potentials similar to those seen in motoneurons were not observed in layer 5 pyramidal neurons from neocortex, many of the cortical cells exhibit more complex firing patterns than spinal motoneurons when their soma is depolarized. Those layer 5 neurons classified as regular spiking respond to constant suprathreshold current injection with repetitive single action potentials, similar to motoneurons, whereas those classified as intrinsic bursters respond with initial or even repetitive bursts of action potentials (reviewed by Connors and Gutnick 1990). However, if the injected current is maintained the initial burst firing is followed by regular spiking in most recorded cells (see Schwindt et al. 1997 and references therein).

In a previous study we depolarized sites on the apical dendrite of layer 5 pyramidal cells to examine the effect on regular spiking evoked by the simultaneous injection of depolarizing current into the soma (Schwindt and Crill 1996). In that study we obtained results similar to those obtained in motoneurons (Granit et al. 1966; Powers et al. 1992), namely, the steady current transmitted from dendrite to soma summed with the intrasomatic injected current to cause the steady-state input–output relation (which reflected only regular spiking) to undergo a parallel shift along the current axis. Voltage-gated dendritic channels caused a greater transmission of dendritic current to the soma than expected for a passive dendrite.

During a subsequent study (Schwindt and Crill 1997a) we found that stronger dendritic depolarization could evoke dendritic Ca$^{2+}$-dependent action potentials. Brief dendritic depolarizations were used in most cells studied. The use of longer dendritic depolarizations of the same strength produced puzzling results. When a long-lasting dendritic depolarization was applied during voltage clamp of the soma at potentials that prevented Na$^+$ spike initiation in the soma or axon, action currents representing Ca$^{2+}$-dependent dendritic spikes were observed only during the onset of the iontophoresis. In contrast, when the soma of the same cell was not voltage clamped,
repetitive bursts of action potentials occurred for the duration of the dendritic depolarization, not just at its onset. No such repetitive (or even single) bursts could be evoked in the same cell by depolarization of the soma. We hypothesized that the repetitive burst firing evoked by dendritic depolarization was caused by some sort of interaction between somatic Na\(^+\) spikes and dendritic Ca\(^{2+}\) spikes.

We investigated this question further. We confirmed that the firing patterns evoked by dendritic depolarization and somatic depolarization in the same cell can be quite different. These differences were seen more frequently in this study in which we specifically examined the firing patterns evoked by somatic and dendritic depolarization. We also found that depolarizations caused by an intrasomatic current pulse or a synaptically evoked excitatory postsynaptic potential (EPSP) could evoke a burst of spikes if the dendrite was depolarized to a critical subthreshold potential. In addition to the burst firing, stronger dendritic depolarization evoked regular spiking with peculiar properties. We used electrophysiological methods and the application of channel blocking agents to investigate the mechanisms underlying the firing patterns evoked by dendritic depolarization. These mechanisms consisted of Ca\(^{2+}\)-dependent dendritic action potentials whose nature varied with the degree of dendritic depolarization.

**METHODS**

Most methods were similar to those described previously (Schwindt and Crill 1995, 1996, 1997a,b). Sprague-Dawley rats of either sex (28–35 days postnatal) were anesthetized with ketamine (150 mg/kg) and xylazine (10 mg/kg) and killed by carotid section. A coronal slice from midline, corresponding to layer 5 of area HL of sensorimotor cortex, was prepared and maintained as described. Recorded cells lay 1.12–1.26 mm below the pial surface and 2.59–3.15 mm from midline, corresponding to layer 5 of area HL of sensorimotor cortex (Zilles and Wree 1985).

Intracellular recordings were made with the slice submerged in a chamber maintained at 33 ± 0.1°C. Slices were perfused with physiological saline consisting of (in mM) 130 NaCl, 3 KCl, 2 CaCl\(_2\), 2 MgCl\(_2\), 1.25 Na\(_2\)HPO\(_4\), and 10 dextrose saturated with 95% O\(_2\)-5% CO\(_2\), pH 7.4. In some experiments TTX (1 μM, Sigma Chemical, St. Louis, MO) was added to this saline or 200 μM CdCl\(_2\) was substituted for an equimolar amount of CaCl\(_2\), and NaH\(_2\)PO\(_4\) was omitted to avoid precipitation.

Cells were impaled with sharp microelectrodes made from standard 1.0 mm OD borosilicate tubing and filled with 2.7 M KCl (DC resistance 30–40 MΩ). An Axoclamp-2A amplifier (Axon Instruments, Foster City, CA) was used to record membrane potential and inject current in active bridge mode or to control somatic membrane potential in single electrode voltage-clamp mode with a switching rate of 3.5–4.2 kHz (30% duty cycle). Membrane potential and injected current were monitored and usually were filtered at 10 kHz. Membrane potential and current measured in voltage clamp were filtered at 0.1–1 kHz. Signals were amplified and recorded on a multichannel videocassette recorder with pulse code modulation (Neuro-Data, New York, NY). Resting potential was taken as the difference between the intracellular and extracellular DC potentials recorded on a chart recorder.

The second current-clamp amplifier and headstage of the same Axoclamp-2A was used to pass a constant iontophoretic current through a second glass microelectrode that was broken to a tip diameter of ~2 μm and filled with 0.5 M Na glutamate adjusted to pH 7.4 with NaOH. A separate micromanipulator was used to position this iontophoretic electrode, as described previously (Schwindt and Crill 1995, 1996, 1997a,b). The distance between recording and iontophoretic electrodes was measured at the slice surface with a calibrated eyepiece on a dissecting microscope.

Extracellular electrical stimulation near the pial surface employed an optically-coupled, constant-current, stimulus isolation unit coupled to a bipolar electrode consisting of a twisted pair of 0.1 mm O.D. stainless steel wires insulated except for their tips. Pulse durations of 0.1 ms were used. This electrode was positioned 200–500 μm from the intersection of the apical dendrite of the recorded cell with the pial surface as estimated from the extension of a line defined by the positions of the recording and iontophoretic electrodes.

