JOURNAL OF NEUROPHYSIOLOGY
Vol. 74, No. 4, October 1995. *Printed in U.S.A.*

RAPID PUBLICATION

Inhibitory Control of Excitable Dendrites in Neocortex

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SUMMARY AND CONCLUSIONS

1. Many dendrites of pyramidal cells in mature neocortex express active Na^+ and Ca^{2+} conductances. Dendrites are also the target of numerous inhibitory synapses. We examined the interactions between the intrinsic excitability of dendrites and synaptic inhibition using whole cell recordings from the apical dendrites of layer 5 pyramidal cells. Experiments were performed on slices of somatosensory cortex from mature rats. Slices were bathed in the glutamate receptor antagonists 2-amino-5-phosphonopentanoic acid and 6,7-dinitroquinoxaline-2,3-dione, and maintained at 32–36°C.

2. In agreement with previous findings, intradendritic current injection evoked two distinct types of dendritic firing. Type I dendrites generated monophasic fast spikes, whereas type II dendrites showed more complex firing patterns, consisting of fast and slow spike components.

3. Stimulation of cortical layers 2/3 evoked fast inhibitory postsynaptic potentials (IPSPs) in all dendrites tested. IPSP reversal potentials were bimodally distributed, with means of about −53 and −85 mV when recorded with high-Cl^--concentration-filled electrodes. Interestingly, IPSP reversal potentials were correlated with the type of dendritic spiking pattern.

4. IPSPs were able to delay, completely block, or partially block spiking in dendrites, depending on the relative timing between inhibition and dendritic spiking. Slow, Ca^{2+}-dependent spike components could be blocked selectively by IPSPs. Furthermore, inhibition could either phase advance or phase delay repetitive patterns of dendritic spiking, depending on the timing of the IPSP.

INTRODUCTION

Most synapses in the vertebrate brain terminate on dendrites (Shepherd 1990). The dendritic trees of many neurons express voltage-dependent conductances (Amitai et al. 1993; Llinás and Sugimori 1980; Wong et al. 1979), thereby dramatically affecting the rules by which synaptic inputs are transformed to generate neuronal output (for review see Mel 1994). Most dendrites also receive a dense inhibitory innervation, yet physiological studies of dendritic inhibition have remained sparse (Llinás and Nicholson 1971; Masukawa and Prince 1984). Very few studies have directly examined the interactions between spiking and inhibitory postsynaptic potentials (IPSPs) in dendrites (Callaway et al. 1995; Traub et al. 1994).

The apical dendrites of pyramidal cells in neocortex are electrically excitable. Patch-clamp recordings reveal a low Na^+ channel density in the somadendritic membrane (Huguenard et al. 1989; Stuart and Sakmann 1994). Direct intradendritic recordings using sharp (Amitai et al. 1993; Pockberger 1991) and whole cell recordings (Kim and Connors 1993; Stuart and Sakmann 1994) have demonstrated that the apical dendrites can generate spikes mediated by Na^+ currents or a combination of Na^+ and Ca^{2+} currents. Pyramidal cells have a variety of Ca^{2+} currents (Brown et al. 1993), and labeling with specific antibodies (Westenbroek et al. 1992) and intracellular Ca^{2+} imaging (Yuste et al. 1994) suggests that dendritic Ca^{2+} channels may have a nonuniform distribution. The active ion channels in apical dendrites may enhance the efficacy of distal excitatory synapses (Cauller and Connors 1992; Kim and Connors 1993) and may mediate bidirectional propagation of electrical signals (Stuart and Sakmann 1994).

Synaptic inhibition has a profound influence on neocortical excitability. Up to 80% of the inhibitory synapses on pyramidal cells terminate on dendrites, and various inhibitory cell types each innervate different regions of the pyramidal cell (Somogyi 1990). GABAergic synapses can powerfully control the somatic excitability of cortical neurons (Connors et al. 1988), yet the form and effects of IPSPs on dendritic spiking have not been directly examined for neocortical neurons.

To investigate the interaction between intrinsic spiking and synaptic inhibition, we obtained whole cell recordings from dendrites in slices of mature rat neocortex while blocking excitatory synaptic transmission pharmacologically. Some of the results have appeared in abstract form (Beierlein et al. 1994).

