Slow Afterhyperpolarization Governs the Development of NMDA Receptor–Dependent Afterdepolarization in CA1 Pyramidal Neurons During Synaptic Stimulation

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Wu, Wendy W., C. Savio Chan, and John F. Disterhoft. Slow afterhyperpolarization governs the development of NMDA receptor–dependent afterdepolarization in CA1 pyramidal neurons during synaptic stimulation. J Neurophysiol 92: 2346–2356, 2004. First published June 9, 2004; 10.1152/jn.00977.2003. CA1 pyramidal neurons from animals that have acquired a hippocampus-dependent task show a reduced slow postburst afterhyperpolarization (sAHP). To understand the functional significance of this change, we examined and characterized the sAHP activated by different patterns of synaptic stimuli and its impact on postsynaptic signal integration. Whole cell current-clamp recordings were performed on rat CA1 pyramidal neurons, and trains of stratum radiatum stimuli varying in duration, frequency, and intensity were used to activate the AHP. At −68 mV, a short train of subthreshold stimuli (20–150 Hz) generated only the medium AHP. In contrast, just two suprathreshold stimuli (>50 Hz) triggered a prominent sAHP sensitive to bath-applications of isoproterenol, carbachol, or intracellularly applied BAPTA, suggesting that the underlying current is the Ca2+-activated K+ current, the s\textsubscript{AHP}. The sAHP magnitude was positively related to stimulus train duration and frequency, consistent with its dependence on intracellular Ca2+ accumulation for activation. About 20% of neurons recorded did not have a sAHP. In response to high-frequency suprathreshold stimuli, these neurons developed a pronounced afterdepolarization (ADP) and multiple action potential firing. The ADP magnitude increased with successive stimuli and was positively related to stimulus intensity and frequency. It was sensitive to bath-applications of thapsigargin and nitrendipine, and abolished by d-AP5, indicating that it is supported by intracellular Ca2+ release, the L-type Ca2+ influx, and N-methyl-d-aspartate (NMDA) receptor–mediated influx. In the presence of d-AP5, we were unable to trigger an ADP with maximal stimulus intensity. Pharmacologically eliminating the sAHP allowed neurons to develop an ADP with the original stimulus train. We propose that the slow AHP acts to facilitate Mg2+ development and NMDA receptor–mediated ADP with the original stimulus train. We propose that the intensity. Pharmacologically eliminating the sAHP allowed neurons to develop an ADP with the original stimulus train. We propose that the slow AHP acts to facilitate Mg2+ development and NMDA receptor–mediated ADP with the original stimulus train. We propose that the slow AHP acts to facilitate Mg2+ development and NMDA receptor–mediated ADP with the original stimulus train.

INTRODUCTION

Action potentials in CA1 pyramidal neurons are followed by a slow, prolonged postburst afterhyperpolarization (sAHP), mediated by a Ca2+-activated K+ current, s\textsubscript{AHP}. We have previously shown a reduction in the sAHP in CA1 and CA3 pyramidal neurons from animals after acquisition of a hippocampus-dependent temporal task, trace eyelink condition.

The sAHP limits cell firing in response to sustained depolarization, a phenomenon known as spike frequency adaptation (Lancaster and Nicoll 1987; Madison and Nicoll 1984; for reviews, see Sah 1996; Storm 1990). There is evidence that the sAHP shapes temporal integration of synaptic inputs by shunting excitatory postsynaptic potentials (EPSPs) arising in the stratum radiatum (Sah and Bekkers 1996). Steady-state activation of s\textsubscript{AHP} with intracellular application of diazo-2, a photolabile BAPTA derivative, also decreases EPSP temporal summation (Lancaster et al. 2001). Since the sAHP decreases the overall neuronal responsiveness to stimulation, it is considered an index of neuronal excitability (Sah 1996).

The sAHP can be activated by Ca2+ derived from many sources. In most studies, it was activated by Ca2+ influx associated with somatic depolarization. However, under certain conditions, voltage-gated Ca2+ influx triggered by synaptic stimuli, even in the absence of action potential generation, or N-methyl-d-aspartate (NMDA) receptor–mediated Ca2+ influx evoked by focal applications of NMDA may be sufficient to activate the sAHP (Lancaster et al. 2001; Shah and Haylett 2002). Although the functional implication for the sAHP has been generally accepted as a dampening in neuronal excitability (Sah 1996), how and to what extent this afterpotential affects the processing of different patterns of EPSPs, such as those likely to be involved during learning, are questions that remain to be determined. Thus to further our understanding of the functional significance of the sAHP reduction, our objectives in this study were 1) to characterize the sAHP activated by different patterns of synaptic stimuli and 2) to examine the...
interaction between the sAHP and the neuronal response to these inputs.

**METHODS**

All experiments were conducted in strict accordance with a protocol approved by the Animal Care and Use Committee of Northwestern University and the USDA. The data represented here are based on recordings from 87 neurons obtained from 29 animals.

