Selective shunting of the NMDA EPSP component by the slow afterhyperpolarization in rat CA1 pyramidal neurons

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Fernández de Sevilla D, Fuenzalida M, Porto Pazos AB, Buño W. Selective shunting of the NMDA EPSP component by the slow afterhyperpolarization in rat CA1 pyramidal neurons. J Neurophysiol 97: 3242–3255, 2007. First published February 28, 2007; doi:10.1152/jn.00422.2006. Pyramidal neuron dendrites express voltage-gated conductances that control synaptic integration and plasticity, but the contribution of the Ca2+-activated K+-mediated currents to dendritic function is not well understood. Using dendritic and somatic recordings in rat hippocampal CA1 pyramidal neurons in vitro, we analyzed the changes induced by the slow Ca2+-activated K+-mediated afterhyperpolarization (sAHP) generated by bursts of action potentials on excitatory postsynaptic potentials (EPSPs) evoked at the apical dendrites by perforant path-Schaffer collateral stimulation. Both the amplitude and decay time constants of EPSPs (τEPSP) were reduced by the sAHP in somatic recordings. In contrast, the dendritic EPSP amplitude remained unchanged, whereas τEPSP was reduced. Temporal summation was reduced and spatial summation linearized by the sAHP. The amplitude of the isolated N-methyl-D-aspartate component of EPSPs (EPSPNMDA) was reduced, whereas τNMDA was unaffected by the sAHP. In contrast, the sAHP did not modify the amplitude of the isolated EPSPAMPA but reduced τAMPA both in dendritic and somatic recordings. These changes are attributable to a conductance increase that acted mainly via a selective “shunt” of EPSPNMDA because they were absent under voltage clamp, not present with imposed afterhyperpolarization simulating the sAHP, missing when the sAHP was inhibited with isoproterenol, and reduced under block of EPSPNMDA. EPSPs generated at the basal dendrites were similarly modified by the sAHP, suggesting both a somatic and apical dendritic location of the sAHP channels. Therefore the sAHP may play a decisive role in the dendrites by regulating synaptic efficacy and temporal and spatial summation.

INTRODUCTION

Hippocampal pyramidal neurons receive thousands of synaptic inputs coordinated via complex processes of dendritic integration where the location of the synapse and the activation of dendritic conductances at specific sites play key roles (Lipowsky et al. 1996; Magee and Coke 2000; reviewed in Johnston et al. 1996, 2003; Magee 1998; Spruston et al. 1994; Yuste and Tank 1996). Because of its voltage independency, slow kinetics and regulation by neuronal activity, neurotransmitters and hormones (Borde et al. 1995, 1999, 2000; Carrer et al. 2003; Krause et al. 2002; reviewed in Sah 1996; Storm 1989; Stocker 2004), the contribution of the slow Ca2+-activated K+-mediated afterhyperpolarization (sAHP) could have a significant impact on integration of synaptic input arriving at the dendrites of CA1 pyramidal neurons.

The sAHP regulates synaptic efficacy in hippocampal pyramidal neurons via shunting of excitatory postsynaptic potentials (EPSPs) (Borde et al. 1999; Lancaster et al. 2001; Sah and Bekkers 1996) or by facilitation of the Mg2+ re-block of N-methyl-D-aspartate (NMDA) receptors (Wu et al. 2004). However, those publications did not analyze the relative contribution of NMDA versus α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) components of EPSPs to the synaptic regulation by the sAHP in the apical and basal dendrites of pyramidal neurons that may be of key importance in information processing leading to memory formation because Ca2+ inflow through NMDA receptors (NMDARs) is a proposed mechanism for the induction of long-term potentiation (LTP). In addition, the participation of the sAHP in dendritic function is not well understood mainly because the uncertain molecular identity of the Ca2+-activated K+ channels that mediate the sAHP (Bond et al. 2004; Sah and Faber 2002; reviewed in Sah 1996; Stocker 2004; Vogalis et al. 2003) has complicated their analysis. As a result of this uncertainty, contradictory data have been provided on the localization of the sAHP and also on its “shunting” effects on EPSPs, and it has either been assumed that the channels are uniformly distributed over the neuron’s surface (Jaffe et al. 1994), predominate in proximal apical (Sah and Bekkers 1996), or basal dendrites of CA1 pyramidal cells (Bekkers 2000).

Using somatic and apical dendritic recordings in CA1 pyramidal neurons in vitro, we analyzed the impact of the sAHP evoked by brief bursts of action potentials on the amplitude, waveform, and propagation of EPSPs evoked by stimulation of perforant path-Schaffer collaterals (PP-SC) at the apical dendrites and by stratum oriens (SO) stimulation at the basal dendrites. We show that the peak amplitudes of EPSPs evoked at apical and basal dendrites and recorded in the soma were reduced by the sAHP. In contrast, the amplitude of EPSPs in apical dendritic recordings was not modified by the sAHP. In addition, the decay time constant of EPSPs (τEPSP) was decreased at both somatic and apical dendritic sites. These EPSP modifications markedly reduced temporal summation and linearized spatial summation. We provide original evidence indicating that under pharmacological isolation of AMPA and NMDA EPSP components (EPSPAMPA and EPSPNMDA, respectively) the sAHP acted mainly via a selective “shunt” of EPSPNMDA. In addition, we show that the contribution of the

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hyperpolarization activated H-current ($I_h$) and of voltage-gated sodium currents ($I_{Na}$) are irrelevant in our conditions. Therefore the sAHP during a brief interval following the burst of action potentials may serve as a mechanism for metaplasticity controlling induction of LTP by regulating the temporal and spatial summation of EPSPs, the relative contribution of AMPA and NMDA components, their propagation to the soma, and finally by controlling action potential generation.

**METHODS**

Procedures of animal care, surgery, and slice preparation were in accordance with the guidelines laid down by the European Communities Council. The procedures will be described briefly because they have been extensively detailed previously (Borde et al. 1995, 2000; de Sevilla et al. 2002).

**Slice preparation**

Young Wistar rats (14–16 day old) were decapitated, and the brain was removed and submerged in cold artificial cerebrospinal fluid (ACSF; containing, in mM, 124.00 NaCl, 2.69 KCl, 1.25 KH$_2$PO$_4$, 2.00 MgSO$_4$, 26.00 NaHCO$_3$, 2.00 CaCl$_2$, and 10.00 glucose). In Mg$^{2+}$-free solutions MgSO$_4$ was equimolarly replaced with CaCl$_2$, or MgSO$_4$ was simply removed achieving the same results. The pH of the ACSF was stabilized at 7.4 by bubbling carbogen (95% O$_2$, 5% CO$_2$) and the temperature maintained at ≈4°C. Transverse hippocampal slices (300–350 μm) were cut with a Vibratome (Pelco 101, St Louis, MO) and incubated in the ACSF (1 h, at room temperature, 20–22°C). Slices were transferred to a 2-ml chamber fixed to an upright microscope stage (Olympus BX51WI, Tokyo, Japan) equipped with infrared differential interference contrast video microscopy and a ×40 water-immersion objective. Slices were superfused with carbogen-bubbled ACSF (2 ml/min) and maintained throughout the experiments at room temperature and in some cases at 32–34°C. Picrotoxin (50 μM) was added to the ACSF to eliminate GABA$_A$ inhibition. Abnormal epileptiform activity was not observed in our recording conditions.