METHODS

Male rats were anesthetized with pentobarbital sodium (60 mg/kg ip). The brain was quickly removed into cold physiological solution containing (in mM) 126 NaCl, 3 KCl, 1.25 NaH_2PO_4, 2.6 $NaHCO_3$, 2 $MgSO_4$, 20 dextrose, and 2 $CaCl_2$. Coronal slices 400 μm thick were cut from the primary somatosensory area using a vibratome, and maintained in an interface chamber at 32–36°C. The bathing solution contained both the *N*-methyl-D-aspartate (NMDA) receptor antagonsist 2-amino-5-phosphonopentanoic acid (AP-5) (50–100 μM) and the non-NMDA receptor antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX) (10–20 μM). Solutions were saturated with 95% O_2-5% CO_2.

Electrodes were pulled from borosilicate tubing with resistance of 4–15 MΩ and filled with either a high-Cl^--concentration (high-[Cl^-]) intracellular solution containing (in mM) 130 KCl, 10 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, 5 ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid, 2 $MgCl_2$, 4 MgATP, 0.5 $CaCl_2$, and 0.3 guanosine 5′-triphosphate, pH 7.0, or a control filling solution containing 120 mM potassium gluconate in place of equivalent KCl. KCl electrodes were used for most recordings except the ones shown in Figs. 1*C* and 3*D*; Cl^- and gluconate solutions yielded similar forms of dendritic excitability. Gigaohm seals were made onto the apical dendrites of pyramidal cells whose somata were in layer 5, and whole cell recordings were obtained using standard protocols (Blanton et al. 1989). Recording sites were within layer 4 (Fig. 1*A*), and den-