**Hippocampus slice preparation**

Six to eight mo-old male F344XBN rats were anesthetized with halothane and killed by decapitation. The brain was rapidly removed, and a block containing the left hippocampus and surrounding structures was dissected out, attached to a mounting tray with cyanoacrylate glue, and immersed in chilled artificial cerebrospinal fluid (aCSF; −1°C) consisting of the following (in mM): 119 NaCl, 26 NaHCO3, 2.5 KCl, 1.3 MgCl2, 6 H2O2, 2 CaCl2, 2 H2O, and 11 glucose. The aCSF used throughout the experiments was continuously aerated with carbogen (95% O2-5% CO2). Transverse hippocampus slices (300 μm) were prepared along the dorsal-ventral axis using a vibratome (TPI, O’Fallon, MO). Slices were transferred to and maintained in a holding chamber filled with aCSF at room temperature (−22°C). Only slices from the middle one-third of the left hippocampus were used for this study. All experiments were conducted ≥1.5 hours after slice preparation at room temperature.

**Electrophysiology**

Patch electrodes were made from filamented, thick-walled borosilicate glass pipettes (Sutter Instrument, Novato, CA), using a Flaming-Brown horizontal puller (P-97, Sutter Instrument), and heat-polished with a microforge (model MF-930, Narishige International, East Meadow, NY) to a resistance of 2–4 MΩ when filled with two internal solutions consisting of the following (in mM): 1) 140 KMeSO4, 10 KCl, 10 HEPES, 4 Mg2+,ATP, and 0.4 Na2,GTP or 2) 130 KMeSO4, 10 KCl, 10 HEPES, 10 BAPTA, 4 Mg2+,ATP, and 0.4 Na2,GTP. In some experiments, 30 mM BAPTA was used to chelate Ca2+ maximally. In a subset of neurons, 0.3% biocytin or 2% Lucifer yellow was added to the patch solution to label the recorded neurons for further morphological identification. The pH of these solutions was adjusted to 7.25 with KOH; the final osmolarities of these solutions were ~290 mOsm. Liquid junction potential (~8 mV) was not corrected.

A stimulating electrode was placed in the s. radiatum, roughly 300 μm distal and 100 μm lateral from the perpendicular axis of the cell layer and recorded neurons. Area CA3 was cut away to prevent recurrent excitation of area CA1 due to repeated synaptic stimulation. The AHP in CA1 pyramidal neurons contains a component mediated by the aminobutyric acid (GABA)A receptor. The amplitude of the AHP was analyzed by determining the time to peak of the current identified as the AHP (IAHP), which is the time from the first voltage pulse to the time that the membrane potential returned to baseline. Statistical significance was determined using ANOVA, Fisher’s PLSD, and Mann-Whitney U test. Pooled data from electrophysiological recordings are expressed as means ± SE.

**Histology for light microscopy**

Some cells were recorded with 0.3% biocytin for later morphological identification. Following recording, hippocampus slices containing labeled neurons were fixed for 48 h at 4°C in 2–4% paraformaldehyde and 15% picric acid in 0.1 M phosphate buffer (pH 7.3–7.4). They were subsequently reacted by 1:100 dilution of avidin-biotin complex conjugated to horseradish peroxidase (ABC Elite kit, Vector Laboratories, Burlingame, CA) for 2 h at room temperature and incubated in 0.1 M Tris-buffered saline containing 0.025% 3,3-diaminobenzidine tetrahydrochloride (DAB, Sigma, St. Louis, MO), 0.05% nickel chloride, and 0.006% hydrogen peroxide. Slices were incubated in 0.1 M Tris-buffered saline containing 0.025% 3,3-diaminobenzidine tetrahydrochloride (DAB, Sigma, St. Louis, MO), 0.05% nickel chloride, and 0.006% hydrogen peroxide. Slices were incubated in 0.1 M Tris-buffered saline containing 0.025% 3,3-diaminobenzidine tetrahydrochloride (DAB, Sigma, St. Louis, MO), 0.05% nickel chloride, and 0.006% hydrogen peroxide.
dehydrated, coverslipped, and permanently mounted for morphological examination.

**Drugs**

KMeSO₄ was purchased from ICN (Aurora, OH); d-AP5 was purchased from Tocris (Ellisville, MO). All other drugs were purchased from Sigma.