**Recordings and analysis**

Single or dual whole cell recordings from soma and dendrites of CA1 pyramidal cells were performed with somatic (4–8 MΩ) and dendritic (10–12 MΩ) patch pipettes (BF150-86-10, Sutter Instruments, Novato, CA) pulled with a P-87 Flaming/Brown Puller (Sutter Instruments) and filled with an internal solution that contained (in mM) 135 KMeSO$_4$, 10 HEPES, 2 Na$_2$-ATP, and 0.4 Na$_3$-GTP, buffered to pH 7.2–7.3 with KOH. Recordings were both in the current- and voltage-clamp modes connected to an Axoclamp-2B amplifier (Axon Instruments, Foster City, CA) (Borde et al. 2000; Martín et al. 2001). For paired recordings, a Cornerstone PC-ONE amplifier (DAGAN, Minneapolis, MN) was also used. In voltage-clamp experiments, the holding potential ($V_h$) was adjusted to −60 or −50 mV to increase the driving force of the sAHP. In current-clamp conditions, the membrane potential ($V_m$) was set to the same values by injecting DC current as needed except when indicated otherwise. In the voltage-clamp configuration, the series resistance was compensated to ≈70 and to ≈80% when Axoclamp-2B and Cornerstone PC-ONE amplifiers were used, respectively. Neurons were accepted only when the seal resistance was >1 GΩ and the series resistance (10–20 MΩ for somatic recordings and 30–50 MΩ for dendritic recordings) did not change >20% during the experiment. Current-clamp recordings were rejected if the resting $V_m$ depolarized to values >−50 mV. Visually identified dual somatic/dendritic recordings were possible with the dendritic electrode placed up to ≈200 μm from the soma (Fig. 1C). However, prolonged single dendritic record-
Experiments started after a 15- to 20-min stabilization period after the establishment of the whole cell configuration. The sAHP was activated by a depolarizing current pulse (intensity: 0.5 nA, duration: 200 ms) applied through the somatic or dendritic recording electrode. The pulse induced a burst of action potentials (APs) followed by the AHPs. Experiments in which the average number of APs or the amplitude of the sAHP changed >20% from initial controls were rejected; both values usually remained stable for the duration of the experiment (≤1 h). In some experiments, the sAHP was induced by AP barrages (200 ms, 30 Hz) evoked either by SC stimulation (close to the soma) or by antidromic stimulation (in the alveus). The sAHP was activated under voltage clamp by a depolarizing voltage command pulse (duration: 200 ms, from the V_r to 0 mV) either applied through the somatic or dendritic electrodes. The sAHP remained stable for the duration of the recording and experiments were rejected when the amplitude of the sAHP changed >20%. All these procedures minimized errors due to inadequate voltage control both in somatic and dendritic recordings. Cells were rejected if the resting V_m depolarized to values more than −50 mV throughout an experiment.

Afferent PP-SC stimulation (Gray S88 and SIU, Quincy, MA) was performed using bipolar nickel-chrome electrodes (80 μm diam, tip separation: ≈100 μm) placed at the stratum radiatum near the CA2 region (≈500 μm from somatic layer). In some cases, a second electrode (as in the preceding text) was placed closer to the somatic layer (≈50 μm) to stimulate a separate group of SC axons. Stimulation in the SO at the basal dendrites was performed with a similar bipolar electrode. For antidromic bipolar stimulation a similar electrode was placed in the alveus and stimulation intensity and electrode placement were adjusted to avoid the generation of synaptic potentials. The voltage- and current-clamp data were low-pass filtered at 3.0 kHz and sampled at >10.0 kHz, either through a Digidata 1200B or 1322A (Axon Instruments). The pClamp programs (Axon Instruments) were used to generate stimulus timing signals and transmembrane current pulses and to record and analyze data. Statistical analysis was performed using the SigmaPlot program (SPSS, Chicago, IL). Results are given as means ± SE in text and figures, and statistical significance was calculated by Student’s t-test for unpaired or paired data. Differences were expressed in percentage from control values in all cases. The threshold level of significance was set at P < 0.05 (*); P < 0.01 (**) and P < 0.001 (***) levels are also indicated.

**Intracellular Ca^{2+} variations**

Cells were loaded with the Ca^{2+} indicator Fluo-3 (Molecular Probes, Eugene, OR) added to the internal pipette solution at a concentration of 100 μM. Fluorescence measurements of changes in intracellular Ca^{2+} concentration were made with excitation at 490 nM with the Polychrome IV (TILL Photonics) and a filter set optimized for Fluo 3 (Chroma Technology). Recordings were made after 15- to 20-min stabilization period after breaking in to allow the equilibration of the dye, which uniformly filled the soma and the apical dendritic shaft. In most cases, recordings were also performed later (>20 min) and no significant differences in the intracellular Ca^{2+} variation in the soma and apical dendritic shaft were observed. Cells were illuminated at 490 nm during 40 ms at a rate of 0.33/s. The Cohu camera was adjusted to obtain three images per second. Recording and data analysis were performed with the Imaging Workbench software (version 3.0, INDEC Systems, Santa Clara, CA). The changes in fluorescence signals were expressed as the proportion (%) of relative change in fluorescence (∆F/∆F_0) where F_0 is the prestimulus fluorescence level when the cell is at rest and ∆F is the change in fluorescence during activity. Corrections were made for indicator bleaching during trials by subtracting the signal measured under the same conditions when the cell was not stimulated. Cells were recorded in current-clamp conditions at −50 mV, and a depolarizing current pulse (intensity: 0.5 nA, duration: 200 ms) was applied to evoke the Ca^{2+} signal and the subsequent sAHP. Recordings of the intracellular Ca^{2+} concentration changes versus time were obtained “off-line” from specified regions of interest in the soma and apical dendrite.

**Modeling the shunting effects of the sAHP on EPSPs**

The simulation was performed using the NEURON environment (v5.6, Carnevale and Hines 1997; Hines 1994). The simplified model neuron was constructed according to the electrophysiological characteristics of the CA1 pyramidal neurons (Mainen et al. 1996). It consisted of four parts: a simplified apical dendritic compartment, comprising 80 segments (length: 500 μm, diameter from 0.5 to 6 μm from the farthest to the nearest segment); a spherical soma with a 10 μm diam; a basal dendrite (length: 100 μm, diameter: 1.5 μm); and two simplified presynaptic compartments (length: 150 μm, diameter: 1.5 μm), which simulated both the presynaptic PP-SC input that established a synaptic contact at 500 μm from the soma and the presynaptic compartment at the basal dendrites at 100 μm from the soma.

These presynaptic compartments could generate APs and comprised equally distributed leak channels that generated a leak current according to the equation

\[ I_{\text{leak}} = g_{\text{leak}}(E_m - E_{\text{leak}}) \]

where \( I_{\text{leak}} \) is the leak current, \( g_{\text{leak}} \) the conductance, \( E_m \) the membrane potential, and \( E_{\text{leak}} \) the reversal potential of the leak current (\( E_{\text{leak}} = -60 \text{ mV} \)). Active channels, based on Hodgkin and Huxley (1952)-type kinetics, were simulated in the presynaptic compartment (\( \gamma_{\text{Na}} = 2 \text{ μS/cm}^2; \gamma_K = 0.363 \text{ μS/cm}^2 \)).

The sAHP channels were either uniformly distributed from first proximal 250 μm of the apical dendrite and in the soma (the sAHP conductance (\( g_{\text{sAHP}} \)) was 100 μS/cm²), only in the first proximal 250 μm of the apical dendrite (Lancaster and Zucker 1994; Sah and Bekkers 1996); and only in the soma (Bekkers 2000). The Ca^{2+}-influx that triggered the sAHP was through simulated L-type voltage-gated Ca^{2+} channels activated by a depolarizing current pulse and located in the apical dendritic segments and soma (Magee et al. 1998). The L-channels were based on Goldman Hodgkin Katz-type kinetics (Yamada et al. 1989) and had a calcium permeability of 0.000025 cm/s. Following Ca^{2+}-influx through the L-channels the resting Ca^{2+} concentration was recovered via extrusion by Ca^{2+} pumps (Eakin et al. 1995), aided by radial and longitudinal diffusion mechanisms, and intracellular Ca^{2+}-buffering systems (Regeh and Tank 1992).