**RESULTS**

**Responses evoked by dendritic depolarization**

Recordings were obtained from 12 cells, each from a different animal, whose resting potential varied from ~68 to ~77 mV (mean: ~72 mV). Input resistance measured with 1-s duration hyperpolarizing current pulses was relatively low (mean: 20 MΩ; range: 11–29 MΩ), suggesting that our recordings were restricted to large layer 5 neurons. In each cell repetitive firing was evoked first by a series of 1-s duration depolarizing somatic current pulses up to 2-nA amplitude. The firing patterns evoked by somatic depolarization were as observed previously in low-input resistance layer 5 cells by Schwindt et al. (1997). Most cells (n = 8) responded to somatic depolarization with a regular-spiking, fast-adapting firing pattern. Four cells responded with a single burst of action potentials at the onset of the current pulse. This single burst was followed by regular spiking for the duration of the pulse. Two of these cells were low-threshold bursters, and two were high-threshold bursters as defined by Schwindt et al. (1997). However, all cells responded differently to dendritic depolarization or to combined somatic and dendritic depolarization, as described below.

Figure 1 illustrates the firing patterns evoked by dendritic depolarization in 9 of 12 recorded cells. Depolarizations that were subthreshold for spike initiation were devoid of periodic synchronous depolarizations (Fig. 1A). The smallest depolarization that evoked any action potentials evoked repetitive bursts of action potentials that were separated from each other by large hyperpolarizing afterpotentials (Fig. 1B). Among the cells tested, the burst consisted of two to four spikes. If the last spike of a burst failed an underlying delayed depolarization (DD) was revealed (DD in Fig. 1C). When the dendritic depolarization was increased above the minimum that evoked any action potentials initial burst firing was followed by regular spiking (Fig. 1D). The epoch of burst firing decreased in duration, and the duration of regular spiking increased as iontophoretic strength was increased (Fig. 1, D–F). Only one or two initial bursts were evoked by the largest iontophoretic current employed, and most of the response consisted of regular spiking (Fig. 1F). Although the duration of regular spiking increased with iontophoretic current strength, the firing rate during the regular spiking did not change significantly. For example, the mean rate during the last 300 ms of regular spiking only varied between 41 and 47 Hz in Fig. 1, D–F.

These responses to dendritic depolarization contrasted with those evoked by somatic depolarization in the same cells. The cell of Fig. 1, like most cells tested (8/12), responded to somatic depolarization only with regular spiking (Fig. 1G).
whose rate increased with current strength (cf. Schwindt et al. 1997). The four additional cells that exhibited a single burst at the onset of somatic depolarization also responded similarly to the cell of Fig. 1 during dendritic depolarization, namely, repetitive bursts were evoked for the duration of the iontophoresis at the minimum suprathreshold dendritic depolarization, and bursting and regular spiking epochs were evoked by larger dendritic depolarizations.

The distinctive, contrasting firing patterns evoked by dendritic and somatic depolarization that are illustrated in Fig. 1 were reported in a previous study (Schwindt and Crill 1997a). In that study, these contrasting firing patterns were observed incidentally in 29% of the cells that were being studied for a different purpose. Our current results indicate that the distinctive firing pattern evoked by dendritic depolarization can be observed much more frequently in layer 5 pyramidal neurons when they are looked for specifically. The rest of this study was directed toward answering the following questions. What causes repetitive burst firing to occur in response to dendritic but not somatic depolarization? Why does burst firing cease and regular spiking commence during larger dendritic depolarizations, and why does the duration but not the rate of regular spiking increase as the dendrite is depolarized further? Most data were collected when the iontophoretic electrode was placed between 370 and 500 μm from the soma (mean distance: 417 μm). In two cells that exhibited responses similar to those shown in Fig. 1 we moved the iontophoretic electrode to ≤150–200 μm of the soma after recording the responses at a more distal location. Only regular spiking, similar to that evoked by somatic depolarization, was evoked by dendritic depolarization. All records are from same cell whose apical dendrite was depolarized by glutamate iontophoresis 407 μm from soma. A, B, and D–F. bottom traces: 1-s duration iontophoretic currents of indicated amplitudes. A: subthreshold response. B: repetitive bursts of action potentials were evoked by the smallest iontophoretic current that caused spiking. C: superposition of the 4 bursts of B aligned on the first spike of each burst. Numbers indicate spike number within a burst; DD, delayed depolarization. Bottom trace: resting potential (~74 mV). D–F: increasing iontophoretic current evoked shorter period of burst firing, which was followed by longer period of regular spiking. G: regular spiking evoked by 1-s duration depolarizing intrasomatic current. H: trace CC shows (at higher gain) single 3-spike burst evoked by iontophoresis of ~20 nA for 300 ms (spikes are truncated). Superimposed horizontal traces (labeled 1 and 2) are membrane potentials recorded when same iontophoretic current was applied during somatic voltage clamp to indicated potentials. Like-numbered middle traces show corresponding membrane current. Voltage calibration in A applies to all but C and H; time calibration to all but C. Vertical bar in A is 1.6 nA for H.
depolarization at these proximal locations. These observations suggest that the burst response may be evoked most readily by depolarizing dendritic membrane ≥300 μm from the soma, but the precise distance required for burst generation was not examined systematically.

It is conceivable that the glutamate iontophoresis excited presynaptic elements whose synchronous firing evoked EPSPs that triggered the bursts of action potentials. According to this idea, the recorded bursts do not result from the activation of a postsynaptic burst firing mechanism but are merely the normal repetitive response to the large postsynaptic current provided by the synchronized EPSPs. These hypothetical EPSPs would have to be large to account for the high-rate firing observed during the bursts (cf. Fig. 1C). Three sets of observations rule out this possibility. First, we always recorded glutamate-evoked depolarizations that were just subthreshold for spike initiation (i.e., for repetitive burst firing), as in Fig. 1A, to ensure these responses were smooth and without sign of periodic synchronous depolarizations. It seemed unlikely that iontophoresis of a few nanoamperes more would suddenly and consistently trigger large synchronized postsynaptic potentials. Second, when applying the same iontophoresis that evoked repetitive burst firing, we usually employed DC hyperpolarization of the soma to bring somatic membrane potential below Na+ spike threshold to ensure that the underlying depolarization lacked periodic synchronous depolarizations. It is difficult to imagine how hyperpolarization of the postsynaptic cell could suppress the firing of synchronized presynaptic neurons. Finally, in four of the cells we voltage clamped the soma to subthreshold potentials while applying the same iontophoretic current that evoked burst firing. During an iontophoresis that evoked a burst the somatic membrane current recorded during voltage clamp was smooth over the whole range of subthreshold membrane potentials (Fig. 1H). Although depolarizing amplification of the current was apparent (cf. Schwindt and Crill 1995), there was no sign of periodic, synchronous, inward currents that would be expected from periodic, synchronous, excitatory synaptic input. The glutamate-evoked dendritic depolarizations are completely abolished by blockade of ionotropic excitatory amino acid receptors (Schwindt and Crill 1998), and specific metabotropic glutamategic agonists evoke quite different responses in these cells (Greene et al. 1994). Therefore the responses of Fig. 1 are best explained as the result of postsynaptic voltage-gated mechanisms triggered by dendritic depolarization caused by current flowing through ionotropic glutamategic channels.