**RESULTS**

**Pharmacological properties and stimulus-dependence of synaptically activated AHP**

A train of five suprathreshold stimuli delivered to the s. radiatum at 50 Hz triggered corresponding numbers of action potentials that rode on top of a depolarizing envelope, followed by a pronounced postburst AHP in CA1 pyramidal neurons (Figs. 1 and 2). Previous studies have yielded conflicting data regarding the ionic mechanisms underlying the hyperpolarizing potential following different forms of stimulation protocols (i.e., somatic depolarization, glutamate application, and synaptic stimulation) (Lancaster and Wheal 1984; Nicoll and Alger 1981). Thus we first wanted to identify the current mediating the slow component of the AHP (sAHP) activated by synaptic stimulation. The sAHP was sensitive to bath applications of isoproterenol (5–10 μM), a β-adrenergic receptor agonist (Fig. 1A; n = 5), and carbachol (2–5 μM), a muscarinic receptor agonist (Fig. 1B; n = 5). When BAPTA (10 mM), a high affinity Ca²⁺ chelator, was included in the patch solution, the sAHP was never observed (Fig. 1C; n = 12). The sensitivity of the sAHP to β-adrenergic and muscarinic modulations, as well as its dependence on Ca²⁺ for activation, indicated that the current underlying the synaptically activated sAHP is the classically defined slow Ca²⁺-activated K⁺ current, IₐHₛ (Benardo and Prince 1982; Cole and Nicoll 1984; Haas and Rose 1987; Hotson and Prince 1980; Lancaster and Adams 1986; Lancaster et al. 2001; Madison and Nicoll 1986; for review, see Storm 1990). Normally, the apamin/bicuculline-sensitive IₐHK partially overlaps with IₐHₛ in CA1 pyramidal neurons. However, as bicuculline (10 μM) was a standard component of our recording aCSF, IₐHK was suppressed in our recordings (Debarbieux et al. 1998; Johnson and Seutin 1997; Khawaled et al. 1999).

The sIₐHₛ can be activated by Ca²⁺ derived from many sources. Past studies have shown that this current can be activated by NMDA receptor–mediated Ca²⁺ influx alone (Shah and Haylett 2002) or by voltage-gated Ca²⁺ influx when Na⁺ action potentials were suppressed with low concentrations of QX-314 in the patch solution (0.5–2 mM; Lancaster et al. 2001). In these studies, the membrane potentials were maintained at more depolarized levels, presumably to increase the K⁺ driving force and thereby optimize the conditions to trigger sIₐHₛ. However, at a more hyperpolarized resting membrane potential, it is not clear whether a measurable sAHP can be triggered by synaptic stimuli that do not result in cell firing. To answer this question, we compared the total AHPs evoked at −68 mV by five subthreshold and five suprathreshold synaptic stimuli delivered at 50 Hz. When action potentials were not generated, only the faster AHP was observed (Fig. 2A; n = 50). In contrast, both the faster and the slow AHP were activated when suprathreshold stimuli that resulted in action potentials

![FIG. 1.](image-url) Pharmacological properties of the synaptically activated slow afterhyperpolarization (sAHP). A: representative voltage traces in response to trains of 5 synaptic stimuli at 50 Hz, before and after bath-application of isoproterenol (10 μM) or carbachol (2–5 μM) or with patch solution containing BAPTA (10 mM). Square voltage pulses are indicated below the voltage traces. Isoproterenol and carbachol completely abolished sAHP [for isoproterenol, AHP integral in control artificial cerebrospinal fluid (aCSF) = 13.3 ± 2.5 mV · s; in isoproterenol-aCSF = 0.4 ± 0.1 mV · s; P < 0.01, Mann-Whitney U test; n = 5; for carbachol: AHP integral in control aCSF = 9.9 ± 1.9 mV · s; in carbachol-aCSF = 0.2 ± 0.04 mV · s; P < 0.01, Mann-Whitney U test; n = 5]. Bath-application of atropine (1 μM) reversed the effect of carbachol (data not shown), indicating that reduction of sAHP by carbachol was mediated through the muscarinic pathway. When the patch solution contained BAPTA, none of the cells recorded exhibited sAHP (n = 12). Control and BAPTA traces were acquired from different neurons. These properties indicate that the current underlying the synaptically activated sAHP is the apamin-insensitive, Ca²⁺-activated K⁺ current, sIₐHₛ were presented. In most cases, a small sAHP was seen following just two suprathreshold synaptic stimuli at 50 Hz (Fig. 2B).

The sAHP evoked by somatic depolarizing steps is known to summate with successive action potentials (Lancaster and Adams 1986; Madison and Nicoll 1984), reflecting its sensitivity to intracellular Ca²⁺ accumulation (Sah 1992; Sah and Clements 1999). Given the heterogeneous distribution of Ca²⁺ channels and Ca²⁺-permeable receptors along the somato-dendritic axis (Benke et al. 1993; Christie et al. 1995; Yin et al. 1999; for review, Magee 1998) and the differential contribution of Ca²⁺ influxes via different Ca²⁺ channel subtypes to activate the sAHP (Shah and Haylett 2000; Tanabe et al. 1998), we next examined the summation profile for the sAHP triggered by different patterns of synaptic stimuli (Fig. 2, B and C). At all frequencies examined, the integral of the total AHP, largely reflecting the sAHP (Fig. 3),
AHP integrals were fit by 2, 3, and 5 suprathreshold stimuli at 50 Hz. Action potentials were truncated for clarity. A train of subthreshold stimuli evoked an AHP that decayed back to baseline within 0.5 s; in contrast, a train of suprathreshold stimuli evoked a prolonged AHP that lasted for 5 s. The maximal AHP integral reached was significantly greater for the 50- and 100-Hz trains than for a 20-Hz train ($P < 0.01$; ANOVA), suggesting that the limiting factors for maximal sAHP are frequency-dependent. Correspondingly, the total AHP integral triggered by a train of 15 somatically activated action potentials at 100 Hz was also significantly larger than that triggered by the same train at 20 Hz ($100 \text{ Hz} = 12.3 \pm 1.1 \text{ mV} \cdot \text{s}; 20 \text{ Hz} = 7.1 \pm 0.8 \text{ mV} \cdot \text{s}; P < 0.01$; ANOVA).