The presynaptic elements were stimulated by a brief suprathreshold current pulse (1 nA, 2 ms) that triggered an AP that simulated the activation of PP-SC terminals and terminals contacting basal dendrites. The AP-evoked glutamate release matching the time course of transmitter action based on the minimal kinetic model of Destexhe et al. (1994, 1998). The released glutamate activated NMDARs and AMPARs that simulated the PP-SC EPSPs. Simple kinetics were used to simulate the binding-unbinding of glutamate (\( G \)) to the postsynaptic AMPARs and NMDARs at the apical dendrite (close) + G ⇄ (open)

The corresponding equations were

\[ \frac{dr}{dt} = \alpha[G](1 - r) - Br I = g_{\text{max}}[\text{open}]B(V_m)(V_m - E_{\text{rev}}) \]

where \([G] \) is the glutamate concentration (in mM), \( r \) the fraction of receptors in the open state, \( \alpha \) and \( \beta \) the binding and unbinding rates, respectively, and \( g_{\text{max}} \) the maximum conductance. In addition, \( B(V_m) \) represents the voltage-dependent Mg^{2+} block of NMDARs and \( E_{\text{rev}} \) the reversal potential of the corresponding synaptic conductances (Jahr and Stevens 1990a,b). The term \( B(V_m) \) was absent when the Mg^{2+}-free condition was simulated.

The model was fit to simulate experimental recordings of the EPSP with amplitude and \( \tau_{\text{EPSP}} \) as recorded both in control conditions and during the sAHP and recorded simultaneously at 350 μm in the
sAHP regulates dendritic integration

The sAHP controls the amplitude, decay time-constant and "spread" of EPSPs.

RESULTS

sAHP controls the amplitude, decay time-constant and "spread" of EPSPs.

The sAHP regulates synaptic inputs by "shunting" EPSPs at the apical dendrites of CA1 pyramidal neurons (Borde et al. 1999; Lancaster et al. 2001; Sah and Bekkers 1996). Therefore we tested whether the sAHP modified EPSPs evoked by PP-SC stimulation far from the soma (≈500 μm) and recorded both at the soma and the apical dendrite up to ≈350 μm from the soma (Fig. 1A, left). The sAHP was evoked by a depolarizing current pulse (0.5 nA, 200 ms) applied at a low rate (0.1 Hz) at the corresponding somatic (Fig. 1D, left) or dendritic recording sites and exceptionally by generating AP barrages via synaptic or antidromic stimulation (see following text). Stimulations used to elicit the sAHP were adjusted to evoke on the average 7.4 ± 0.5 APs (n = 41) (Fig. 1D, left). The sAHP was measured at the soma and dendrite 250–300 ms after the depolarizing pulse end, when the medium AHP (mAHP) is negligible and the sAHP has attained its peak amplitude (Borde et al. 1999, 2000; Storm and Spruston 1989) of 5.3 ± 0.7 mV (n = 10) at the soma and of 5.1 ± 0.8 mV (P > 0.05 in both cases; n = 10) at the dendrite. The sAHP decayed slowly with a similar time-constant (τsAHP; fits to single exponentials, as in all other cases) of 3.5 ± 0.3 and 3.8 ± 0.4 s (P > 0.05; same cells) in somatic and dendritic recordings, respectively. These measurements were performed at −60 mV (Fig. 1D, middle).

We compared EPSPs evoked by PP-SC stimulation before and during the sAHP, 250–300 ms after the depolarizing pulse end (Fig. 1D, middle). In those conditions, the somatic EPSP peak amplitude decreased from 2.9 ± 0.4 to 2.2 ± 0.3 mV, a 23.0 ± 3.5% reduction, P < 0.01; n = 10) during the sAHP (CC, Fig. 1A, top, and B). In contrast, the peak amplitude of the EPSP recorded at the dendrite was unaffected by the sAHP (P > 0.05; n = 10); CC, Fig. 1A, bottom, and B). Therefore the drop in τEPSP was similar at both recording sites (P > 0.05). Note that both in the control and during the sAHP dendritic EPSPs were faster than somatic ones (CC, Fig. 1A top and bottom, and B).

The preceding results are consistent with the sAHP controlling the amplitude, duration and the “spread” of the EPSPs from apical dendrites to the soma. Because τEPSC is about one order of magnitude faster (3–5 ms) (Hestrin et al. 1990) than the membrane time constant (20–30 ms) (Spruston and Johnston 1992), the EPSP peak amplitude reflects the local input resistance (i.e., the Rm at the EPSP generation site) rather than the whole cell resistance (Carlen and Durand 1981; Sah and Bekkers 1996). In addition, during the sAHP, the EPSPs peak amplitude was unaffected at far dendritic sites (≈350 μm from the soma) but was reduced in dendritic recordings closer to the soma (≈200 μm) and at the soma (Fig. 1C), consistent with the channels mediating the sAHP being located in the first 200 μm of the apical dendritic shaft and also in the soma. In agreement with this location, the decrease in the EPSP amplitude induced by the activation of the sAHP was similar in somatic and dendritic recordings at 100 and 200 μm (Fig. 1C).

Similar experiments were performed under voltage clamp. The depolarizing command pulse evoked a prolonged outward "tail current" with an initial mAHP followed by the slower sAHP (Fig. 1D, right). The mean peak amplitude of the sA HP measured 250–300 ms after pulse end when the mAHP had disappeared, was 91.8 ± 7.5 pA. The peak conductance gAHP was 5.2 ± 0.9 nS and the τAHP 2.8 ± 0.2 s (n = 10). It is noteworthy that the peak conductance of the sAHP and the τAHP were essentially identical in somatic and apical dendritic voltage-clamp recordings (P > 0.05; n = 7). The EPSC was measured before and during the sAHP (250–300 ms after pulse end), and neither the peak amplitude nor the τEPSC of somatic EPSCs was modified by the sAHP (P > 0.05; in both cases; n = 10; VC, Fig. 1A). The same was true for dendritic recordings where neither EPSCs amplitude nor τEPSC changed (P > 0.05; in both cases; n = 7; VC, Fig. 1A). The peak amplitude and τEPSC values of the dendritic and somatic EPSCs were similar (see preceding values), indicating that in our experimental conditions, an acceptable space clamp was achieved in the apical dendrite at least up to ≈400 μm from the soma (Fig. 1A) because a perfect space clamp cannot be achieved in these cells (Spruston et al. 1993). These results indicate that under voltage clamp, the conductance of synapses were not modified by the opening of channels mediating the sAHP. Therefore we conclude that the insensitivity of the EPSCs to the activation of the sAHP suggests that the changes in the properties of EPSPs observed during the sAHP under current clamp were either due to an increased membrane conductance (gAHP) or to the membrane hyperpolarization or to a combination of both.

EPSP changes were induced by the rise in gAHP associated with the sAHP activation.

