Protein Kinase C Activation Decreases Activity-Dependent Attenuation of Dendritic Na^+ Current in Hippocampal CA1 Pyramidal Neurons

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Colbert, Costa M. and Daniel Johnston. Protein kinase C activation decreases activity-dependent attenuation of dendritic Na^+ current in hippocampal CA1 pyramidal neurons. *J. Neurophysiol.* 79: 491–495, 1998. Action potentials recorded from the soma of CA1 pyramidal neurons remain relatively uniform in amplitude during repetitive firing. In contrast, the amplitudes of back-propagating action potentials in dendrites decrease progressively during a spike train. This activity-dependent decrease in amplitude is dependent on the frequency of firing during the train and distance from the soma. Previously, we described a property of Na^+ channels that provides a plausible mechanism for the activity dependence of the amplitude of the dendritic action potentials: available Na^+ current decreases during trains of action potentials through an inactivation, distinct from fast inactivation, that appears rapid in onset, but slow and voltage-dependent in its recovery. In this study we found that activation of protein kinase C by phorbol esters decreased this activity-dependent inactivation of pharmacologically isolated Na^+ current in cell-attached dendritic, but not somatic, patches. Similarly in whole cell recordings phorbol esters decreased the attenuation of back-propagating dendritic action potentials during trains. These results indicate a novel effect of protein kinase C on the dendritic Na^+ channel and further support the hypothesis that the activity dependence of the dendritic action potentials is derived from the inactivation properties of Na^+ channels.

INTRODUCTION

Somatic action potentials maintain a relatively constant amplitude at moderate rates of firing (<100 Hz), whereas action potentials back-propagating into the dendrites of CA1 pyramidal neurons decrease in amplitude in an activity-dependent manner (Callaway and Ross 1995; Spruston et al. 1995). This decrease in amplitude greatly reduces the influx of Ca^{2+} associated with the back-propagating action potential (Jaffe et al. 1992; Spruston et al. 1995) and thus provides a potential target for modulation of Ca^{2+}-dependent processes by activity or neuromodulatory systems (Magee and Johnston 1997; Markram and Tsodyks 1996; Tsubokawa and Ross, 1997).

Previously, we and others investigated the mechanisms underlying this specific attenuation of dendritic action potentials (Colbert et al. 1997; Jung et al. 1997). By using manipulations that should alter Na^+ and A-type K^+ channels, we found that both types of channels could contribute to the larger attenuation in the dendrites. Observing Na^+ channels in cell-attached patches, we found that low-frequency trains of brief depolarizations induced an inactivation, apparently distinct from fast inactivation, that recovered very slowly and in a voltage-dependent manner. The attenuation of current because of this inactivation was approximately twofold greater in dendritic patches than somatic patches (Colbert et al. 1997). Although the K^+ currents did not show any activity-dependent changes in amplitude, their presence in the dendrites at a high density enhanced the effects of Na^+ current attenuation in the dendrites.

What underlies the differences in recovery from inactivation between somatic and dendritic Na^+ channels? No previous evidence supports the idea of different channel subtypes: other properties such as conductance, activation, and fast inactivation are all similar in the soma, dendrites, and initial segment (Colbert and Johnston 1996; Magee and Johnston 1995; Stuart and Sakmann 1994). Thus we wondered whether local modulation of Na^+ channels, rather than different types of channels, might determine the regional differences in slow recovery from inactivation. In the present study we found that phorbol esters decreased both the activity-dependent inactivation of dendritic Na^+ channels and the decline in spike amplitude during trains of back-propagating dendritic action potentials.

METHODS

Preparation and solutions

Slice methods are essentially those described in Colbert et al. (1997). Briefly, the experiments used 4 to 10-wk-old male Sprague-Dawley rats. Animals were anesthetized with a combination of ketamine, xylazine, and acepromazine. Once deeply anesthetized, they were perfused through the heart with cold modified artificial cerebrospinal fluid (ACSF) containing (in mM) 110 cholineCl, 2.5 KCl, 1.2 NaH_2PO_4, 25 $NaHCO_3$, 0.5 $CaCl_2$, 7.0 $MgCl_2$, and 20 dextrose. After removal of the brain, 400-μm-thick slices were cut with a Vibratome (Lancer) and stored submerged at room temperature.