Bursts evoked by combined somatic and dendritic depolarization

Although long-lasting somatic depolarization could not duplicate the firing pattern evoked by long-lasting dendritic depolarization, a somatic depolarization combined with a subthreshold dendritic depolarization could duplicate the response obtained by a larger dendritic depolarization. For example, the combination of a subthreshold dendritic depolarization (Fig. 2A) and a subthreshold somatic depolarization (Fig. 2B, trace 1) could evoke repetitive burst firing (Fig. 2B, trace 2). In fact, in each cell tested (n = 7) firing patterns similar to those evoked solely by dendritic depolarization in Fig. 1, B–F, could be duplicated by combining adequate subthreshold dendritic depolarization with increasing, but still subthreshold, somatic depolarization, although firing caused solely by somatic depolarization evoked only regular spiking in these same cells.

In addition, the combination of somatic and dendritic depolarization could evoke firing patterns not elicited by either stimulus alone. Neither somatic nor dendritic depolarization alone could elicit burst firing in 3 of 12 cells. The responses of each of these cells to dendritic depolarization were similar to those illustrated in Fig. 2, C and D, namely, only regular spiking could be evoked as iontophoretic current was increased to the largest value tested. In contrast, a combination of subthreshold dendritic depolarization (Fig. 2E) and subthreshold somatic depolarization (Fig. 2F, trace 1) evoked repetitive bursts (Fig. 2F, trace 2). Increasing either the dendritic depolarization or the somatic depolarization evoked initial bursts followed by regular spiking, similar to the responses in Fig. 1, D–F (data not shown). These observations indicate that adequate dendritic depolarization can enable burst firing even if it does not trigger it directly.

We found previously that adequate dendritic depolarization can evoke broad dendritic Ca2+ spikes (Schwindt and Crill 1997a). The depolarizing current that reaches the soma during these Ca2+ spikes might cause a somatic depolarization that was large and long enough to trigger the several Na+ spikes that constitute a burst. Thus the simplest hypothesis to explain the burst firing is that directly evoked, repetitive, dendritic Ca2+ spikes underlie the repetitive bursts of Na+ spikes. However, the results illustrated in Fig. 2 suggest that a more complicated mechanism is involved because the dendritic depolarization in those experiments was subthreshold for the initiation of any spikes. The results illustrated in Fig. 3 provide further evidence that neither Ca2+ spikes nor burst firing is triggered directly by the dendritic depolarization. Rather dendritic depolarization enables the generation of burst firing by a less direct mechanism.

In the experiment of Fig. 3 a subthreshold dendritic depolarization was employed together with a brief intrasomatic current pulse. A suprathreshold current pulse triggered a single spike followed by an afterhyperpolarization (AHP) when injected at resting potential (Fig. 3, A and B) or during the early portion of the subthreshold dendritic depolarization (Fig. 3C). When the current pulse was injected during the peak of the subthreshold dendritic depolarization, it evoked a burst of action potentials instead of a single spike (Fig. 3D). The dendritic depolarization alone evoked no spikes, but some minimum dendritic depolarization was required to enable the intrasomatic pulse to evoke a burst of spikes. Only the single spike and AHP were evoked if the somatic current pulse was applied during the peak of a smaller dendritic depolarization (data not shown) or if the soma was depolarized to the same membrane potential by DC injected current in the absence of iontophoresis (Fig. 4A). Similar results were obtained in each cell tested with either an intrasomatic current pulse (n = 8) or an EPSP evoked by electrical stimulation near the pial surface (see METHODS, n = 3, data not shown).

During repeated trials of the somatic current pulse (or the EPSP in other experiments), spikes comprising the burst sometimes failed, and an underlying DD was revealed (DD indicated by arrow in Fig. 4B), as was also observed during bursts evoked by suprathreshold dendritic depolarization (Fig. 1C). Figure 4B shows that the burst and DD were triggered in an
all-or-none manner. In the five superimposed sweeps of Fig. 4B, membrane potential either decayed back to baseline if the current pulse-evoked depolarization did not reach spike threshold or the whole burst and DD were triggered together with the first Na+ spike. The all-or-none nature of the DD itself is shown in a different way in Fig. 4C. In these experiments (n = 3) the depolarizing current pulse was always large enough to trigger the first Na+ spike. When the depolarized pulse was
followed by a sufficiently large hyperpolarizing current pulse, the DD itself failed in an all-or-none manner. These results indicate that the DD is a kind of small action potential. It is triggered by a Na\(^+\) spike when the dendrite is adequately depolarized, and it provides the depolarization that triggers the “extra” Na\(^+\) spikes that form the burst.