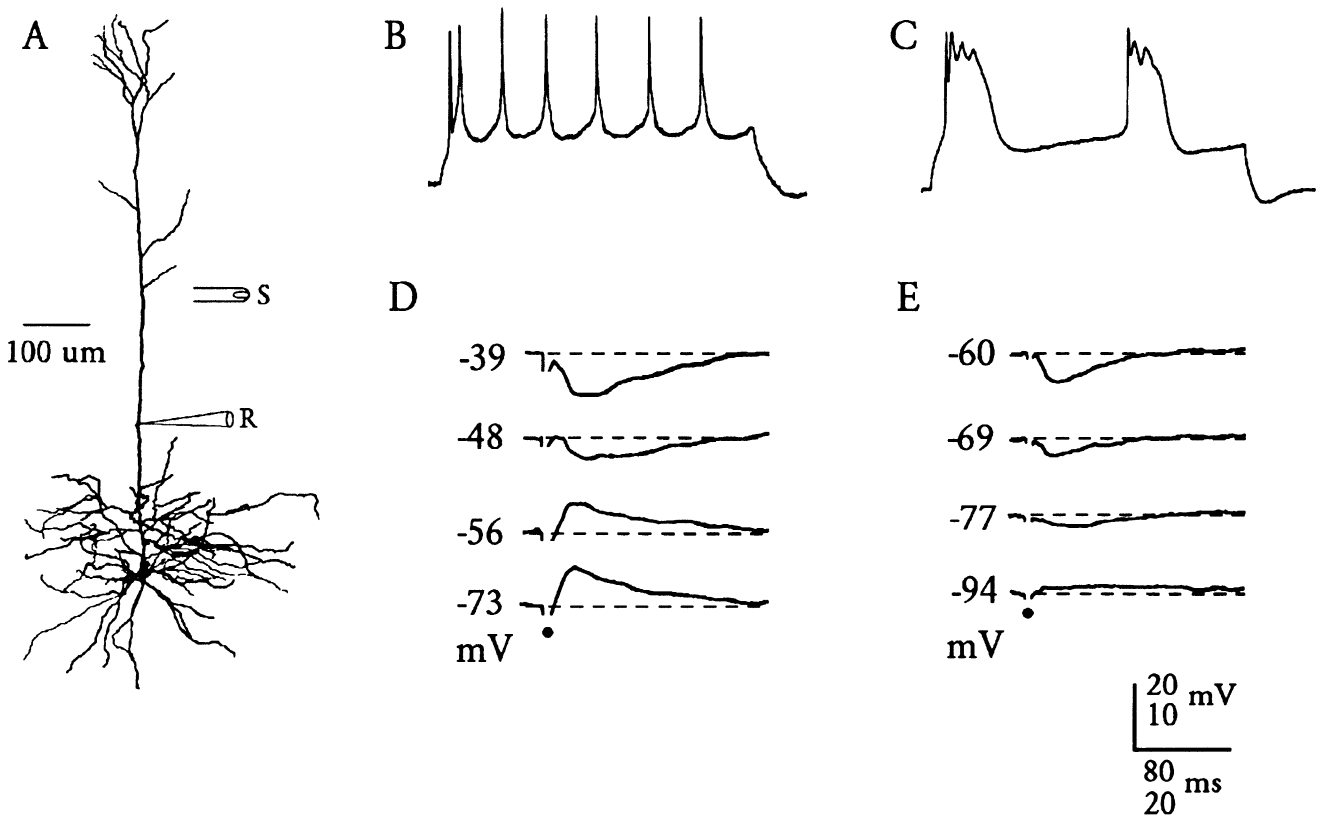

FIG. 1. Inhibitory postsynaptic potentials (IPSPs) in apical dendrites. *A*: layer 5 pyramidal neuron showing the approximate recording site within layer 4, and extracellular stimulus site in layer 2/3. The cell image was reconstructed from a dendritically recorded, biocytin-filled neuron. *B*: repetitive Na^+ spikes from a type I dendrite, elicited by an 0.5-nA intradendritic current step. Resting membrane potential (V_m) = −73 mV. *C*: complex spikes from a type II dendrite with fast, initial Na^+-dependent components and longer-lasting Ca^{2+} components, elicited by an 0.7-nA intradendritic current step. V_m = −65 mV. *D*: IPSPs generated in a type I dendrite; V_m was varied with constant current injections; IPSP reversal potential occured between −48 and −56 mV. *E*: IPSPs generated in a type II dendrite, with reversal between −77 and −94 mV. Dot: times of extracellular stimuli.

drites were identified by physiological criteria that have been confirmed with biocytin dye injections as described previously (Kim and Connors 1993). Only recordings with stable resting potentials were analyzed. The distances between soma and dendritic recording sites varied from ~100 to 500 μm. Extracellular stimuli (up to 250 μA in intensity, 100 μs in duration) were delivered via a concentric, bipolar electrode (200 μm diam) placed in the upper part of layer 2/3. All data are reported as means ± SD.

RESULTS

Intradendritic current injections revealed two general forms of dendritic spiking: simple, monophasic fast spikes, designated type I (n = 19; Fig. 1*B*), and more complex patterns of fast and slow spikes, called type II (n = 24; Fig. 1*C*). As shown previously, type I spikes are mediated by tetrodotoxin-sensitive Na^+ currents, whereas type II spikes are generated by a combination of fast Na^+ currents and slow, longer-lasting high-threshold Ca^{2+} currents (Kim and Connors 1993); the same study also showed a correlation of the electrophysiological types with different anatomic classes of apical dendrites from layer 5 neurons.

We evoked pure IPSPs on dendrites by electrically stimulating layer 2/3 in the presence of AP-5 and DNQX. Recording electrodes in layer 4 contained high [Cl^-], which was intended to shift the reversal potential of γ-aminobutyric acid-A ($GABA_A$)-mediated IPSPs toward more depolarized levels. IPSPs were recorded in all dendrites tested. Most IPSPs had a single reversal potential, although a few IPSPs had mixed reversal potentials. Those with one well-measured reversal level fell into two nonoverlapping groups (Figs. 1, *D* and *E*, and 2). IPSPs in the first group (Fig. 1*D*; n = 8) reversed at −53 ± 6 (SD) mV (range −61 to −44 mV), whereas IPSPs in the second group (Fig. 1*E*; n = 6) reversed at −85 ± 4 mV (range −91 to −80 mV). However, both groups of IPSPs had similar time courses; the 10–90% rise