**A subpopulation of CA1 pyramidal neurons did not exhibit a sAHP**

The duration of the total AHP activated by a train of five suprathreshold stimuli at 50 Hz ranged from 88.1 ms to

![Image](https://www.jn.org/content/92/10/2349/F2.large.jpg)

**FIG. 2.** AHPs triggered by different patterns of synaptic stimuli. A: activation of sAHP from a resting membrane potential of $-68 \text{ mV}$ required Ca$^{2+}$ influx associated with action potential generation. Representative voltage traces in response to 5 subthreshold and 5 suprathreshold synaptic stimuli at 50 Hz. Action potentials were truncated for clarity. A train of subthreshold stimuli evoked an AHP that decayed back to baseline within 0.5 s; in contrast, a train of suprathreshold stimuli evoked a prolonged AHP that lasted for 5 s. B: for a given stimulation frequency, AHP increased with each successive suprathreshold stimulus presentation. Representative voltage traces of AHPs triggered by 2, 3, and 5 suprathreshold stimuli at 50 Hz. C: for a given number of stimuli, the slow component of AHP increased with increasing stimulation frequency. Representative voltage traces of AHPs evoked by 3 suprathreshold stimuli at 20, 50, and 100 Hz. D: frequency- and stimulus-dependence of AHP. Total AHP integral as a function of number of stimuli (1, 2, 5, 10, and 15) is plotted against stimulation frequencies (20 Hz, 50 Hz, 100 Hz). Growth of AHP integrals were fit with monoeXponential functions in the following form: $A_{\text{max}} \times (1 - \exp[-(\text{no. stimuli} - 1/k)])$, where $A_{\text{max}}$ is the maximal total AHP integral, and $k$ is the growth constant. The duration of the total AHP activated by a train of five suprathreshold stimuli at 50 Hz ranged from 88.1 ms to

![Image](https://www.jn.org/content/92/10/2349/F3.large.jpg)

**FIG. 3.** A small population of CA1 pyramidal neurons did not have a sAHP. A: distributions of total AHP durations and integrals revealed 2 distinct populations: 20% ($n = 10$) of the neurons had a total AHP duration $<1 \text{ s}$ (range, 0.09–0.9 s) and an AHP integral $<0.7 \text{ mV} \cdot \text{s}$ (range, 11.8–0.6 mV · s); the majority of the neurons ($n = 40$) had a total AHP duration $>3.3 \text{ ms}$ (range, 3.7–18.3 s) and an AHP integral $>2 \text{ mV} \cdot \text{s}$ (range, 2.3–20.3 mV · s). In this study, we categorized the neurons with a total AHP duration $<1 \text{ s}$ as ones without a sAHP, whereas the neurons with a total AHP duration $>1 \text{ s}$ were categorized as the ones with a sAHP. B: graphical illustration for these 2 categories of neurons. C: total AHP duration and integral mostly reflect the presence or absence of the slow AHP. ***$P < 0.001$ as determined by ANOVA. D: representative biocytin-filled CA1 pyramidal neurons. Neurons $a–c$ exhibited a prominent sAHP in response to synaptic stimuli; neurons $d$ and $e$ had no detectable sAHP. All of the CA1 pyramidal neurons without a sAHP that we recovered have apical dendrites that bifurcate rather close to the soma ($n = 3$). We have also observed 1 neuron with a robust sAHP with an early apical dendrite ($n = 1/17$); however, based on this limited sampling, it would appear that the majority of CA1 pyramidal neurons with a large sAHP do not have this morphology. Scale bar is 50 μm for $a$ and $c$, 100 μm for $b$ and $d$, and 40 μm for $e$.
18.3 s (6.6 ± 0.5 s; n = 50). The distributions for the duration and integral of total AHP revealed two distinct, nonoverlapping peaks (Fig. 3A): 20% of the neurons (n = 10) showed a total AHP duration <1 s and an AHP integral <1 mV·s, whereas 80% of the neurons showed a total AHP duration >3.7 s and an AHP integral >2.3 mV·s. When BAPTA (10 mM) was included in the patch solution, the duration of the total AHP that was Ca2+-insensitive was determined to be 0.7 ± 0.03 s (n = 12). Thus we considered the presence of a hyperpolarizing potential lasting beyond 1 s after pulse offset to be indicative of the sAHP (Fig. 3B).

No statistical difference was observed in the resting membrane potentials (with a sAHP = −65.9 ± 0.7 mV, n = 40; without a sAHP = −69.0 ± 1.5 mV, n = 10) or membrane resistances (with a sAHP = 120.8 ± 5.7 MΩ, n = 40; without a sAHP = 112.6 ± 10.3 MΩ, n = 10) between these two categories of neurons (P > 0.05, ANOVA; Table 1). The total AHP duration and integral were significantly larger for neurons with a sAHP than for those without (P < 0.0001, ANOVA), indicating that they predominantly reflect the sAHP (Fig. 3C).