**Na\(^+\) spike-triggered dendritic Ca\(^{2+}\) spike underlies the bursts**

We hypothesized that the DD represented a Na\(^+\)–spike-triggered dendritic spike. This was tested with the use of somatic voltage clamp, as illustrated in Fig. 5. Figure 5A, traces 1, shows at high gain and fast sweep a portion of the same iotophoretically evoked, subthreshold depolarization as Fig. 4B and the burst that could be triggered in an all-or-none manner by a brief somatic injected current pulse (traces 2 and 3). In Fig. 5B the mode-switching capability of the Axoclamp amplifier was used to suddenly switch from current- to voltage-clamp recording after the first Na\(^+\) spike of the pulse-evoked burst. To reduce noise and better visualize the recorded membrane current it was convenient to use the Axoclamp’s built-in low-pass filter, which filters voltage as well as current. This filtering accounts for the apparently attenuated spikes and rounded current pulses in Fig. 5B. When voltage clamp was initiated after the first spike of the burst, a slow inward current was recorded (Fig. 5B, traces 2), but this inward current was absent when the current pulse was subthreshold for spike initiation (Fig. 5B, traces 1). (The initial downward deflection is capacitative current artificially lengthened by the 100-Hz filtering.) Thus the inward current was dependent on the occurrence of the preceding spike, and it was generated in an all-or-none manner similar to the preceding Na\(^+\) spike, that is, the inward current represents the all-or-none action current responsible for the DD. Because somatic (thus axonic) membrane potential was kept well below Na\(^+\) spike firing level during the somatic voltage clamp (see voltage traces in Fig. 5B), this action current must have arisen in the dendrite.

A slow, all-or-none inward current that depends on the generation of a preceding Na\(^+\) spike is unusual. Control experiments of the type illustrated in Fig. 5C and D, were carried out on each cell to ensure that this current was not an artifact, i.e., that the voltage clamp was working properly. When the voltage clamp was delayed until the hyperpolarizing afterpotential after the burst, the expected outward membrane current was recorded (Fig. 5C, traces 2). The same was true when the voltage clamp was imposed after single or multiple spikes evoked from resting potential by an intrasomatic current pulse in the absence of iotophoresis. Only the expected outward membrane current was recorded (Fig. 5D, traces 2). This latter result shows that the spike-linked action current is triggered only when the dendrite is depolarized, as also was found for the pulse-evoked burst and the DD.

To learn if a Na\(^+\) spike-linked dendritic spike also was responsible for the repetitive bursts evoked by suprathreshold dendritic depolarization, we employed the same experimental strategy. Figure 6 illustrates our results. Figure 6A shows a single, three-spike burst evoked during a short iotophoresis. A prolonged iotophoresis of the same strength evoked repetitive bursts (cf. Fig. 1D). The voltage clamp was switched on at different delays before and after this burst. In Fig. 6B, traces 1, the voltage clamp was turned on just before the burst was expected to occur, and no inward current was evoked. (Again note that the initial downward deflection is the filtered capacitative current.) When the voltage clamp was delayed until after the first spike of the burst (Fig. 6B, traces 2), a slow inward action current, similar to that of Fig. 5B, was recorded. This result also shows that the action current depended on the occurrence of the preceding Na\(^+\) spike and, because somatic membrane potential was kept well below spike threshold by voltage clamp, the action current arose in the dendrites. When the voltage clamp was turned on after the second spike of the burst (Fig. 6C, traces 2), the inward current also was observed, but it was smaller than after the first spike. Probably the inward current is triggered only by the first Na\(^+\) spike and decays subsequently because the current-generating mechanism is refractory. No inward current was observed when the voltage clamp was turned on after spikes that comprised the burst. Figure 6D is a control to show that the expected outward current was observed when the voltage clamp was turned on during the hyperpolarizing afterpotential.

Results identical to those illustrated in Figs. 5 and 6 were obtained in three cells tested in voltage clamp, except that the amplitude of the action current varied from cell to cell (from...
Na$^+$ spike-linked action current is carried by Ca$^{2+}$

Because the Na$^+$ spike-linked action current was smaller and slower than that expected from a Na$^+$ spike, we suspected that it was caused by a Ca$^{2+}$ spike. This idea was tested by blocking voltage-gated Ca$^{2+}$ currents with Cd$^{2+}$ (200 μM; see METHODS). The addition of Cd$^{2+}$ did not abolish the bursting evoked by dendritic depolarization, but the burst changed character. As illustrated in Fig. 7A, the bursts evoked in Cd$^{2+}$ contained more spikes, were of longer duration, and exhibited less repolarization between spikes than the bursts evoked in physiological saline. In addition, a somatic current pulse that evoked only a single spike in control solution evoked a burst in an all-or-none manner in the Cd$^{2+}$-containing solution. This increased excitability in Cd$^{2+}$-containing solution probably results from the concomitant reduction of Ca$^{2+}$-dependent K$^+$ currents (Friedman and Gutnick 1989).

Although burst firing was enhanced rather than eliminated by blocking voltage-gated Ca$^{2+}$ channels, it seemed possible that a different depolarizing current sustained the bursts. This idea was tested by repeating the voltage-clamp experiments in the Cd$^{2+}$-containing solution. The records in Figs. 5 and 6. Figure 7C shows, in bridge mode, the burst that was evoked all-or-none by a somatic current pulse applied during a subthreshold depolarization in the Cd$^{2+}$-containing saline. In contrast to results obtained in physiological saline, turning on the voltage clamp after the first spike of the burst revealed no inward current (Fig. 7D). The postspike membrane current was...
the same as when no burst was evoked. We obtained the same result when the burst was evoked by suprathreshold dendritic depolarization. Figure 7E shows the evoked burst in bridge mode. Figure 7F shows that voltage clamp after the first spike evoked a membrane current (traces 2) that was not significantly different from that evoked by clamping just before the spike was expected (traces 1). The same result was obtained in a second cell tested in both normal saline and Cd\(^{2+}\) alternative mechanism, a likely candidate is the persistent Na\(^+\) dendrite because bursts were evoked both by dendritic and spike was expected (significantly different from that evoked by clamping just before the first spike of the burst (traces 1) and just after the first spike (traces 2) to maintain somatic membrane potential below Na\(^+\) spike threshold. A slow inward membrane current (trace 2, bottom) was recorded only when the spike occurred. C: similar to B, but voltage clamp (VC) was turned on after the second spike of the burst (traces 2), and a smaller inward membrane current than in B (trace 2, bottom) was recorded. D: turning on the voltage clamp during the AHP after the burst resulted in an outward membrane current. Voltage calibration in A applies to A–D; current calibration applies to B–D.