The EPSP amplitude reduction and the decreased τEPSP could either be caused by the increased gAHP associated with the opening of the Ca2+-activated K+ channels that mediate the sAHP or to membrane hyperpolarization and Mg2+ re-block of NMDA channels. To distinguish between the two possibilities, we performed different tests. First, we compared the effects of the "real" sAHP (Fig. 2A) on responses evoked both by brief "test" depolarizing pulses (0.01 nA and 200 ms) applied through the somatic recording electrode and by EPSPs evoked by PP-SC stimulation far from the soma (≈500 μm) and of a "simulated" sAHP (Fig. 2C). The simulated sAHP was generated by ap-
The preceding results taken together are consistent with a scenario where the EPSP modifications are caused by the increase in $g_m$ induced by the opening of the Ca$^{2+}$-activated K$^+$ channels that mediate the sAHP. Those results are also in harmony with the linear functional relationship between the changes in the $g_m$ estimated with responses evoked by test current pulses, and the amplitude of the sAHP (Fig. 2C, bottom). In addition, both the EPSP amplitude and $\tau_{\text{EPSP}}$ also showed a linear relationship with the $g_m$ during the sAHP both at $-60$ and $-80$ mV (Fig. 2, A and B, bottom), consistent with an ohmic behavior and with the parallel change in membrane time constant associated with the number of open Ca$^{2+}$-activated K$^+$ channels controlling those variables. Therefore the increased $g_m(\approx 150\%)$ reduced the transmembrane voltage drop induced by the synaptic current (see following text) and shifted the cutoff frequency of the membrane to higher frequencies (Carlen and Durand 1981; Mainen et al. 1996). These results are consistent with the functional relationship between the membrane capacitance ($C_m$) and $R_m$ that control the membrane time constant $\tau_m$ (where $\tau_m = C_m \times R_m$) and determine the cutoff frequency filtering properties of the membrane. Therefore the Mg$^{2+}$-re-block of NMDA channels and the activation-deactivation of voltage-gated conductances by the hyperpolarization mediated by the activation of the sAHP were not engaged in our experimental conditions (see following text).

The rise in intracellular Ca$^{2+}$ caused by the AP burst that elicited the sAHP could activate Ca$^{2+}$-mediated second-messenger cascades that might trigger the EPSP reduction independently of the change in membrane $g_m$. However, the effects induced by the activation of second-messenger cascades usually outlast the Ca$^{2+}$ elevation (reviewed in Frick and Johnston 2005), whereas the sAHP time course closely follows the kinetics of the $s_{\text{AHP}}$ and the increased $g_m$ (Martín et al. 2001). Therefore we analyzed whether the “shunting” effect on the EPSPs and the sAHP decayed with similar kinetics and paralleled the associated $g_m$ modifications. We also simultaneously monitored the EPSP changes and the intracellular Ca$^{2+}$ elevation induced by the AP burst that elicited the sAHP both in control conditions (Fig. 3A) and during inhibition of the sAHP with 10 μM isoproterenol (Fig. 3B). We found that the EPSP amplitude followed the time course of the sAHP (Fig. 3, A and C). In addition 10 μM isoproterenol inhibited the sAHP, the associated $g_m$ rise and the EPSP amplitude reduction, without changing the peak amplitude of the intracellular Ca$^{2+}$ signal in the soma and the apical dendrite ($P > 0.05$ in both cases; $n = 6$; Fig. 3, A, B, and D). Although isoproterenol may increase Ca$^{2+}$ influx through L-type channels (Fisher and Johnston 1990), we observed no changes of the intracellular Ca$^{2+}$ signal in the soma and dendrite, implying that increases in the intracellular Ca$^{2+}$ concentration were not contributing to the observed effects.

These results taken together indicate that the EPSP reduction was independent of the activation of Ca$^{2+}$-mediated second-messenger cascades. In addition, these results suggest that there is a threshold rise in $g_m (\approx 150\%)$ required to effectively shunt the EPSPs because the statistically significant EPSP modifications only occurred when the $g_m$ rise attained that value and the sAHP amplitude was large (Fig. 3C).
The sAHP is temperature-sensitive and raising the bath temperature reduces its amplitude and its decay $\tau_{\text{sAHP}}$ in neocortical and hippocampal CA1 pyramidal neurons (Lee et al. 2005). This temperature sensitivity may be important functionally because effects induced by the activation of the sAHP could be different at physiological temperatures. Therefore we tested the effects of increasing the temperature to 32–34°C and found that the sAHP was reduced (by 18.5 ± 4.6%, $P < 0.05; n = 5$) and the decay $\tau$ of the sAHP was decreased (by 10.5 ± 2.8%, $P < 0.05$; same cells; Fig. 4A, bottom right). The drop in $g_m$ caused by the activation of the sAHP at 32–34°C (i.e., the difference in $g_m$ between both temperatures) was 5.8 ± 2.1% ($P < 0.05$; same cells). In addition, the modifications in EPSP amplitude and the decay $\tau_{\text{EPSP}}$ induced at the soma by the activation of the sAHP at 32°C (a 19.3 ± 3.2% amplitude reduction and a 39.5 ± 10.4% $\tau_{\text{EPSP}}$ reduction, $P < 0.05$ in both cases; same cells) were essentially identical to those recorded at room temperature (Fig. 4B). Therefore the interactions of EPSP with the sAHP may be functional in the natural condition.

Neither the H-current nor voltage-gated Na current contributed to EPSP changes during the sAHP

The dendrites of CA1 pyramidal neurons contain a variety of voltage-gated ion channels that could contribute to the modulation of EPSPs (Lipowsky et al. 1996; Magee and Johnston 1995; reviewed in Johnston et al. 2003; Spruston et al. 1994). The dendritic hyperpolarization-activated H-current ($I_h$) accelerates EPSP kinetics (Otmakhova and Lisman 2004; reviewed in Johnston et al. 2003; Magee 1998) and may contribute to the decreased $\tau_{\text{EPSP}}$ observed during the sAHP because the hyperpolarization induced by the sAHP could activate the H-current.

To test this possibility, we analyzed the EPSP before and during the sAHP in control solution and after blocking the $I_h$ with extracellular ZD7288 (50 μM). The EPSP amplitude and $\tau_{\text{EPSP}}$ modifications induced by the sAHP were not modified by blocking the $I_h$ with ZD7288 neither in somatic recordings (Fig. 5A) nor in apical dendritic recordings (Fig. 5B; $P < 0.05$ in both cases; n = 8). Therefore these results imply that the hyperpolarization induced by the sAHP did not modify the H-current, suggesting that the observed effects were most likely exclusively mediated by the $g_m$ drop associated with the activation of the sAHP. Our results also indicate that $I_h$ was activated at the resting $V_m$ because ZD7288 increased the EPSP amplitude in the soma (a 37.3 ± 2.1% increase; $P < 0.05$; same cells) and increased $\tau_{\text{EPSP}}$ both in soma and dendrite (Fig. 5C; a 25.3 ± 3.2% and a 72.8 ± 37.3% increase, respectively; $P < 0.05$ in both cases; same cells; see DISCUSSION).

Finally, dendritic voltage-gated sodium channels mediating the $I_{\text{Na}}$ could amplify EPSPs in hippocampal CA1 pyramidal cells (Lipowsky et al. 1996). To test this possibility, we blocked $I_{\text{Na}}$ with intracellular QX-314 (5 mM) (Talbot and Sayer 1996). In these conditions, a Ca$^{2+}$ spike was triggered by the depolarizing pulse that activated a sAHP that did not change during the experiment ($P > 0.05$; n = 6). The sAHP
Evoked under QX-314 modified somatic and dendritic EPSPs in essentially the same manner as in control conditions \((P < 0.05\) in both cases; \(n = 8\); Fig. 5B). These results suggest that \(I_{\text{Na}}\) is not involved in the sAHP-mediated changes of the EPSPs. Although QX-314 affects a wide range of membrane conductances including \(K^+\), \(Ca^+\)-mediated (Svoboda et al. 1997) and hyperpolarization-activated conductances (Perkins and Wong 1995) it did not modify the sAHP, suggesting that the described effects on EPSPs are mediated by the activation of the sAHP without a relevant contribution of other membrane conductances.