The external solution contained (in mM) 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 2.0 $CaCl_2$, 1.0 $MgCl_2$, and 25 dextrose bubbled continuously with 95% O_2-5% CO_2. The pipette solution used for whole cell recordings contained (in mM) 120 K-gluconate, 20 KCl, 10 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), 0.4 ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′*N*′-tetraacetic acid (EGTA), 4.0 NaCl, 4.0 Mg_2ATP, 0.3 Mg_2-guanosine 5′-triphosphate (GTP), and 14 phosphocreatine (pH was adjusted to 7.25 with KOH). The pipette solution for cell-attached patch recordings of Na^+ currents contained (in mM) 120 NaCl, 30 tetraethylammonium (TEA) chloride, 10 HEPES, 2 $CaCl_2$, 3 KCl, 1 $MgCl_2$, and 10 4-aminopyridine (4-AP; pH was adjusted to 7.4 with NaOH).

Phorbol 12,13-diacetate (PDA), phorbol 12,13-dibutyrate (PDBu), and 4α-phorbol were obtained from Sigma Chemicals. Phorbol stock solutions of 1–10 mM were made in dimethylsulfox-

ide (DMSO) and diluted to final concentrations before each experiment. When included in patch pipettes, tips were filled with solution containing the drug.

Recording techniques

Recordings were made from somata and dendrites of hippocampal CA1 pyramidal neurons. Neurons were visualized by using infrared-illuminated, differential interference contrast optics (Axioskop, Ziess) according to standard techniques (Stuart et al. 1993). Whole cell patch recordings were made with an Axoclamp 2A (Axon Instruments) in bridge mode. Cell-attached patch recordings were made with a patch-clamp amplifier with a capacitive headstage (Axopatch 200A, Axon Instruments). Pipettes (3–5 MΩ for whole cell, 7–12 MΩ for cell-attached) were made from borosilicate glass and pulled with a P-87 Flaming-Brown pipette puller (Sutter Instruments). Recordings were made submerged at room temperature (~25°C). Membrane potential was determined by rupturing the patch at the end of the recording session and was in the range of −63 to −68 mV. Whole cell series resistance was 15–25 MΩ. Whole cell recordings were low-pass filtered at 3 kHz (6 dB/octave) and digitized at 10 kHz. Cell-attached patch recordings were filtered at 2 kHz (8-pole Bessel filter) and sampled at 10 kHz. Data were digitized at 16-bit resolution (ADC488/16, IOTech) and stored by computer for off-line analysis (Next Computer). Ensemble waveforms were constructed from 25–30 individual sweeps acquired at 0.1 Hz. Antidromic action potentials were stimulated by constant-current pulses (Neurolog, Digitimer or WPI Instruments) through tungsten electrodes (AM Systems) placed in the alveus. Significance was determined by Student's *t*-test with $P < 0.05$ considered significant. Data are reported as mean ± SE.

RESULTS

PDA decreases attenuation of Na^+ current in cell-attached patches

To investigate the effects of phorbol esters on sodium currents we measured pharmacologically isolated Na^+ currents in cell-attached patches from somata and apical dendrites of CA1 pyramidal neurons. To mimic activation of Na^+ channels by back-propagating action potentials, patches were stepped from the resting potential to a test potential of approximately −5 mV (Fig. 1*A*). Trains of 10 steps of 2 ms duration were given with an interval of 50 or 100 ms between steps. Changes in the evoked Na^+ current during a train were expressed as the ratio of the amplitudes of the 10th current in the train to the amplitude of the first current. Dendritic patches were made in the range of 80–280 μm from the soma, with the great majority between 120–200 μm. In control cell-attached patches (i.e., no phorbols in the pipette), Na^+ current amplitude decreased to 35 ± 3% (SE; $n = 4$) by the 10th step. In patches where PDA (10 μM) was included in the pipette solution, Na^+ current decreased significantly less to 74 ± 5% ($n = 7$) by the 10th step in the train. Control patches were made within 30 μm of a test patch in the same slices. Example cell-attached patch recordings are shown in Fig. 1. Holding the patches at −20 mV from rest had no further effect on attenuation: 80 ± 4% ($n = 4$) of the current remained in the 10th step. This was similar to our previous finding that modest hyperpolarizations did not greatly alter attenuation of Na^+ current in the dendrites (Colbert et al. 1997).