Long-lasting dendritic action potential underlies late regular spiking

To gain insight into the mechanism underlying the transition to regular spiking, we applied an iontophoresis large enough to evoke initial bursts followed by regular spiking (Fig. 8A), and we then hyperpolarized the soma by DC-injected current to force somatic membrane potential below Na\(^+\) spike threshold. During intermediate hyperpolarizations, illustrated in Fig. 8B, bursting ceased and firing rate slowed. During the larger hyperpolarization of Fig. 8C, membrane potential was maintained below Na\(^+\) spike threshold, and an early oscillation and a late prolonged response (which we will refer to as the “plateau”) were revealed. Both of these responses were identified as action potentials because both could be evoked in an all-or-none manner. For example, decreasing the iontophoretic current from −70 to −60 nA evoked only a subthreshold depolarization (Fig. 8D, trace 1). The all-or-none initiation of plateau potentials is also shown in Figs. 10, B–D, and 11.

The plateau evoked by the −90-nA iontophoresis in Fig. 8D was of longer duration than the plateau evoked by the −70-nA iontophoresis in Fig. 8C, but both plateaus had the same amplitude. In contrast, the depolarization preceding the action potentials was graded with iontophoretic strength (compare membrane potentials at arrow in Fig. 8D, traces 1 and 2). Figure 10B illustrates in another cell how the duration but not the amplitude of a plateau evoked at resting potential increased with iontophoretic strength. The duration of the plateau in Fig. 8C is shorter than the period of regular spiking in Fig. 8A, but this reflects the influence of somatic polarization on plateau duration. Figure 11C shows the opposite effect. Somatic depolarization caused a longer plateau. The effect of somatic polarization on plateau duration could be countered by altering calcium current. Voltage calibration in A applies to A–D; current calibration applies to B–D.
FIG. 7. Spike-linked, slow, inward, dendritic current is absent after Cd2+ channel blockade. Records from same cell as Fig. 1 taken 7.5–9.5 min after changing to saline containing 200 μM Cd2+. A: burst evoked by iontophoresis (−25 nA) just before Cd2+ application (trace 1) and 7.5 min in Cd2+ -containing saline (trace 2). Cell hyperpolarized in Cd2+, and a larger (~32 nA), longer iontophoretic current was required to evoke the burst. B: burst evoked all-or-none by intrasomatic current pulse in Cd2+. C: fast sweep, high gain record of current pulse-evoked burst (recorded in bridge mode with 10-kHz filtering) during a subthreshold iontophoresis (~15 nA) in Cd2+ (spikes are clipped). D: records obtained in discontinuous mode at 3.6 kHz with both voltage and current filtered at 100 Hz. Trace 1 (top): pulse-evoked burst with no subsequent voltage clamp. Trace 2: voltage clamp (VC) was turned on after first spike of burst. Trace 3: membrane potential during subthreshold iontophoresis with no evoked burst. Voltage also was clamped to this potential to compare evoked membrane currents with and without preceding burst. Like-numbered lower records show corresponding currents. E: fast sweep, bridge mode (10-kHz filter) recording of burst evoked by iontophoresis in Cd2+ (as in A, trace 2). F: discontinuous mode recording with filtering as in D. In trace 1 voltage clamp (VC) was turned on just before the burst was expected. In trace 2 voltage clamp was turned on after first spike of burst. In trace 3 voltage clamp was turned on during repolarization of membrane potential after spikes. Like-numbered lower records show corresponding membrane current. No spike-linked inward current is apparent in D or F. Calibrations in C apply to C–F.

FIG. 8. Long-lasting dendritic action potential underlies late regular spiking evoked by dendritic depolarization. Records from cell whose apical dendrite was depolarized by glutamate iontophoresis 389 μm from soma. A: iontophoresis-evoked initial bursts followed by regular spiking. B and C: soma was hyperpolarized to eliminate Na+ spikes (truncated in all panels) by injection of DC hyperpolarizing current while same iontophoresis was repeated. When membrane potential was maintained below Na+ spike threshold in C, a short and long action potential was revealed. D: responses evoked at same hyperpolarized membrane potential as C by smaller (~60 nA) and larger (~90 nA) iontophoretic currents, which evoked responses numbered 1 and 2, respectively. Calibrations in B apply to all panels.
evoked the epoch of regular spiking. The shorter action potential that preceded the plateau in Fig. 8C was not observed in every cell tested (see Fig. 10). Because of their amplitude and duration, we assume the shorter action potential is a Ca\(^{2+}\) spike of the same type that we evoked in isolation with a shorter iontophoresis in an early study (Schwindt and Crill 1997a). When it was present (6/8 cells), its amplitude was similar to that of the subsequent plateau (see Figs. 8C and 11A). Mean plateau amplitude (taken as the difference between the plateau and the just-subthreshold response) was 7.6 mV (range 4.7–12.0 mV, n = 8). These plateaus were evoked by iontophoresis between 389 and 481 mC.

Our observations suggest that the plateau potential underlies the epoch of regular spiking because the plateau was evoked only by dendritic depolarization of sufficient strength to cause burst firing to cease and regular spiking to commence. On the basis of plateau properties, it is easy to understand why the duration but not the rate of regular spiking would increase with further dendritic depolarization (cf. Fig. 1, D–F). Increased dendritic depolarization increased plateau duration but did not alter plateau amplitude (Figs. 8, C and D, and 10B). Thus the amplitude of the depolarizing current that the plateau delivers to the soma is unchanged, and a constant current results in a constant firing rate. However, why do not the Na\(^{+}\) spikes evoked by the plateau trigger burst as they do during smaller dendritic depolarizations? The answer to this question was provided by experiments illustrated in Fig. 9.

As shown in Fig. 9, A and B, an intrasomatic current pulse applied during the plateau triggered only a single spike. This spike was followed by an AHP instead of a DD and a burst. When the spike was evoked during the smooth depolarization before the plateau developed, it was followed by a DD (arrow in Fig. 9C). This DD did not evoke a burst in Fig. 9C only because DC hyperpolarization kept membrane potential during the DD below Na\(^{+}\) spike threshold. This result was obtained in each of four cells tested. Thus the Na\(^{+}\) spike-linked Ca\(^{2+}\) spike burst mechanism is unavailable during the plateau depolarization, perhaps because the Ca\(^{2+}\) channels are already fully activated to cause the plateau.