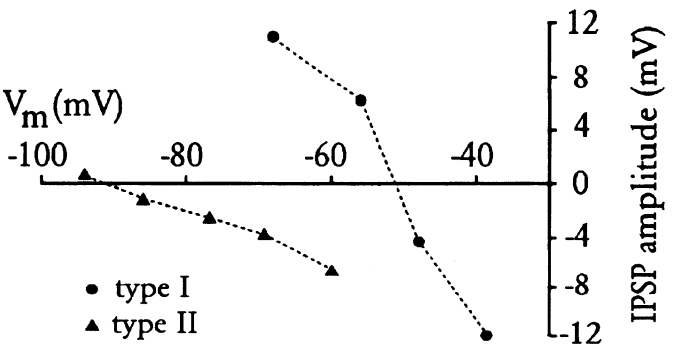

FIG. 2. Graph of IPSP amplitude vs. V_m for dendritic responses shown in Fig. 1, *D* (●) and *E* (▲).

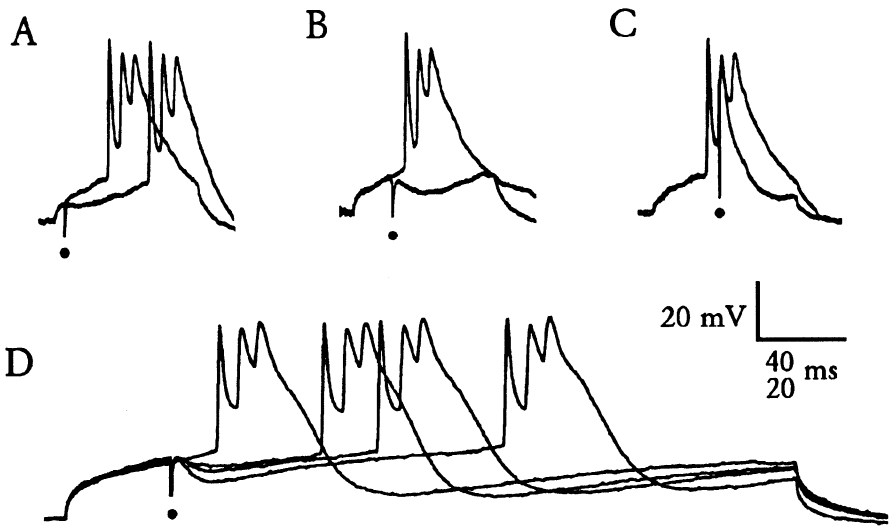

FIG. 3. Effects of dendritic IPSPs on spiking in type II dendrites. In each case, spikes were evoked by a constant-amplitude intradendritic current step. *A–C*: as the relative timing between the IPSP and dendritic spikes changed, the spikes were either delayed (*A*), completely blocked (*B*), or partially blocked (*C*). Resting $V_m = -60$ mV. *D*: in a different type II dendrite, IPSPs were evoked by increasingly strong stimulus intensities. Superimposed traces show the control response with no IPSP (earliest spike complex) and for IPSPs evoked by stimuli of 140, 160, and 180 μA. Scale bar: 40 ms for *A–C* and 20 ms for *D*. Dot: times of extracellular stimuli.

times were 11 ± 7 ms ($n = 8$) and half-widths were 24 ± 13 ms ($n = 7$) for the first group, and 12 ± 6 ms and 20 ± 10 ms ($n = 7$) for the second group, respectively.

The two groups of IPSPs, as defined by reversal potential, were not randomly distributed among the two types of dendrites. Of 10 type I dendrites, 6 reversed at the relatively depolarized potentials, 2 at the more hyperpolarized potentials, and 2 showed IPSPs with mixed reversal potentials. Of 13 type II dendrites, 10 had IPSPs reversing at the more hyperpolarized levels, only 1 showed an IPSP reversing at the more depolarized potentials, and 2 had mixed responses.

IPSPs strongly influenced dendritic spikes by shifting their latency, decreasing their probability, and changing their form. Figure 3 shows recordings from type II dendrites in which a spike complex was evoked with a suprathreshold current step, and an IPSP was triggered by a shock of varying latency or strength. As the onset of the IPSP was progressively delayed relative to dendritic spiking, the entire spike complex was first delayed by 20 ms (Fig. 3*A*), then blocked entirely (Fig. 3*B*), and finally the IPSP eliminated only the later, slower phases of the spike complex (Fig. 3*C*). In another dendrite (Fig. 3*D*), gradually increasing the strength of the stimulating current yielded a progressively stronger IPSP. As the strength of the IPSP increased, the dendritic spike complex was progressively delayed by up to 65 ms without influencing the spiking waveform. Similar observations were made in eight recordings.