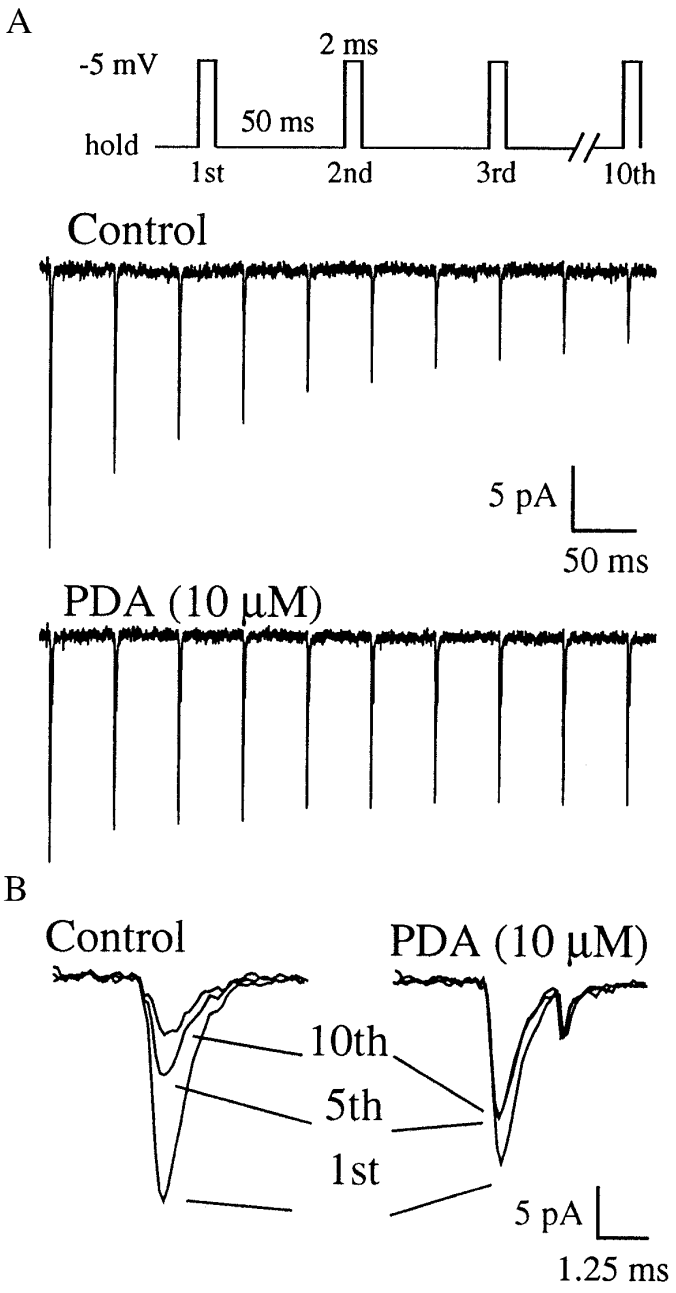

FIG. 1. Phorbol ester 12,13-diacetate (PDA) decreases activity-dependent attenuation of Na^+ current. *A*: to simulate action potentials trains of 10 depolarizing steps to approximately −5 mV and 2 ms in duration were given to cell-attached patches at a frequency of 20 Hz. Between depolarizations patch was held at neuron's resting potential. 4-aminopyridine (4-AP) and tetraethylammonium (TEA) were included in patch pipette to block K^+ channels. Control: waveform is leak subtracted ensemble average (30 sweeps) of currents evoked by trains applied to a dendritic cell-attached patch ~150 μm from soma. Amplitude of 10th evoked current is greatly attenuated. Resting potential recorded after rupturing patch was −65 mV. PDA: waveform is leak-subtracted ensemble average of currents evoked by trains applied to a dendritic patch in same slice and dendritic region as control patch. Pipette solution was similar to that in control patch but additionally contained 10 μM PDA. Amplitude of 10th evoked current was attenuated much less than in control patch. Resting potential recorded after rupture of patch was −65 mV. *B*: 1st, 5th, and 10th evoked currents from *A* at expanded (1.25 ms) time base.

Previously, we reported that Na^+ currents in somatic patches decrease much less than in dendritic patches (~25%). Including PDA (10 μM) in the patch pipette did not significantly alter the the ratio of the amplitudes of the 10th current in the train to the amplitude of the first current in somatic patches (79% ± 2%, $n = 4$).