**sAHP had different effects on EPSP\(_{\text{AMPA}}\) and EPSP\(_{\text{NMDA}}\)**

Glutamate released by PP-SC terminals activates both AMPARs and NMDARs evoking a fast EPSP\(_{\text{AMPA}}\) and slow EPSP\(_{\text{NMDA}}\) component, respectively (Collingridge et al. 1983; Hestrin et al. 1990). Therefore the sAHP could decrease the peak amplitude of the EPSP recorded at the soma as a consequence of reductions of EPSP\(_{\text{AMPA}}\) or EPSP\(_{\text{NMDA}}\) or of both. We tested the possible differential modulation of the isolated EPSP\(_{\text{AMPA}}\) and EPSP\(_{\text{NMDA}}\) by the sAHP with an \(Mg^{2+}\)-free solution that abolished the voltage dependence of the NMDA conductance and separately blocked NMDARs and AMPARs with AP5 (50 \(\mu M\)) and CNQX (20 \(\mu M\)), respectively (Fig. 6).

During the sAHP, the peak amplitude of the isolated EPSP\(_{\text{NMDA}}\) was reduced both in somatic sites (from 1.8 \(\pm\) 0.2 to 1.0 \(\pm\) 0.1 \(mV\), a 44.5 \(\pm\) 8.5\% reduction, \(P < 0.01\); \(n = 8\)) and apical dendritic sites (from 2.6 \(\pm\) 0.2 to 1.2 \(\pm\) 0.2 \(mV\), a 53.9 \(\pm\) 8.5\% reduction, \(P < 0.01\); \(n = 8\); Fig. 6, A and B). In contrast, \(\tau_{\text{NMDA}}\) did not change during the sAHP neither in dendritic nor somatic recordings \((P > 0.05\) in both cases; \(n = 8\)), implying that the slower EPSP\(_{\text{NMDA}}\) was affected by the “shunt” caused by the increased \(g_{\text{m}}\), but not by the frequency-filtering change that paralleled the activation of the sAHP (Fig. 6, A and B).

The \(g_{\text{m}}\) rise reduced EPSP\(_{\text{NMDA}}\) both at dendritic and somatic recording sites. This occurred because the NMDA current \((I_{\text{NMDA}})\) has slow ON-OFF kinetics as compared with the membrane time constant, and therefore its peak amplitude depends on the whole cell \(g_{\text{m}}\) that was drastically increased \((\approx 150\%)\) during the sAHP. Therefore \(I_{\text{NMDA}}\) tends to flow exclusively through the reduced \(R_{\text{m}}\) but not through \(C_{\text{m}}\), thus obeying the Ohm law where the membrane voltage change \((V_{\text{NMDA}})\) induced by the \(I_{\text{NMDA}}\) flowing through \(R_{\text{m}}\) is given by \(V_{\text{NMDA}} = I_{\text{NMDA}} \cdot R_{\text{m}}\) (Carlen and Durand 1981). In contrast, the peak EPSP\(_{\text{AMPA}}\) amplitudes were unmodified during the sAHP both in somatic (Fig. 6A) and apical dendritic (Fig. 6B) recordings \((P > 0.05\) in both cases; \(n = 8\)). In addition, \(\tau_{\text{AMPA}}\) decreased during the sAHP both in the dendrite \((52.0 \pm 5.7\%\) \(P < 0.01\); \(n = 8\)) and the soma \((47.9 \pm 5.7\%\) reduction; \(P < 0.01\); \(n = 8\)), suggesting an effect mediated by a change in frequency-filtering properties that paralleled the \(g_{\text{m}}\) increase associated with the activation of the sAHP.

The peak amplitude of EPSP\(_{\text{AMPA}}\) in apical dendritic and in somatic recordings was not modified by the “shunt” induced by the sAHP activation. This occurs because \(I_{\text{AMPA}}\) has extremely

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**FIG. 5.** Neither the H-current nor the Na-current contribute to the effects of the sAHP. A: summary data showing that neither ZD7288 (50 \(\mu M\)) nor QX314 (5 \(\mu M\)) modified the effects of the sAHP on somatic EPSP amplitude and \(\tau_{\text{EPSP}}\); B: same as A but dendritic recording. C: averaged somatic and dendritic EPSPs before (control) and during superfusion with ZD7288 (50 \(\mu M\)) in the absence of sAHP activation.

**FIG. 6.** The sAHP specifically shunts EPSP\(_{\text{NMDA}}\). A, left: isolated somatic EPSP\(_{\text{AMPA}}\) (APV, 50 \(\mu M\)) and EPSP\(_{\text{NMDA}}\) (CNQX, 20 \(\mu M\)) averages recorded in Mg\(^{2+}\)-free solution, before and during the sAHP. Right: summary data showing the effects (%) of the sAHP on average EPSP\(_{\text{AMPA}}\) and EPSP\(_{\text{NMDA}}\) amplitudes and corresponding \(\tau_{\text{AMPA}}\) and \(\tau_{\text{NMDA}}\). B, left and right: same as A but dendritic recordings.
rapid “on” kinetics (Jonas 2000) as compared with the membrane time constant (Agmon-Sir and Segev 1993; Redman 1973), and at the onset of the EPSP, the AMPA current (I_{AMPA}) tends to flow through C_m but not through R_m and therefore is not reduced by the g_m rise caused by the opening of the channels that mediate the sAHP. However, the decay slope of I_{AMPA} has components with kinetics that are closer to the membrane time constant and the current tends to flows both through R_m and C_m with relative magnitudes that depend on the frequency components of the decay slope of I_{AMPA}. Consequently, \( \tau_{AMPA} \) is reduced because the R_m drop associated with the opening of the sAHP channels effectively decreases the membrane time constant (\( \tau_m \)) according to the functional relationship \( \tau_m = R_m \cdot C_m \).

The preceding results suggest a key role of the increased g_m and of the associated changes in frequency-filtering properties caused by the activation of K’’ channels mediating the sAHP in the regulation of the EPSP waveform, the contribution of NMDA versus AMPA components and of their spread along the apical dendrite. In addition, the experiments support the absence of a contribution of membrane hyperpolarization and Mg^{2+} reblock of the NMDA channels because the voltage dependence of NMDA channels was eliminated in the Mg^{2+}-free experiments. Therefore these results would indicate that the decreased somatic EPSP amplitude (Fig. 1A) was caused by a selective shunt of the slower EPSP_{NMDA} component. Consistent with this view the dendritic EPSP peak amplitude was unaffected by APV (113.5 \pm 30.2% of control; \( P > 0.05; n = 6 \)) but profoundly antagonized by CNQX (33.4 \pm 5.2% of control; \( P < 0.001; n = 6 \)). In contrast, the somatic EPSP peak amplitude was reduced both by APV (75.2 \pm 10.2% of control; \( P < 0.01; n = 6 \)) and CNQX (28.4 \pm 5.2% of control; \( P < 0.01; n = 6 \); data not shown). These results match those published by Otmakhova and Lisman (2004) showing that 30% of the somatic perforant pathway EPSP peak amplitude is mediated by a NMDA component. However, \( \tau_{EPSP} \) changed both at apical dendritic and somatic recording sites in harmony with the combined effects of the amplitude reduction of the slower EPSP_{NMDA} and the accelerated \( \tau_{AMPA} \) mediated by the change in frequency-filtering properties.