To date, there has been no report of CA1 pyramidal neurons lacking a sAHP. Thus some neurons were subsequently recorded using patch solutions containing 2% Lucifer yellow or 0.3% biocytin for further identification. All labeled neurons, with (n = 17) and without the sAHP (n = 3), were identified as CA1 pyramidal neurons based on morphology, with somata situated in the CA1 cell layer, and the apical dendrites extending far into the s. radiatum (Fig. 3D).

Effect of the sAHP on temporal summation

The depolarizing integral triggered by one suprathreshold synaptic stimulus was not different for these two categories of neurons (with a sAHP = 934.9 ± 94.5 mV·ms; without a sAHP = 900.0 ± 114.4 mV·ms; P > 0.05); neither was the temporal summation of EPSPs triggered by a short train of subthreshold stimuli at 50 Hz (Fig. 4A). In contrast, the depolarizing envelope triggered by a train of suprathreshold stimuli was much shorter for neurons with a sAHP (Fig. 4, B and C). This difference became more pronounced as the stimulus trains lengthened, with the total depolarizing integral and duration triggered by 10–15 suprathreshold stimuli significantly curtailed for neurons with a sAHP compared with neurons without (P < 0.05 and 0.005, respectively, ANOVA; Fig. 4C). For the latter group, the depolarizing envelope would overcome the fast component of the AHP and evolve into a pronounced ADP (Fig. 5A), which could be further prolonged by lengthening the stimulus train or increasing the stimulation frequency (Fig. 5, A and B).

Multiple action potential firing was commonly observed during the ADP (Fig. 6A).

The development of an ADP for neurons without a sAHP was rather unexpected. Bath application of CNQX (20 μM) and d-AP5 (50–100 μM) after baseline recordings for a few cells from each group (n = 3 for each; data not shown) abolished the EPSPs and the associated Na+ action potentials as well as the sAHP or ADP. Corresponding somatic suprathreshold current injections never induced an ADP at all frequencies tested (≤150 Hz; Fig. 5C). These data indicate that the development of ADP in response to high-frequency inputs was a result of ionotropic glutamate receptor activation and not due to direct electrical stimulation of the recorded neurons.

### Table 1. Neurons with and without the sAHP

<table>
<thead>
<tr>
<th></th>
<th>With sAHP (n = 40)</th>
<th>Without sAHP (n = 10)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMP (mV)</td>
<td>−65.9 ± 0.7</td>
<td>−69.0 ± 1.5</td>
<td>NS</td>
</tr>
<tr>
<td>MR (MΩ)</td>
<td>120.8 ± 5.7</td>
<td>112.6 ± 10.3</td>
<td>NS</td>
</tr>
<tr>
<td>AHP integral (mV·s)</td>
<td>9.6 ± 0.8</td>
<td>0.3 ± 0.06</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total AHP duration (s)</td>
<td>8.1 ± 0.4</td>
<td>0.5 ± 0.09</td>
<td>&lt;0.0001</td>
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Values are means ± SE. sAHP, slow afterhyperpolarization; RMP, resting membrane potential; NS, not significant; MR, membrane resistance.
Ionic mechanisms underlying ADP

To examine the ionic mechanisms underlying ADP, we searched for and recorded from additional CA1 neurons without a measurable sAHP ($n = 11$). Neurons were discarded if they exhibited a postburst AHP duration $>1$ s in response to five suprathreshold synaptic stimuli at 50 Hz. All neurons that met this selection criterion developed an ADP of varying magnitude and duration when presented with 15 suprathreshold stimuli at 150 Hz (ADP integral with $\sim1.5$ times suprathreshold stimulus intensity = $75.0 \pm 17.8$ mV $\cdot$ s; ADP duration = $7.1 \pm 0.9$ s). At this stimulation frequency, the magnitude and duration of the ADP increased exponentially in response to increases in stimulus intensity (Fig. 6A).

It has previously been shown that an afterdepolarizing potential can be induced by exogenous muscarinic receptor agonist application (Egorov et al. 1999; Fraser and MacVicar 1996). To determine whether the ADP reported in this study is also the result of muscarinic receptor activation, we triggered an ADP with 15 synaptic stimuli at 150 Hz, using $\sim1.5$ times

![FIG. 5. Development and properties of the afterdepolarization (ADP). A: for neurons without a slow AHP, the depolarizing envelope increased with each successive suprathreshold stimulus presentation and eventually evolved into a pronounced ADP. Representative voltage traces from 1 neuron in response to trains of suprathreshold stimuli at 50 Hz; numbers denote number of stimuli presented. Action potentials are truncated for clarity. B: for a given number of synaptic stimuli, the ADP increased with increasing stimulation frequency. Representative voltage traces in response to 15 suprathreshold synaptic stimuli at 50 and 150 Hz. Voltage traces were aligned at the end of the stimulus train for clarity. C: ADP reported in this study required synaptic activation. Suprathreshold somatic depolarizing steps $\leq$150 Hz never resulted in an ADP. Representative voltage traces in response to 15 suprathreshold synaptic stimuli at 150 Hz recorded from the same neuron showing that ADP could only be triggered by synaptic stimuli.](image)