Next we tested whether the effects on attenuation of Na^+ current were mediated by activation of kinases or by a nonspecific effect of the particular phorbol molecule. Such nonspecific effects have been reported on Ca^{2+} channels (Doerner et al. 1990). Thus we tested an additional phorbol ester that activates protein kinase C (PDBu, 10 μM), and a phorbol ester that does not activate protein kinase C (4α-phorbol, 10 μM). In patches where 4α-phorbol was included, the amplitude of the Na^+ current attenuated to 45 ± 4% ($n = 4$) by the 10th step, which was not significantly different from the attenuation in control patches. In the experiments with PDBu, to allow a single patch to serve as its own control, we applied the drug to the bath after obtaining control data (Fig. 2, $n = 2$). Additionally, we recorded from one other patch after PDBu was already present in the bath. In this set of patches with PDBu in the bath, the Na^+ current attenuated to 78 ± 2% ($n = 3$) by the 10th step. Together these results suggest that the effects of PDA on Na^+ channels are the result of activation of protein kinase C and not some nonspecific effect of the phorbol molecule.

PDA decreases attenuation of back-propagating dendritic action potentials

Previously, we proposed that slow recovery from inactivation of Na^+ channels underlies, in part, the activity-dependent attenuation of dendritic action potentials during a train (Colbert et al. 1997). If PDA decreases the activity-dependent attenuation of Na^+ current in the dendrites, then we hypothesized that the attenuation of the dendritic action potentials should decrease as well. We tested this hypothesis by comparing the attenuation of the dendritic spikes with and without bath-applied PDA. Dendritic spikes were observed in whole cell recordings from the apical dendrites ~200 μm from the soma. Trains of action potentials (10 Hz) were evoked by antidromic stimulation in the alveus once every 10 s (Fig. 3, *A* and *B*) or by a depolarizing current step through the recording electrode. The amplitude of the first action potential in the train was 54 ± 13 mV ($n = 3$). Normalized to this first action-potential amplitude, the 10th action potential in the train decreased to 47 ± 6% ($n = 3$). Subsequent bath application of PDA (10 μM) resulted in a nonsignificant increase in the first spike in the train to 57 ± 13 mV ($n = 3$), but a significant decrease in the attenuation; the amplitude of the 10th action potential decreased to 75% ± 12% ($n = 3$) of the amplitude of the first action potential. Application of PDA also reduced the decrease in the maximum rate of rise of the dendritic action potential. The rate of rise of the 10th action potential in the train, expressed as a percentage of the maximum rate of rise of the first action potential, increased 21% ± 3% ($n = 3$, from ~44 to ~65%) after the application of PDA. These results, taken together with the effect of PDA on the Na^+ channels, support the hypothesis that attenuation of the dendritic spikes results, in part, from the inactivation properties of the Na^+ channels.