The plateau is a Ca\(^{2+}\) action potential

That the plateau and the preceding Ca\(^{2+}\) spike were of similar amplitude suggested that the plateau was simply a prolonged Ca\(^{2+}\) spike. This idea was tested by blocking voltage-gated Ca\(^{2+}\) currents with Cd\(^{2+}\). In the cell of Fig. 10 adequate iontophoresis at resting potential evoked regular spiking, and a DC hyperpolarization of the soma revealed an underlying plateau potential (Fig. 10A). The duration and amplitude of the plateau were stable at a fixed suprathreshold iontophoresis (Fig. 10C, Control), and the plateau was abolished by adding 200 \(\mu\)M Cd\(^{2+}\) to the perfusate (Figs. 10C, +Cd). No plateau could be evoked with iontophoretic currents up to –100 nA in the presence of Cd\(^{2+}\) (not shown), whereas plateaus similar to those evoked by the same iontophoretic currents in control solution were recorded after washout of Cd\(^{2+}\) (Fig. 10D). The abolition of plateau potentials by blockade of voltage-gated Ca\(^{2+}\) channels was observed in two other cells tested in this study and in cells recorded by a different method in a subsequent study (Oakley et al. 1998).

Further evidence that the plateau depends on Ca\(^{2+}\) influx through voltage-gated channels is provided by the observation that they can be evoked in an all-or-none manner by dendritic depolarization in the presence of TTX (Oakley et al. 1998; Schwindt and Crill 1997a) and after the blockade of N-methyl-D-aspartate–preferring glutamate channels (Oakley et al. 1998). Nevertheless, inward currents through both Ca\(^{2+}\) and Na\(^{+}\) channels are sometimes necessary either for the generation of the plateau or for its observation at the soma, as shown in Fig. 11.

Figure 11A shows the slow spikes and the subsequent plateau evoked by dendritic depolarization during DC hyperpolarization of the soma in physiological saline. After TTX application (1 \(\mu\)M), both the slow spikes and plateau were abolished, and they could not be triggered by depolarization of the soma during the iontophoresis (Fig. 11B). (This somatic depolarization was an attempt to increase dendritic depolarization indirectly and thereby test whether the dendritic Ca\(^{2+}\) spikes were actually blocked or whether the threshold for their initiation had risen above the depolarization provided by the iontophoresis alone.) After return to physiological saline, the slow spike and plateau reappeared and again could be triggered in an all-or-none manner (Fig. 11C, traces 1 and 2). DC depolarization of the soma caused the plateau to become longer and larger (Fig. 11C, –71 mV). Sufficient depolarization resulted in the generation of small (partially recovered) Na\(^{+}\) spikes fol-

**FIG. 9.** Burst-generating mechanism is unavailable during plateau action potential. All records from cell of Fig. 8. A: 2 superimposed plateau action potentials evoked by iontophoresis of –50 nA during DC hyperpolarization of soma to same value (–86 mV) as in Figs. 8, C and D; intrasomatic current pulse injected during the plateau on 1 sweep evoked only a single spike followed by AHP. B: spike of A shown at faster sweep speed. C: current pulse injected before the onset of the plateau evoked a spike followed by a DD. Voltage and injected current calibrations in A apply to all panels.
lowed by repetitive oscillations (Fig. 11C, −65 mV). When Cd\(^{2+}\)-containing saline (200 μM) was applied, the small spike and plateau were abolished rapidly (Fig. 11D, traces 1 and 2). They could not be evoked by simultaneous DC somatic depolarization (Fig. 11D, −70 mV), although adequate somatic depolarization evoked larger (more-recovered) Na\(^{+}\) spikes (Fig. 11D, −65 mV). Because the spike and plateau of Fig. 11 had small amplitudes, it is possible that they were more vulnerable to Na\(^{+}\) current blockade than more robust Ca\(^{2+}\)-dependent responses would be.

**DISCUSSION**

We confirmed our earlier finding (Schwindt and Crill 1997a) that the firing patterns evoked by dendritic and by somatic depolarization in the same layer 5 pyramidal neuron can be quite different. Specifically, most (5/8) cells in this study that exhibited no bursting whatsoever during somatic depolarization exhibited the repetitive bursts illustrated in Fig. 1 during dendritic depolarization. Four additional cells that responded with a single initial burst (followed by regular spiking) during somatic depolarization also exhibited repetitive bursts and the

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**FIG. 10.** Plateau action potential is Ca\(^{2+}\)-dependent. Records from cell whose apical dendrite was depolarized by glutamate iontophoresis 444 μm from soma. A: superimposed records of repetitive firing evoked by iontophoresis at resting potential (−76 mV) and plateau potential revealed by DC somatic hyperpolarization to −79 mV during same iontophoresis. B: superimposed responses evoked by indicated iontophoretic currents. Currents >25 nA triggered plateaus that arose earlier and were of longer duration. Na\(^{+}\) spikes (truncated) were triggered during the plateau on 2 sweeps. C: superimposed records showing four consecutive sweeps (Control 4X) of plateau evoked by −40 nA iontophoresis just before switching to perfusate containing 200 μM Cd\(^{2+}\) and 2 responses (+Cd\(^{2+}\) ×2) recorded 3 min apart in Cd\(^{2+}\). D: superimposed records taken 15 min after return to control perfusate showing responses evoked by indicated iontophoretic currents. Calibrations in B apply to all panels.