![FIG. 6. Characteristics of ad ionic mechanisms underlying ADP. A: increasing the stimulus intensity increased the magnitude and the duration of ADP. Representative voltage traces of ADPs triggered by 15 synaptic stimuli at 150 Hz, using different stimulus intensities. $X =$ threshold stimulus intensity (that consistently triggered 5 action potentials when presented in a train of 5 stimuli at 50 Hz). Inset: normalized ADP integral plotted against the normalized stimulus intensity. Regression analysis shows that the ADP integral increased exponentially to increases in stimulation intensity. Data were fit with an exponential regression model with equation $Y = 0.206 \times e^{(1.735 \times X)}$. B: sequential bath applications of thapsigargin (1 $\mu$M) and nitrendipine (10–20 $\mu$M) reduced ADP, and further addition of d-AP5 (50 $\mu$M) abolished ADP. Representative voltage traces of ADPs triggered by 15 suprathreshold synaptic stimuli at 150 Hz using 1.5 $\times$ stimulus intensity in various drug cocktails. C: bar graph showing percentage of residual ADP in various drug cocktails. ***$p < 0.001$ as determined by Fisher’s PLSD. D: applications of d-AP5 alone abolished ADP. Representative voltage traces triggered by 15 suprathreshold synaptic stimuli at 150 Hz using 1.5 $\times$ stimulus intensity. In the presence of d-AP5, further increasing the stimulus intensity could not trigger an ADP mediated by the L-type Ca$^{2+}$ current or thapsigargin-sensitive intracellular Ca$^{2+}$ release.](image)
suprathreshold stimulus intensity. Bath application of atropine (1 μM), an antagonist of the muscarinic receptors, had no effect on the ADP, indicating that muscarinic receptor activation is not involved in generating this afterpotential (n = 2; data not shown). In the geniculate nucleus, an afterdepolarizing plateau potential mediated by the L-type Ca²⁺ channels (Ping and Shepard 1999). In the cells of the lateral geniculate nucleus, an afterdepolarizing plateau potential mediated by the L-type Ca²⁺ channels and NMDA receptors has also been described (Lo et al. 2002). Thus we next examined the effects of nitrendipine, an L-type Ca²⁺ channel blocker, and D-AP5 on the ADP. Because Ca²⁺ influxes through the L-type Ca²⁺ channels, the NMDA receptors, as well as that associated with action potential firing, have all been shown to trigger intracellular Ca²⁺ release (CICR; Emptage et al. 1999; Sandler and Barbara 1999), we also examined the effect of thapsigargin, an inhibitor for intracellular Ca²⁺ store release, on the ADP. Bath applications of thapsigargin (1 μM) caused a 20.3 ± 19.6% reduction in the ADP integral (Fig. 6, B and C; n = 5; P < 0.0001; Fisher’s PLSD). Additional applications of nitrendipine (10–20 μM) to the bath caused a 52.1 ± 7.07% reduction in the ADP integral relative to control (Fig. 6, B and C; n = 5; P < 0.0001 compared with control and thapsigargin’s effect; Fisher’s PLSD). Further applications of D-AP5 (50 μM) caused a 89.8 ± 1.6% reduction in the ADP integral relative to control (Fig. 6, B and C; n = 5; P < 0.0001 compared with control, thapsigargin’s, and nitrendipine’s effects, Fisher’s PLSD). Thus Ca²⁺ influxes via the L-type Ca²⁺ channels and NMDA receptors, as well as CICR, all contribute to the generation of the ADP. When D-AP5 was applied alone, it caused a 90.9 ± 2.3% reduction in the ADP integral (Fig. 6, C and D; n = 4; P < 0.0001 Fisher’s PLSD). In neurons that the ADPs were abolished by bath-applications of D-AP5 alone, increasing the stimulus intensity to the maximum allowed by the stimulus isolator without damaging the fiber path was ineffective in triggering an ADP (Fig. 6D), suggesting that the NMDA receptor-mediated potential is a necessary trigger to initiate the ADP.

*Modulation of the sAHP leads the development of an ADP*

The depolarizing envelope associated with synaptic stimuli was much smaller when the sAHP was present. Thus we next examined the consequence of suppressing the sAHP on postsynaptic processing of high-frequency suprathreshold synaptic inputs. When BAPTA (10–30 mM) was included in the patch solution, all neurons recorded developed a pronounced ADP lasting ≤5 s that could be blocked by D-AP5 (50–150 Hz; n = 20; Fig. 7A). Bath applications of carbachol (2–5 μM; n = 3; Fig. 7B) or isoproterenol (5 μM; n = 5; Fig. 7C) abolished the sAHP, and in response to a train of high-frequency synaptic stimuli, the neurons instead developed an ADP. In the presence of carbachol, two neurons showed a decrease in the number of action potentials triggered by the same train of suprathreshold stimuli compared with control. Therefore stimulus intensity was increased to maintain the same number of action potentials generated. The effect of carbachol was mostly reversed by additional application of atropine (1 μM) to the bath solution (Fig. 7B). Together, these data indicate that neuromodulation of the sAHP can powerfully modulate the postsynaptic response to synaptic inputs.