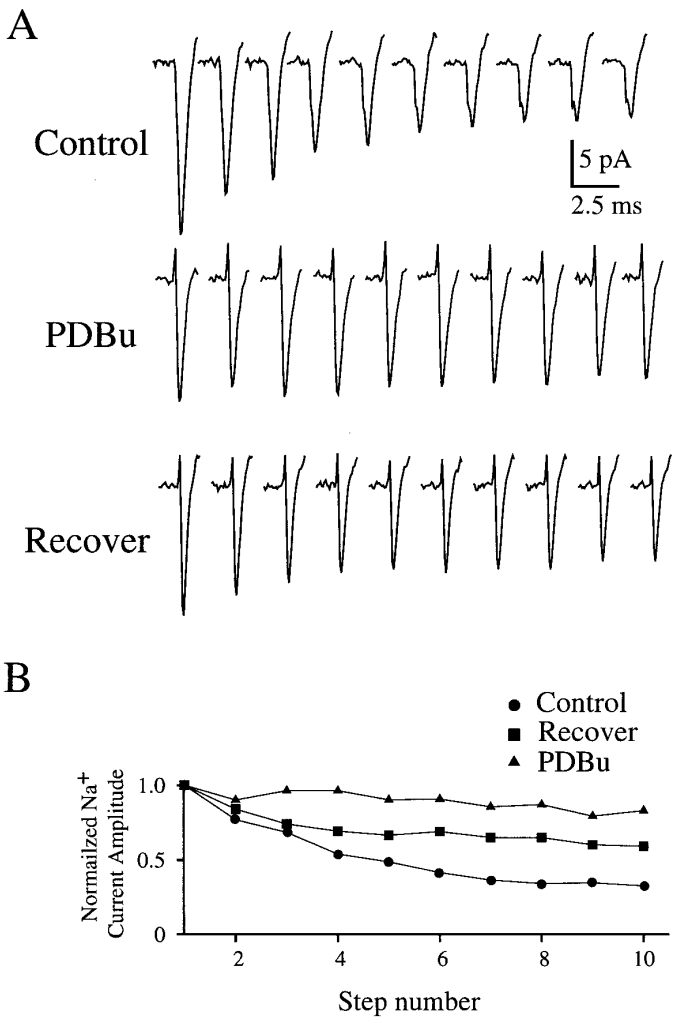

FIG. 2. Phorbol ester 12,13-dibutyrate (PDBu) decreases activity-dependent attenuation of Na^+ current. *A*: trains of voltage steps were given to a dendritic cell-attached patch ~210 μm from soma as in Fig. 1*A* (except for an interval of 100 ms rather than 50 ms). Control: waveform is leak subtracted ensemble average of currents (30 sweeps). Breaks in waveform represent 100 ms intervals between command steps. PDBu: currents from same patch 10 min after addition of PDBu (10 μM) to bath. Recover currents from patch after 45 min of washout of PDBu. *B*: normalized amplitudes of Na^+ currents as a function of number of command steps. Amplitudes are normalized to that of 1st current in each train. Note that PDBu greatly decreases activity-dependent attenuation and that attenuation increases again as PDBu washes out. Pipette solution as described in Fig. 1. Resting potential measured after rupture of patch was −62 mV.

DISCUSSION

The present study demonstrates that the activity-dependent attenuation of Na^+ current in the dendrites of CA1 pyramidal neurons (Colbert et al. 1997; Jung et al. 1997) is modulated by phorbol esters that activate protein kinase C. The present results further support the hypothesis that the attenuation of Na^+ current and its modulation determines the activity-dependent properties of the back-propagating dendritic action potential.