**FIG. 11.** Blockade of Na\(^{+}\) or Ca\(^{2+}\) current eliminated slow spikes and plateau action potentials. Records from cell whose apical dendrite was depolarized by glutamate iontophoresis 481 μm from soma. A: initial slow spikes and plateau evoked all-or-none by iontophoresis during DC hyperpolarization of soma just before TTX application. B: responses evoked by larger iontophoresis after TTX application at different soma potentials maintained by DC current injection. C: responses evoked 13.5 min after return to physiological saline at different soma potentials maintained by DC current injection. Two iontophoretic currents were applied at −76 mV; −25 nA evoked a subthreshold depolarization (traces 1), and −30 nA evoked a slow spike and plateau action potential (trace 2). Spikes (marked by asterisks) on first two oscillations at −65 mV are small, partially recovered Na\(^{+}\) spikes. D: slow spike and plateau (traces 1) evoked just before changing to Cd\(^{2+}\)-containing saline. Trace 2 is response at same somatic membrane potential 8.5 min after Cd\(^{2+}\) (200 μM). Responses evoked in Cd\(^{2+}\) at two depolarized soma potentials are also shown. Na\(^{+}\) spikes (still attenuated from TTX washout) were evoked during largest (−65 mV) depolarization. Calibrations in A apply to all panels.
other features illustrated in Fig. 1 during dendritic depolarization. Three other cells that exhibited no bursting whatsoever during either dendritic or somatic depolarization did exhibit the repetitive bursts when the two types of stimuli were combined.

Although our recorded population consisted of only 12 cells we are confident that the reported behavior is representative of a significant fraction of layer 5 pyramidal neurons in the area of cortex studied. In an earlier study (Schwindt and Crill 1997a) we observed responses similar to those illustrated in Fig. 1 in 12 of 41 cells that exhibited dendritic spikes during dendritic depolarization. However, these were only chance observations in cells that were being studied for a different purpose. We were able to observe the distinctive firing pattern evoked by dendritic (or combined somatic and dendritic) depolarization in every recorded cell when we specifically looked for these responses. In both studies our observations were confined to low-input resistance (presumably large) pyramidal cells, probably because of the sampling bias of our relatively low resistance recording microelectrodes. Results from a subsequent study (Oakley et al. 1998) that used tight-seal recording methods and optics that allow visualization of the recorded neurons suggest that the responses we described here are not restricted to low-input resistance layer 5 pyramidal neurons.

Our voltage-clamp experiments revealed that both the single bursts evoked by current pulses or EPSPs during adequate subthreshold dendritic depolarization and the repetitive bursts evoked by suprathreshold dendritic depolarizations are caused by a dendritic Ca2+ spike that is triggered by the first Na+ spike of the burst. This was the mechanism proposed by Traub et al. (1991), based on their analysis of a computer model, to underlie burst firing in hippocampal pyramidal neurons. In the model of Traub et al. (1991) a backpropagated Na+ action potential triggered a dendritic Ca2+ current that outlasted the Na+ spike and provided the depolarization that evoked additional Na+ spikes to form the burst. Our results appear to provide the first experimental evidence for this burst-generating mechanism. In the study of Traub et al. (1991) repetitive bursts could be triggered by adequate depolarization of the soma of modeled CA3 neurons but not by somatic depolarization of modeled CA1 neurons. Similar to the layer 5 cells we recorded, the CA1 model generated a Ca2+ spike-driven burst only when the dendrites were adequately depolarized. Why should dendritic spikes and bursts be evoked by somatic depolarization in one type of pyramidal neuron and only by dendritic depolarization in another? The difference in the burst-generating ability of the two models depended on differences in the assumed distribution and density of dendritic Ca2+ and K+ channels. It will be important to determine these quantities experimentally in neocortical neurons.

We found that a Na+ spike-linked Ca2+ spike was the mechanism underlying burst generation during adequate subthreshold dendritic depolarization and during suprathreshold dendritic depolarization that evoked repetitive bursts. Adequate dendritic depolarizations can evoke dendritic Ca2+ spikes directly, however (Schwindt and Crill 1997, 1998; this study), and the resultant sudden, prolonged depolarizations clearly are capable of evoking bursts of Na+ spikes. It is possible that some of the bursts that preceded the regular spiking evoked by stronger dendritic depolarization were caused by directly evoked Ca2+ spikes instead of by the Na+ spike-linked Ca2+ spike mechanism. For example, one or two Ca2+ spikes were observed to precede the Ca2+ plateau in most cells (Figs. 8C and 12A). We suspect that the principal mechanism underlying the repetitive bursts (when Na+ spikes are actually evoked) is the Na+ spike-linked Ca2+ spike for the following reasons. We found previously that a Na+ spike evoked by somatic depolarization prevented the occurrence of an iontophoretically evoked Ca2+ spike for tens of milliseconds (Schwindt and Crill 1997a, 1998). We made similar observations in this study. When a current pulse-triggered burst was evoked before the Ca2+ spike, the generation of the iontophoretically evoked Ca2+ spike was prevented or delayed (our unpublished observations). During repetitive bursting the large, long, postburst hyperpolarizing afterpotential also would prevent the generation of a Ca2+ spike (because of the hyperpolarization) until the next Na+ spike was triggered. The initiation of the first Na+ spike probably sets the rhythm of the bursts at all levels of dendritic depolarization.

Stronger dendritic depolarization evoked initial bursts followed by regular spiking. In the model of Traub et al. (1991), stronger somatic depolarization of the CA3 neurons also evoked initial bursts followed by regular spiking. In this model, the transition to regular spiking was caused by tonic activation of a dendritic Ca2+–dependent K+ conductance, which increased dendritic membrane shunt and thereby prevented a regenerative Ca2+ spike from developing. In the layer 5 neurons the mechanism causing a transition to regular spiking was different from that envisioned in this model. It consisted of the tonic inward dendritic current resulting from the plateau potential rather than a tonic outward dendritic current. Bursts could not be evoked during this Ca2+–dependent plateau potential, whose amplitude remained fixed and similar to that of individual Ca2+ spikes. The simplest explanation for an inability to evoke additional Ca2+ spikes or bursts during the plateau is that the same channels underlie the Ca2+ spikes and the Ca2+–dependent plateau. According to this idea, these Ca2+ channels were already fully activated (to cause the plateau), so that an additional Ca2+ spike (thus a burst) could not be evoked. The plateau potential we observed in the layer 5 pyramidal cells differs in two ways from the plateau potential observed in turtle motoneurons. The motoneuron plateau potential can be evoked by somatic depolarization, and its duration can greatly exceed the duration of the depolarization that triggers it (Kiehn 1991). In the pyramidal neurons, the plateau could be initiated only by dendritic depolarization, and it repolarized when the dendritic depolarization was terminated.