**DISCUSSION**

In this study, we examined the sAHP activated by different patterns of synaptic stimuli and its impact on postsynaptic synaptic integration. Our principle conclusion is that activation of the sAHP profoundly limits the duration and magnitude of the postsynaptic depolarization in response to synaptic stimuli—an effect that is especially prominent during periods of high-frequency, suprathreshold synaptic transmission. When the sAHP is absent or suppressed, the propensity of a postsynaptic neuron to develop an NMDA receptor-dependent ADP in response to such input is enhanced. Neuromodulation of the sAHP may thus be a powerful way to regulate the development of NMDA receptor-mediated plasticity.

The postburst AHP evoked by synaptic stimuli under our recording conditions contained two kinetically distinct components. The faster component is Ca²⁺ insensitive, as it persisted.

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in patch solution containing BAPTA (Fig. 1C), and was activated by both sub- and suprathreshold stimuli. These characteristics suggest that the faster component reported in this study is similar to the classically defined medium AHP mediated by both \( I_M \) and \( I_h \) (Takigawa and Alzheimer 2003; Williamson and Alger 1990). We did not evaluate the relative contribution of these currents to the mAHP. However, \( I_h \) channels are more densely located on the dendrite (Lorincz et al. 2002) and intracellular Ca\(^{2+}\) activation by a 50- or 100-Hz train probably reflects AHP channels. In contrast, the maximal AHP activated by a 50- or 100-Hz train is the saturation of the functional \( I_{Ca} \) L-type Ca\(^{2+}\) influx, and intracellular Ca\(^{2+}\) release. The total depolarizing integral resulting from just one suprathreshold synaptic stimulus for both categories of neurons was not statistically different, suggesting that the number of synapses being activated was not different. Given that suprathreshold synaptic activation leads to widespread elevations of Ca\(^{2+}\) and Na\(^+\) throughout the dendritic tree—an effect caused by backpropagating action potentials and subsequent openings of voltage-gated Ca\(^{2+}\) channels as well as simultaneous activation of the NMDA receptors (Jaffe et al. 1992; Markram et al. 1995; Rose and Konnerth 2001; Spruston et al. 1995; Yuste and Denk 1995), the absence of the sAHP in these cells was unlikely the result of insufficient Ca\(^{2+}\) for channel activation. In the presence of atropine, these cells still developed an ADP, indicating that differential involvement of muscarinic neuromodulation cannot account for the presence or absence of the sAHP in these cells.

**ADP and NMDA receptors**

The ADP reported here differs mechanistically from the slow ADP and plateau potential derived from muscarinic receptor activation (Fraser and MacVicar 1996). It is mediated by influx through the NMDA receptors, the L-type Ca\(^{2+}\) channels, and CICR (Fig. 7, A and B). In the presence of \( \alpha\)-ADP, further increases in the stimulus intensity failed to trigger an ADP mediated by the latter two components (Fig. 7C). These data suggest that the NMDA receptor–mediated influx alone is sufficient to support an ADP and is necessary for the activation of L-type Ca\(^{2+}\) influx and CICR. Similar interactions between the voltage-gated Ca\(^{2+}\) influx or intracellular Ca\(^{2+}\) release and NMDA receptors have previously been reported (Calton et al. 2000; Schiller et al. 1997; Schwindt and Crill 1998). A Ca\(^{2+}\)-activated cation current (\( I_{CAN} \)), activated by the muscarinic or metabotropic glutamate receptors, has been found to underlie an afterdepolarizing potential in hippocampal pyramidal neurons (Caeser et al. 1993; Congar et al. 1997; Fraser and MacVicar 1996; Greene et al. 1994; Young et al. 2004). We did not test for the involvement of \( I_{CAN} \) in the ADP triggered by high-frequency synaptic stimuli. However, an ADP was induced in all neurons loaded with BAPTA. While this does not exclude the involvement of \( I_{CAN} \), it does indicate that \( I_{CAN} \) is not necessary to support this form of ADP.

In the presence of BAPTA, all neurons developed a prolonged ADP that was sensitive to \( \alpha\)-ADP in response to a train of high-frequency synaptic stimuli (Fig. 7A). The inclusion of BAPTA in the patch solution most likely prevented CICR from contributing to the ADP (~20%); however, it would not

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*S. AHP LIMITS NMDAR-DEPENDENT ADP IN CA1 NEURONS*
eliminate the charges flowing through the NMDA receptors and voltage-dependent Ca\(^{2+}\) channels. The pronounced ADP we observed in BAPTA-loaded neurons may also in part be caused by the removal of Ca\(^{2+}\)-dependent inactivation of the NMDA receptors and the L-type Ca\(^{2+}\) channels (Hofer et al. 1997; Imredy and Yue 1994; Legendre et al. 1993; Lu et al. 2000; Neely et al. 1994; Rosenmund and Westbrook 1993).