Dendrites generated repetitive spikes when stimulated with prolonged current steps (Figs. 1, *A* and *B*, and 4). IPSPs could either advance or delay repetitive spiking, depending on the relative timing of the synaptic and initial spiking events. In Fig. 4*A* the IPSP arrived in time to block the late components of the first spike cluster. This resulted in a phase advancement of subsequent spikes (*) by ~37 ms compared with control spikes generated without an IPSP. Varying the IPSP onset relative to the current step (Fig. 4*B*) led to predictable phase shifts of repetitive spiking, both positive and negative, of up to 60 ms ($n = 8$ dendrites).

DISCUSSION

This study demonstrates that fast IPSPs can be evoked on apical dendrites of neocortical pyramidal cells, and that these IPSPs can dramatically modify the probability, shape, and timing of dendritic spiking. In the classical view of synaptic integration in dendrites, the effects of inhibitory synaptic conductances interact with those of excitatory synaptic conductances, modified by the passive cable properties of the dendritic tree (Rall 1977). In this study we have shown that, in excitable dendrites, another dimension must also be considered: the interactions between inhibitory synaptic conductances and voltage-dependent conductances.

The inhibitory circuitry of neocortex is not as well characterized as in some other brain structures, such as the hippocampus (Buhl et al. 1994). By stimulating in upper layer

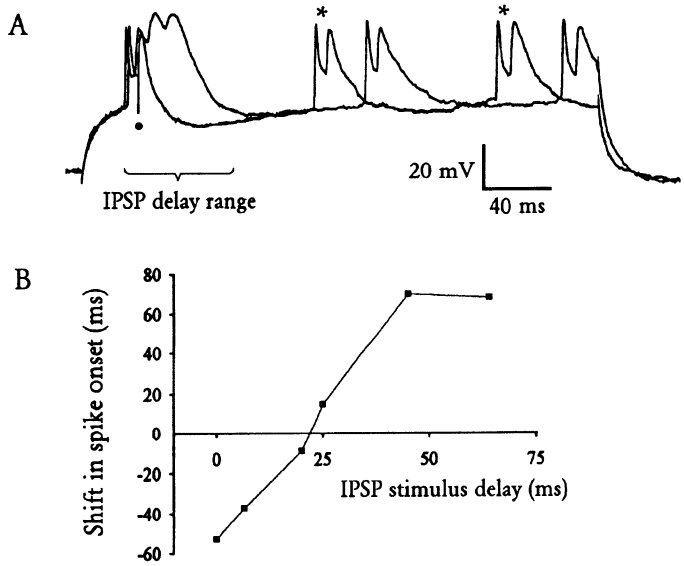

FIG. 4. Repetitive dendritic spikes are phase shifted by IPSPs. *A*: repetitive spikes were evoked in a type II dendrite by injecting long current steps, and an IPSP was evoked at the time of the dot. Two superimposed voltage traces: with the IPSP (spikes marked with ⋆) and without it. In this case the IPSP blocked the late, slow components of the initial dendritic spike and phase advanced the firing of subsequent repetitive spikes (⋆). Bracket: range of IPSP delays tested. *B*: graph plots the change in timing for the 2nd spike complex as a function of IPSP stimulus delay, for the dendrite shown in *A*. Positive values: delayed spikes relative to control. Negative values: advanced spikes. Zero IPSP stimulus delay indicates that the IPSP onset coincides with the initial spike onset.

2/3 we presumably activated inhibitory cells that make synapses with the apical dendrites of deep layer pyramidal cells in the same or an adjacent layer. Kawaguchi (1995) has shown that the vast majority of axon arbors from nonpyramidal cells of layer 2/3 in rat frontal cortex terminate within the same layer. However, we cannot exclude the possibility that somata of pyramidal cells also received substantial synaptic inhibition, which might underlie some of the effects we have observed in this study.