Recapitulating, we show that both at somatic and dendritic recordings the peak amplitude of the slower EPSP_{NMDA} was shunted, whereas the peak of the faster EPSP_{AMPA} was insensitive to the g_m rise that paralleled the sAHP. These results could clarify why the peak amplitude of the dendritic “compound EPSP” (i.e., EPSP_{AMPA} + EPSP_{NMDA}) was unchanged, whereas the compound somatic EPSP was reduced by the sAHP. These somato-dendritic differences in the effects of the sAHP could result if the dendritic EPSP peak is mainly mediated by AMPARs, whereas both AMPARs and NMDARs contribute appreciably to the peak of the somatic EPSP. To test this hypothesis, we compared the time course of averaged and scaled somatic and dendritic isolated EPSP_{AMPA} and EPSP_{NMDA} with the time course of the compound EPSPs, mediated both by AMPARs and NMDARs (Fig. 7). The analysis confirmed our predictions, revealing that EPSP_{AMPA} contributed more than EPSP_{NMDA} to the peak compound dendritic as compared with the peak compound somatic EPSP (D and S, Fig. 7B). This occurred because the membrane frequency filtering properties delayed (\( \Delta t \)) the “spread” of EPSP_{AMPA} to the soma (AMPA, Fig. 7B), whereas EPSP_{NMDA} was not affected by the membrane filter and was not delayed (NMDA, Fig. 7B) (Carlen and Durand 1981; Mainen et al. 1996). Therefore the g_m rise did not affect the peak amplitudes of the compound EPSP in the dendrite, which had little NMDA component, but effectively reduced it in the soma, which had a sizeable NMDA component (AMPA+NMDA, Fig. 7B; see model).

Dendritic recordings in Mg^{2+}-free solution showed substantial shunting of the compound EPSP amplitude during the sAHP as compared with the control solution (bar plots Figs. 1B and 6B). These results are most likely due to the specific increase in the amplitude of EPSP_{NMDA} caused by the absence of block by extracellular Mg^{2+} of the NMDAR channel. This is exemplified in Fig. 7C using the same type of analysis as in B that shows the effective contribution of the larger NMDA component to the dendritic EPSP peak amplitude in Mg^{2+}-free solution. However, in normal Mg^{2+} solution, the amplitude of the NMDA component did not significantly contribute to the compound dendritic EPSP (Fig. 7, B and C). It is noteworthy that although the Mg^{2+}-free solution could also increase Ca^{2+} influx through voltage-gated Ca^{2+} channels, it did not influence the sAHP because in these conditions the sAHP was essentially identical to that induced in control ACSF (\( P > 0.05 \)) and did not contribute to the larger EPSP modifications observed in these experiments.

There is controversy as to where over the somato-dendritic regions sAHP channels are expressed and both dendritic and somatic locations have been reported (Bekkers 2000; Lancaster and Zucker 1994; Sah and Bekkers 1996). Therefore we also tested the effects of the activation of the sAHP on EPSPs evoked at the basal dendrites by stimulation at the SO (\( \approx 100 \) \mu m from the stratum pyramidale) that could provide additional...
FIG. 8. The sAHP also modifies EPSPs evoked in the basal dendrites. A, left: diagram showing recording and stratum oriens (SO) stimulation setup. Right: representative record showing averaged EPSPs in control conditions (top) and under 50 μM APV (middle) and 20 μM CNQX and Mg2+-free solutions (bottom) evoked by the SO stimulation before and during the sAHP. B: summary data showing the changes in the amplitude and τ of the control EPSP, EPSP_{AMPA} and EPSP_{NMDA}, respectively.

-information as to the presence of sAHP channels in the soma (Sah and Bekkers 1996). We found that the sAHP evoked by somatic depolarizing pulses induced a reduction of the SO EPSP amplitude and τ_{EPSP} (amplitude reduction 18.1 ± 5.2%; τ_{EPSP} reduction 45.2 ± 10.1%, P < 0.01 in both cases, n = 6; Fig. 8B) that were similar to those induced by the sAHP on PP-SC EPSPs. These results suggest that the Ca^{2+}-activated K\(^+\) channels that mediate the sAHP are probably located in the soma as well as in the proximal apical dendrite. In addition, at the basal dendrites, the amplitude of the isolated EPSP_{AMPA} remained unmodified, and τ_{AMPA} was reduced whereas the amplitude of EPSP_{NMDA} was reduced by the sAHP (Fig. 8, A and B).

sAHP induced by “physiological” stimulations also modified EPSPs

We investigated if the sAHP induced by AP bursts imitating more physiological conditions also evoked EPSP modifications as those elicited when the sAHP was induced by depolarizing pulses. We recorded EPSPs at the soma and dendrite (≈350 μm) and stimulated SCs near the soma (≈50 μm) with a barrage of high-intensity pulses (30 Hz; 200 ms) that induced EPSPs that triggered a burst of APs followed by a sAHP. We simultaneously evoked EPSPs before and during the sAHP by stimulating another group of PP-SC afferents further from the soma (≈500 μm, Fig. 9A). The amplitude reduction of somatic EPSPs (from 2.3 ± 0.5 to 1.7 ± 0.4 mV, a 25.9 ± 3.5% reduction; P < 0.05; n = 10) and of the τ_{EPSP} (from 80.1 ± 3.8 to 38.2 ± 1.6 ms a 52.4 ± 9.8% reduction, P < 0.05; same cells) during the sAHP were essentially identical to those evoked in the previous experiments. Moreover, the EPSP amplitude was unaffected (P > 0.05; n = 10), whereas the τ_{EPSP} was reduced at dendritic recordings (by 50.0 ± 11.5%, P < 0.05; same cells) as when the sAHP was evoked by depolarizing pulses. Taken together those results suggest that a similar activity-dependent regulation of synaptic signals by the sAHP may be active in the natural conditions. Essentially identical results were also obtained when the sAHP was induced by a barrage of APs evoked by antidromic stimulation of CA1 pyramidal neuron axons at the alveus (data not shown).

In addition, the sAHP evoked by these methods had similar effects on apical dendritic EPSPs as those in which the sAHP was generated by depolarizing pulses injected through the dendritic recording electrode.

FIG. 9. The sAHP generated by PP-SC stimulation shunts EPSPs. The sAHP evoked by depolarizing pulses reduces temporal and spatial summation of EPSPs. A, left: diagram showing recording and PP-SC stimulation setup. Right: representative record showing recording and PP-SC stimulation setup. B: summary data showing the EPSP changes during the sAHP evoked by the SC barrages (as in A). C, left: diagram showing recording and stimulation for the analysis of spatial summation. Right: superimposed averaged responses (top) of the linear sum of the responses evoked by isolated PP-SC stimulation near and far from the soma (linear sum) and by simultaneous stimulation PP-SC stimulations at the same sites (experimental condition), both obtained before and during the sAHPs evoked by somatic depolarizing current pulses. Summary data (bottom, right) showing the summation (as % of expected; linear sum) before and during the sAHP. D, left: diagram showing recording and stimulation used to analyze temporal summation. Right: representative response showing the sAHP evoked somatic by depolarizing pulses and PP-SC EPSPs evoked by paired stimuli (50-ms delay) applied far from the soma; the inset shows averages of summed EPSPs recorded before and during the sAHP.
sAHP reduced both the spatial and temporal summation of EPSPs