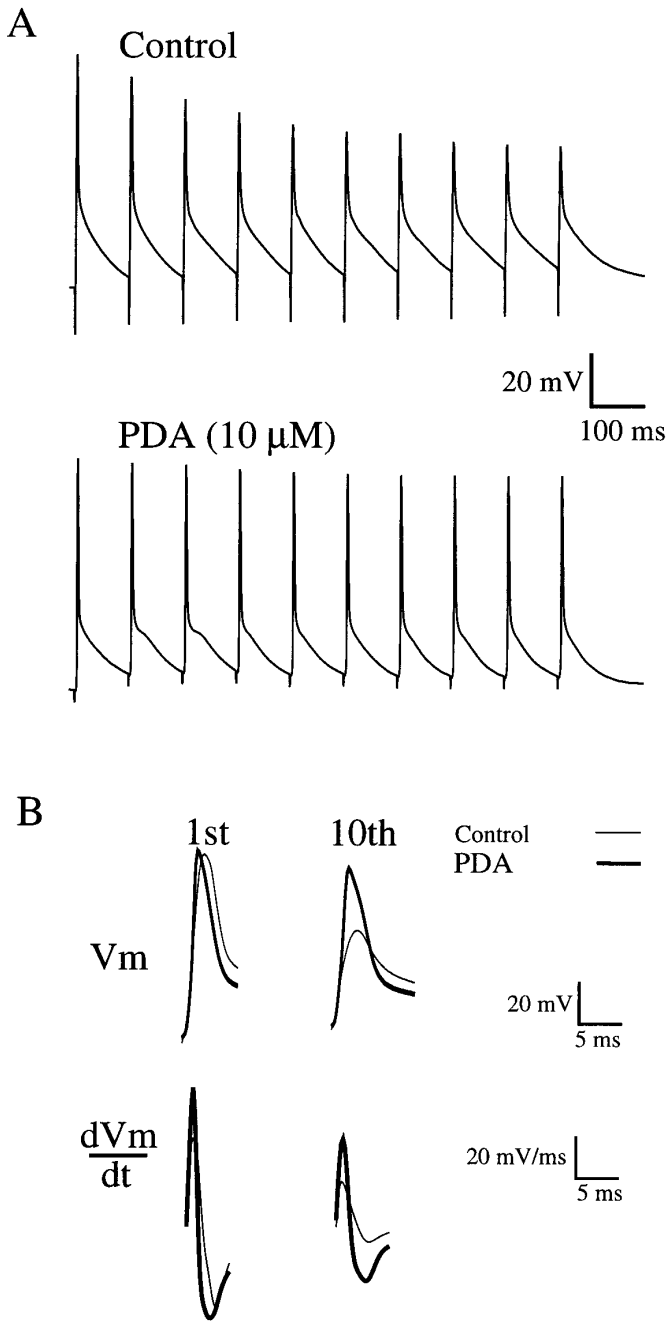

FIG. 3. PDA decreases activity-dependent attenuation of dendritic action potentials. *A*: waveform is a single sweep recorded from a whole cell patch electrode on apical dendrite at 180 μm from soma. A train of antidromic stimuli were evoked at a rate of 10 Hz. Note that amplitude of dendritic action potential attenuates even at this modest frequency. Bath application of PDA (10 μM) greatly decreased attenuation of dendritic action potentials during train. *B*: waveforms are 1st and 10th action potential (V_m) and rate of rise (dV_m/dt) in trains at expanded time base (5 ms) and superimposed for comparison. Light traces are controls (i.e., before application of PDA). Bold traces are after application of PDA. Resting membrane potential was −65 mV.

In addition to its effects on Na^+ currents, PDA shifts the activation curve of the A-type channels to more depolarized potentials (Hoffman and Johnston, in preparation). This effect of PDA on K^+ channels, however, is unlikely to contribute to the activity-dependence of spike amplitude during trains. In our previous study (Colbert et al. 1997), a very significant block of A-type K^+ channels (4-aminopyridine, 3–8 mM) was required to see effects on the activity dependence of dendritic action potential amplitude. Under those conditions, the amplitudes of all the dendritic action potentials were greatly increased. Ca^{2+} spikes occurred unless blocked by Cd^{2+} (Colbert et al. 1997; Hoffman et al. 1997) and yet, the decrease of the maximum rate of rise of the dendritic action potential during the train was unchanged (Colbert et al. 1997). The lack of an effect on the maximum rate of rise is consistent with the idea that the early part of the action potential, where the rate is maximum, is dominated by Na^+ channel activation. Application of PDA here, in contrast, decreased the attenuation of the maximum rate of rise during the train without a large increase in spike amplitude. This result is consistent with the hypothesis that the attenuation of spike amplitude during the train results primarily from Na^+ channel inactivation.

Recently, Tsubokawa and Ross (1997) reported that activation of muscarinic receptors decreases the attenuation of back-propagating action potentials much like the effect of phorbol esters seen here. The mechanism of action of muscarinic activation was not determined in that study. The present results suggest that muscarinic activation, like PDA here, may exert its effect on spike attenuation through modulation of the Na^+ channel. Although it is tempting to postulate that muscarinic receptors and phorbol esters share a common pathway through protein kinase C [as reported in Cantrell et al. (1996) for effects on amplitude and fast inactivation], previous attempts to relate the action of these molecules on I_{AHP} demonstrated pharmacological differences that suggested multiple pathways are involved (Engisch et al. 1996). Some Ca^{2+}-dependent processes in pyramidal neurons, such as long-term potentiation (LTP), may utilize back-propagating action potentials and subsequent Ca^{2+} entry for induction (Jaffe et al. 1992; Magee and Johnston 1997; Markram et al. 1997; Spruston et al. 1995; Tsubokawa and Ross 1997). Modulation of back-propagating action potential amplitude may thus be an important mechanism for control of synaptic plasticity. Furthermore, protein kinase C is persistently activated during LTP expression (Klann et al., 1993) and thus decreases in Na^+ channel inactivation may contribute to some of the postsynaptic changes in excitability associated with LTP (Hu et al. 1987). In vitro, dendritic spike amplitude decreases both with distance from the soma and with repetitive activity (Colbert et al. 1997; Spruston et al. 1995). Previously, we found that A-type K^+ channels contribute strongly to the spatial profile of the amplitude of single back-propagating dendritic spikes (Hoffman et al. 1997). During a train spike amplitude attenuates even further, limiting Ca^{2+} entry to the first few spikes (Spruston et al. 1995; Tsubokawa and Ross 1997). We suggested that the degree of activity-dependent attenuation depends both on Na^+ channel inactivation and the presence of A-type K^+ channels (Colbert et al. 1997). The present results, in which Na^+ channel inactivation appears to be under metabolic control, suggests that modulation of Na^+ channels is sufficient to control the activity dependence of spike amplitude during trains. By modulating Na^+ channels independently of A-type K^+ chan-

nels, the spatial profile of spike amplitude can be maintained even during trains of action potentials.

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