IPSPs were able to control the probability and timing of dendritic spikes. At least some fast Na^+-dependent dendritic spikes are initiated near the axon initial segment and propagate back into the dendritic tree (Stuart and Sakmann 1994). In this case, synaptic inhibition of either dendritic or somatic origin might dictate where and when excitatory synapses are told about the output (spiking) state of the neuron; because at least some neocortical synapses display Hebbian forms of plasticity (Bear and Malenka 1994), inhibition would be an important form of spatial and temporal control over a cell's modifiability. In some cases, distal excitatory synapses may directly trigger dendritic spiking (Kim and Connors 1993; Regehr et al. 1993; Yuste et al. 1994). In these situations, IPSPs might directly control the strength of distal synapses by gating the orthodromic flow of signals from synapse to soma (Vu and Krasne 1992). Na^+-dependent action potentials are generated with relatively low current densities in dendrites, compared with their soma-axonal counterparts (Stuart and Sakmann 1994). Modeling studies show that this difference in Na^+ current density makes dendritic spikes more susceptible to the influences of inhibitory conductances and voltage changes than soma-axonal spikes (Beierlein 1994). Furthermore, properly timed dendritic IPSPs can selectively block the late Ca^{2+}-mediated spike components (Figs. 3*C* and 4*A*), perhaps changing the spatiotemporal pattern of intracellular Ca^{2+} transients without affecting the general pattern of the cell's excitability. A selective suppression of dendritic Ca^{2+} transients by IPSPs was recently demonstrated in cerebellar Purkinje cells (Callaway et al. 1995). Clearly, synaptic inhibition can specifically and delicately control the intrinsic excitability of dendrites.

The pharmacology and kinetics of synaptic inhibition in cortical pyramidal cell dendrites need to be further investigated. The fast time course of all IPSPs recorded in this study suggests that the responses are mediated via $GABA_A$-receptor-coupled Cl^- channels, because no $GABA_B$ receptors have been reported to mediate such rapid responses. The bimodal distribution of IPSP reversal potentials is surprising. It is possible that IPSPs with more hyperpolarized potentials are generated at a greater electrotonic distance from the recording electrode. A more likely explanation is that the dendrites with the most negative reversal potentials have particularly dense and effective Cl^- extrusion mechanisms, leading to very low intracellular $[Cl^-]$ even in the face of Cl^--filled electrodes. Some neocortical neurons appear to have unusually effective Cl^- extrusion pumps (Thompson et al. 1988). Recent work on thalamic neurons showed that reversal potentials for $GABA_A$-receptor-mediated IPSPs were relatively positive when a whole cell recording was first established with a high-$[Cl^-]$ pipette; however, reversal potentials shifted negatively as pipette access resistance increased over several minutes (Huguenard and Prince 1994). In this study we did not evoke IPSPs until several minutes after dendritic whole cell recordings were established, and so would not have observed a shift in IPSP reversal potential that might reflect ion-pump-induced changes in $[Cl^-]$.

We observed a correlation between the type of dendritic spiking and the reversal potential of dendritic IPSPs. The significance of this is unknown, but the morphology of cortical neurons often correlates with intrinsic physiology (Amitai and Connors 1995), and the strength of inhibition may vary among different classes of layer 5 neurons (White et al. 1994).

Inhibitory synapses and voltage-dependent conductances coexist on apical dendrites, and we propose that a primary function of dendritic inhibition is to control the spatial and temporal features of dendritic spiking. By shifting the membrane potential even slightly within the range in which dendritic ion channels are steeply voltage dependent, or by shunting active inward currents, IPSPs can powerfully influence distal spiking. The traditional role of inhibitory dendritic conductances is to interact directly with excitatory synaptic inputs. This certainly may be important; however, the striking nonlinearity of many dendritic membranes forces a reassessment. The most profound effect of GABA, as well as myriad other transmitters that modulate dendritic ion channels, may be to regulate the ebb and flow of active dendritic events.

This work was supported by National Institute of Neurological Disorders and Stroke Grant NS-25983 and Office of Naval Research Grant N00014-90-J-1701.

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Received 6 March 1995; accepted in final form 28 June 1995.

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