The Ca2+ spike and the plateau recorded in a given cell had a similar amplitude. We presented evidence previously (Schwindt and Crill 1997a) that the small apparent size of the Ca2+ spikes is due to their passive, electronic propagation to the soma from the site on the apical dendrite where they are generated. Part of that evidence was the fact that the Ca2+ spikes were smaller when the iontophoretic site was farther from the soma. This observation is inconsistent with active propagation to the soma because spike amplitude at the soma would be independent of iontophoretic distance if propagation was active. We were unable to show a similar clear relationship for the plateaus in this study over the range of iontophoretic distances employed. However, we observed previously that the amplitude of a plateau potential decreased as the iontophoretic site was moved farther from the soma in the same cell (see Fig. 6A).
5 of Schwindt and Crill 1997a), and a similar relation between plateau amplitude and iontophoretic distance in a population of cells was observed in a subsequent study (Oakley et al. 1998). It thus appears that the active propagation of the plateaus is restricted, and their small size at the soma also reflects passive propagation with electrotonic decay. We found that the inability of the Ca\(^{2+}\) spikes to propagate actively to the soma depended on intact dendrite K\(^{+}\) currents (Schwindt and Crill 1997a).

Why does adequate dendritic depolarization evoke a Ca\(^{2+}\) plateau instead of repetitive Ca\(^{2+}\) spikes? The evoked potential will depend on the balance of inward and outward currents. An outward current ultimately must terminate the transient Ca\(^{2+}\) spike. Ca\(^{2+}\) inactivation cannot be the sole mechanism for repolarization of the transient Ca\(^{2+}\) spike because even stronger dendritic depolarization (which should increase inactivation) evokes the plateau. As mentioned previously, we obtained evidence for the existence of voltage-gated, TEA-sensitive K\(^{+}\) channels in the apical dendrite (Schwindt and Crill 1997a,b). K\(^{+}\) currents studied by direct tight-seal recording in layer 5 pyramidal apical dendrites were described as being principally of the delayed rectifier type (Bekkers and Stuart 1998). The tonic of the Ca\(^{2+}\) current throughout the plateau would be aided if the dendritic K\(^{+}\) currents tend to inactivate during long-lasting depolarization. Delayed rectifier-type K\(^{+}\) currents of neocortical neurons do exhibit slow inactivation properties (Foehring and Surmeir 1993; Spain et al. 1991).

A Ca\(^{2+}\)-dependent spike and plateau can sometimes be abolished by blocking Na\(^{+}\) channels (Fig. 11). A similar dependence of other types of Ca\(^{2+}\)-dependent responses on intact Na\(^{+}\) currents was observed previously (Schwindt and Crill 1997a,b). It is possible that the dendritic Ca\(^{2+}\) spikes simply became invisible at the soma after Na\(^{+}\) current blockade. A nonactivating dendritic Na\(^{+}\) current would cause the dendritic current-voltage relation to have an “N” shape, which allows distal events to be less attenuated (better seen at the soma) than in a passive dendrite (Jack et al. 1975). Na\(^{+}\) blockade would abolish the N-shaped current–voltage relation and may thus render the distal events invisible at the soma. This idea is made less likely by the recent finding that identified Ca\(^{2+}\) spikes recorded directly in the distal dendrite were abolished when Na\(^{+}\) currents were blocked (Schiller et al. 1997). The best explanation for the apparent dependence of dendritic Ca\(^{2+}\) spikes on intact Na\(^{+}\) currents therefore seems to remain as proposed previously. A dendritic Na\(^{+}\) current that is activated by a smaller depolarization than the dendritic Ca\(^{2+}\) current is needed to boost dendritic membrane potential to the level where the Ca\(^{2+}\) current can be activated to cause total membrane current to become net inward (i.e., regenerative). According to this idea, abolition of the Na\(^{+}\) current prevents dendritic membrane potential from depolarizing far enough or rapidly enough to trigger the Ca\(^{2+}\)-dependent response.

Can depolarization by means other than glutamate iontophoresis evoke the dendritic Ca\(^{2+}\) plateaus that we observe? The study of Reuveni et al. (1993) clearly pointed to the existence of dendritic Ca\(^{2+}\) plateau potentials, but their results were obtained after reducing K\(^{+}\) currents. Affirmative results in physiological saline are provided by the experiments of Kim and Connors (1993) and Schiller et al. (1997), who made tight-seal recordings from the apical dendrites of layer 5 neurons under experimental conditions similar to ours. Schiller et al. (1997) found that EPSPs or direct depolarization of distal dendrites evoked nonpropagated Ca\(^{2+}\) spikes, which if large enough could trigger multiple Na\(^{+}\) action potentials at the soma. Kim and Connors (1993) found that direct depolarization of more proximal sites on the apical dendrites of their “type 2” layer 5 neurons evoked long-lasting Ca\(^{2+}\)-dependent depolarizations similar to the plateau potentials we recorded. Similar plateau waveforms were triggered by EPSPs evoked by stimulation of layer 1. Kim and Connors (1993) recorded only from the apical dendrite, so it was not clear from their experiments if the dendritic responses could trigger spikes or even be seen at the soma. Our experiments show that similar active dendritic responses can depolarize the soma and trigger spiking there.

The onset of the plateau potential terminated the ability of the cell to fire in bursts, whereas the plateau itself provided a constant current to drive regular spiking. However, it seems possible that the plateau may have a more fundamental significance. In our experiments, stronger glutamate iontophoresis caused no increase of plateau amplitude, suggesting a large membrane conductance and/or depolarization in the plateau-generating region. This result suggests further that any excitatory synaptic input arriving distal to the plateau-generating region would have no influence on the soma once the plateau was initiated. Can dendrites other than the apical (i.e., basal, oblique, or terminal tuft) generate plateaus? If so, would concurrent plateaus on different dendrites act as independent current sources to depolarize the soma, or would the summed depolarization trigger a larger plateau in a more proximal dendritic region? In the latter case, the larger, more proximal plateau would cause a greater depolarization of the soma, but excitatory synaptic input in a greater area of distal dendrite would be prevented from influencing the soma. The answers to these questions are important for understanding the integrative properties of the cell.

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