The preceding described modifications in the amplitude, duration, and spread of the EPSPs over the apical dendrite may have important functional consequences on synaptic integration by modifying the temporal and spatial summation of EPSPs (Borde et al. 1999; Lancaster et al. 2001; Sah and Bekkers 1996). Therefore we tested the modifications in spatial summation induced by the sAHP using somatic recordings and stimulation of PP-SC at the apical dendrites both near (~50 μm) and far (~500 μm) from the soma (Fig. 9C, left). Paired-pulse facilitation tests were made to minimize the possible stimulation of fibers shared by both PP-SC inputs (Le Ray et al. 2004). Distal and proximal PP-SC inputs were stimulated in isolation and both averaged responses were added (Fig. 9C, right, linear sum). Both synaptic inputs were then stimulated simultaneously, and the EPSPs were averaged (Fig. 9C, right, experimental condition). In the control condition, spatial summation was sub-linear (Cash and Yuste 1998, 1999; Skydsgaard and Hounsgaard 1994) because the EPSP was smaller than that expected from the linear sum of EPSPs evoked by the isolated stimulation of both synaptic inputs (amplitude was 6.0 ± 0.5 mV in the experimental condition of EPSPs and 7.3 ± 0.5 mV in the linear sum of EPSPs; a 17.8 ± 3.4% reduction P < 0.01; n = 5; Fig. 9C). This result was most likely caused by interactions between the conductance modifications induced by the EPSPs, where in somatic recordings the far dendritic EPSP is reduced by the g m increase associated with the generation of the EPSP near the soma (Langmoen and Andersen 1983). In contrast, there were no differences between the linear sum of EPSPs and the EPSP evoked by combined stimulation in the experimental condition (P > 0.05; n = 5) when the same stimulations were performed during the sAHP evoked by somatic depolarizing pulses (Fig. 9C, right), indicating that the sAHP linearized the spatial summation of EPSPs in the experimental condition. This result is consistent with the much larger change in g m induced by the activation of the sAHP (g AHP = 5.2 ± 0.9 nS, n = 10) as compared with the smaller change in g m associated with the genesis of the proximal EPSP (g EPSP = 0.5 nS) (see Spruston et al. 1994) that minimizes the interaction between EPSPs observed in control conditions.

To analyze the changes in temporal summation, we used somatic recordings and stimulated PP-SC far from the soma (~500 μm) with paired pulses at 80-ms interval (Fig. 9D). Temporal summation was also markedly reduced during the sAHP (Fig. 9D, right). We estimated the degree of temporal summation, by measuring the ratio of the second to the first EPSP peak amplitude before and during the sAHP. The control difference of 134.3 ± 4.6% (before the AHP) was reduced to 97.6 ± 13.3% during the sAHP (P < 0.05 in both cases; n = 4). Therefore the sAHP induced a ~37% decrease in temporal summation, consistent with the decrease of both EPSPs amplitude and the τ EPSP induced by the g m increase associated with the opening of sAHP channels.

Taken together the preceding results suggest that activation of the sAHP efficiently modifies the integrative properties of pyramidal neurons by regulating the amplitude, time course and spread of EPSPs from the apical dendrite to the soma. The reduced temporal and spatial summation of EPSPs that parallels the sAHP activation may function in physiological conditions when AP bursts are induced in CA1 pyramidal neurons by excitatory synaptic inputs as may occur during the theta rhythm in vivo (Kamondi et al. 1998; Nuñez et al. 1987, 1990) and under activation of NMDARs in vitro where the sAHP plays a key role (Bonansco and Buño 2003).

Computer simulation of sAHP-EPSP interactions

The simplified model neuron (see METHODS) was constructed to simulate the effects of the sAHP on the compound EPSP (AMPA + NMDA) amplitude and τ EPSP both in current- and voltage-clamp conditions. The AMPARs and NMDARs were placed in the apical dendrite 500 μm from the soma, and EPSPs were recorded simultaneously at 350 μm in the apical dendrite and in the soma (Fig. 10A, left). We first simulated the effects of the placing the sAHP both in the dendrite and soma. The activation of the sAHP did not modify the amplitude of the compound EPSP at the dendrite, but reduced it in the soma (16%) and reduced τ EPSP both at the soma (50%) and dendrite (48%; Fig. 10A, right CC). In contrast, there were no changes in the amplitude and waveform of somatic and dendritic EPSCs evoked by stimulation in the dendrite (Fig. 10A, right VC).

Simulations in current-clamp conditions of the effects of the activation of the sAHP placed both in the dendrite and soma were also performed with isolated EPSP NMDA and EPSP AMPA evoked by simulation in the apical dendrite (Fig. 10B, s. radiatum). The sAHP reduced the peak amplitude of EPSP NMDA at dendritic (27%) and somatic (34%) sites (Fig. 10B, EPSP NMDA), whereas the peak amplitude of EPSP AMPA was unaffected at both sites (Fig. 10B, EPSP AMPA). In addition, τ AMPA was reduced by the sAHP both at both somatic (49%) and dendritic sites (48.1%), whereas τ NMDA was not modified.

![Fig. 10](http://jn.physiology.org/DownloadedFrom/10.220.33.2)
We also simulated the effects of the sAHP placed both in the dendrite and soma on isolated EPSP_{NMDA} and EPSP_{AMPA} evoked in the basal dendrite (see METHODS), and effects were essentially identical to those evoked in the apical dendrite (Fig. 10C, SO). Effects of the sAHP as those in the apical dendrite were also observed with the simulated compound EPSP evoked in the basal dendrite. Therefore the simulation closely reproduced our experimental results, supporting the assumptions drawn from the experiments about the mechanisms involved and on the possible somato-dendritic location of the sAHP channels.

Simulations can be extremely useful because they may predict effects that provide important information on the properties of the system that but that are either impossible or very difficult to test experimentally. An open question that remains to be solved is how the EPSP is affected by the placement of the sAHP channels over the somato-dendritic membrane. Therefore we separately simulated the effects of placing the sAHP channels exclusively in the apical dendrite or in the soma on compound EPSPs evoked in the apical dendrite. These simulations confirmed that the best fits to the EPSP amplitudes in the experimental results were obtained by simulating the combined dendro-somatic placement of the sAHP channels (a 4% difference from experimental values) and a dendritic location also provided acceptable fits (a 5% difference), whereas the somatic location gave the poorest results (a 19% difference). Therefore these results are consistent with a model where the sAHP channels that induced the EPSP modifications are most likely located in the dendrite and soma of CA1 pyramidal neurons.

**DISCUSSION**

We have characterized the changes induced by the Ca^{2+}-activated K^+-conductance that mediates the sAHP on the EPSPs evoked by PP-SC and SO stimulations in the apical and basal dendrites, respectively, and their propagation to the soma of CA1 pyramidal neurons. Our main and original findings were that in the apical dendrites: the amplitude of EPSPs was not modified in dendritic recordings close to where EPSPs were generated at distal recording sites of >300 μm; in contrast consistent with depressed dendrite-to-soma propagation, the amplitude of EPSPs was markedly reduced at the soma far from where EPSPs were evoked; the decay time constant τ_{EPSP} was accelerated both in dendritic and somatic recordings, suggesting a change in membrane frequency-filtering properties associated with the activation of the sAHP; and last both spatial and temporal summation were reduced in somatic recordings as a result of the amplitude and duration reductions of EPSPs. These effects can be explained by the interactions between the changes in membrane properties induced by the opening of the Ca^{2+}-activated K^+-channels that mediate the sAHP and the different kinetics of the AMPA and NMDA currents that contribute to PP-SC EPSPs (Collingridge et al. 1983; Hestrin et al. 1990).

We provide original data showing that the “shunting” caused by the increased g_m associated with the activation of the sAHP reduced the peak amplitude of the slower EPSP_{NMDA}. In contrast, the amplitude of the faster EPSP_{AMPA} was insensitive to the changes in g_m caused by the opening of the Ca^{2+}-activated K^+ channels that mediate the sAHP. In addition we show that the selective reduction of the EPSP_{NMDA} was not caused by re-block of NMDA channels by external Mg^{2+} during the sAHP-induced hyperpolarization because membrane hyperpolarization induced by the “simulated” sAHP waveforms did not cause changes in EPSPs, whereas the “real” sAHP did and the action of the sAHP on EPSPs was not preserved in experiments where the voltage dependence of NMDA currents was abolished in Mg^{2+}-free external solution. Moreover, neither the hyperpolarization-activated I_nor the voltage-dependent I_{NMDA} that may modify EPSPs at the apical dendrites (e.g., Lipowsky et al. 1996; Otmakhova and Lisman 2004; reviewed in Johnston et al. 2003; Magee 1998) contributed to the effects of the sAHP on EPSPs because similar results were obtained in the presence of their blockers ZD7288 and QX314, respectively. These negative results are probably related to the small changes in V_m associated with the sAHP activation. Although obviously the significant effect was mediated by the increased g_m associated with the activation of the sAHP, we cannot completely exclude a contribution of the I_n in the experiments performed at ~80 mV.

We also tested the changes induced by the sAHP on EPSPs evoked at the basal dendrites by stimulation at the SO that provide new evidence indicating that the Ca^{2+}-sensitive K^+ channels that mediate the sAHP are localized in the proximal apical dendrite (200 μm) and at the soma. These results contradict those published by Sah and Bekkers (1996) that excluded a somatic location of the sAHP. The differences between our results and those of Sah and Bekkers (1996) could be caused by the significantly slower SO EPSPs recorded in our conditions. Indeed, although the decay τ of SO EPSPs is not indicated in the Sah and Bekkers (1996) paper, the EPSPs shown in their Fig. 3B are much faster than those in the present results and thus would not be significantly modified by a sAHP activated at adjacent sites. The differences in EPSP kinetics between our results and those of Sah and Bekkers (1996) could be caused by the V_m at which they were evoked which was more depolarized in our experiments and thus would show a larger and slower NMDA component. The older age of the animals used by Sah and Bekkers (1996) that may show differences in the dendritic expression of glutamatergic receptor types (Otmakhova et al. 2002) could also underlie the divergence.

Interesting are the changes in τ_{EPSP} because they were essentially identical at both dendritic and somatic recordings and thus independent of the distance of the recording from the EPSP generation site far from the soma. The decreased τ_{EPSP} was caused both by the reduced amplitude of EPSP_{NMDA} and the accelerated τ_{AMPA} that was induced by the shift in frequency-filtering properties associated with the g_m drop that paralleled the sAHP activation.

It has been reported that the propagation of EPSPs from apical dendrites to the soma and their effectiveness in terms of eliciting APs at the axonal generation site can be precisely regulated in pyramidal neurons by dendritic voltage-gated channels that may either boost EPSPs by activation of Na^+ and Ca^{2+} channels (Lipowsky et al. 1996; Magee and Johnston 1995; Stuart and Sakmann 1995) or depress EPSPs by opening voltage-gated K^+ channels (Hoffman and Johnston 1998). In addition, the impact of these conductances in terms of their interactions with EPSPs and their effectiveness in generating APs is critically dependent on their relative position over the
dendritic membrane (reviewed in Johnston et al. 2003; Spruston et al. 1994; Yuste and Tank 1996). Although the slow Ca\textsuperscript{2+}-activated K\textsuperscript{+} conductance has a central impact on postsynaptic excitability (reviewed in Sah 1996; Stocker 2004; Vogalis et al. 2003), much less was known about its influence on synaptic integration at pyramidal neuron dendrites (Bekkers 2000; Borde et al. 1999; Lancaster et al. 2001; Sah and Bekkers 1996). However, present results provide original data on the interaction of the sAHP with EPSPs in the dendrites.

The high-frequency AP bursts used here to evoke the sAHP are a common discharge pattern of CA1 neurons recorded both in immobilized and behaving rats (Kamondi et al. 1998; Nuñez et al. 1987, 1990) and are followed by hyperpolarization that could be mediated by the sAHP. Therefore in the present experiments, the burst-sAHP sequence is likely to be present and exert its effects on excitatory synaptic activity in physiological conditions and could thus have a major functional relevance because dendritic integration of synaptic inputs is effectively controlled by the sAHP. The sAHP could regulate the temporal summation (Borde et al. 1999; Lancaster et al. 2001; Sah and Bekkers 1996) that occurs during repetitive synaptic activity as well as spatial summation that takes place during activation of converging synapses. The briefer EPSPs induced by the sAHP activation could effectively narrow the time window for coincidence detection with back-propagating APs that is known to play an important role regulating the threshold to induce LTP (Kumar and Foster 2002, 2004; Norris et al. 1998; Waters et al. 2005). The possible functional impact of the restricted coincidence time window by the sAHP should be emphasized because it would mainly be caused by a decrease of the NMDA component of PP-SC EPSPs, and it is the coincidence of membrane depolarization caused by the back-propagating AP during the activation of NMDARs that unblocks NMDA channels thus favoring Ca\textsuperscript{2+} influx that plays a major role in the induction of LTP (Collingridge 1987; Lynch et al. 1983; Malenka et al. 1992). Therefore the sAHP may serve as a mechanism for metaplasticity regulating the induction threshold of the LTP during a brief interval after the burst of APs by controlling the relative contribution of AMPA and NMDA components of EPSPs. In addition, a decreased temporal summation would reduce the depolarization (and the ensuing Ca\textsuperscript{2+} influx) induced by high-frequency synaptic inputs (as those used to induce LTP) and this could also hinder LTP induction (Buño et al. 2005; Le Ray et al. 2004).

In summary, we provide evidence demonstrating that the sAHP efficiently regulates the amplitude, shape, and propagation of synaptic inputs arriving at the soma from apical dendrites of CA1 pyramidal cells. This control could represent a postsynaptic mechanism for regulating synaptic efficacy, dendritic integration, and the threshold for LTP induction by decreasing temporal and spatial summation of EPSPs and the relative contribution of AMPA versus NMDA components of EPSPs in triggering APs.

It has been recently reported that the EPSP amplitude recorded at the soma was independent of synapse location on the apical dendrites (Magee and Cook 2000) and that the efficiency of EPSPs in triggering an AP at the axonal generation site was independent of the synapse location over the dendrite (Rudolph and Destexhe 2003). In this scenario, the sAHP would regulate the efficacy of EPSPs to trigger APs if the synapse location on the apical dendrites is far from the soma (≈500 μm) and could thus contribute to segregate the information depending on the location of the synapse on the apical dendrite. The activation of the sAHP can also be conceived as a general cellular mechanism to control information processing both by decreasing and filtering the flow of incoming dendritic synaptic signals to the soma where the AP output is generated. In addition, because the amplitude of the sAHP is controlled in an activity-dependent manner (Borde et al. 1999, 2000; Le Ray et al. 2004), by neurotransmitters (Martin et al. 2001; Stocker 2004; Storm 1990) and hormones (Carrer et al. 2003; Kumar and Froster 2002), it could represent a key cellular mechanism in actions where information processing, synaptic efficacy, integration, and plasticity must be tuned in harmony with the changing functional requirements of the system. The activation of the sAHP, the associated changes in g\textsubscript{m} and its slow kinetics are activity dependent, Ca\textsuperscript{2+} dependent, and voltage insensitive. Therefore the sAHP can exert its prolonged influence on synaptic signals independently of the activation of dendritic voltage-gated conductances, adding its effects to the voltage-gated conductances that may be active at any given time.

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