The magnitude and duration of ADP was dependent on stimulation intensity and frequency. The rate of growth was best fit with an increasing exponential function, consistent with the active properties of the NMDA receptor channels and their activity-dependent amplification (for review, see Schiller and Schiller 2001). Action potentials are typically initiated in the axon and then backpropagate into the dendrites of CA1 pyramidal neurons, initiating dendritic Ca\(^{2+}\) influx (Spruston et al. 1995). Trains of action potentials initiated by somatic depolarizing steps failed to trigger an ADP at all frequencies tested, implying that backpropagating action potentials alone are insufficient. Rather, an ADP required the coincidence of repetitive backpropagating action potentials and NMDA receptor activation. Previous Na\(^{+}\) and Ca\(^{2+}\) imaging studies have shown an accumulation of Na\(^{+}\) transients and supralinear Ca\(^{2+}\) signals mediated by NMDA receptors, detected during coincident occurrence of synaptic potentials and backpropagating spikes (Koester and Sakmann 1998; Magee and Johnston 1997; Markram et al. 1997; Rose and Konnerth 2001; Schiller et al. 1998; Yuste and Denk 1995). Schiller et al. (1998) also showed that the pairing of caged glutamate release with postsynaptic action potentials selectively amplified the NMDA receptor-mediated Ca\(^{2+}\) signals. Similarly, when subthreshold EPSPs were paired with backpropagating action potentials, dendritic action potentials were amplified, Ca\(^{2+}\) influx was evoked near the site of synaptic input, and long-term potentiation (LTP) was observed (Magee and Johnston 1997). Our data suggest that the sAHP places a powerful regulation on this Hebbian-type of association through its interactions with NMDA receptors, thereby affecting synaptic efficacy. In support, an association has been observed between the reduction of the sAHP and the priming of the induction for LTP (Cohen et al. 1999).

The sAHP channels are thought to be situated near the proximal apical dendrite and/or soma (Bowden et al. 2001; Sah and Bekkers 1996; but also see Bekkers 2000). Given the sensitivity of backpropagating action potentials to hyperpolarization (Tsubokawa and Ross 1996), activation of the sAHP can shunt signals propagating both from and toward the dendrite, thereby effectively uncoupling the somato-dendritic compartments and restricting the spread of signals. In addition, the sAHP can limit temporal summation by interacting with the active properties of CA1 pyramidal neurons. During suprathreshold synaptic transmission, Ca\(^{2+}\) influx through the NMDA receptors and voltage-gated Ca\(^{2+}\) channels would activate sI\(_{AHP}\), thereby driving the membrane potential toward the hyperpolarizing direction that facilitates the voltage-dependent Mg\(^{2+}\) re-block of the NMDA receptors. Under conditions that the sAHP is suppressed (e.g., neuromodulation), temporal summation of high-frequency inputs would depolarize the neurons more easily to a membrane potential that favors the removal of the Mg\(^{2+}\) block of the NMDA receptors, plus keep the fast A-type K\(^{+}\) current (I\(_{A}\)) in an inactivated state (Magee and Carruth 1999). Synaptic inputs occurring during this window, with NMDA receptors unblocked by Mg\(^{2+}\) and I\(_{A}\) inactivated, would thus be able to trigger an amplified NMDA receptor–mediated response in the form of an ADP that is capable of initiating multiple action potentials (Fig. 5). In support, we have shown that pharmacologically blocking the sAHP with BAPTA, carbachol, or isoproterenol allowed the neurons to develop an ADP (Fig. 7). We propose that the sAHP is a postsynaptic mechanism that is critical in limiting an NMDA receptor–dependent regenerative ADP during bouts of high-frequency, intense synaptic transmission. Neuromodulation of the sAHP is thus a powerful postsynaptic mechanism in shaping the postsynaptic membrane response to strong inputs.

**sAHP and ADP in the context of cellular plasticity during learning**

Given that the sAHP regulates the propensity of the postsynaptic neuron to develop an NMDA receptor–dependent ADP, the reduction in the sAHP in learning (Moyer et al. 1996, 2000; Oh et al. 2003) may be playing a permissive or facilitating role for further synaptic changes that are also developing during hippocampus-dependent learning. There is evidence suggesting that the neuromodulation of the sAHP participates in establishing various forms of plasticity thought to be involved in learning. Blockade of the sAHP with isoproterenol, an agonist of the β-adrenergic receptors, has been shown to convert short-term potentiation triggered by a weak tetanus train to LTP (Sah and Bekkers 1996). LTP induction triggered by a mild theta burst stimulation protocol was also shown to be “primed” by suppression of the sAHP with isoproterenol and ACPD, an mGlur agonist (Cohen et al. 1999). Together, these studies link the expression of long-term plasticity with the sAHP. We have identified neuroanatomical changes, increases in postsynaptic density area in the s. radiatum, and the total number of multiple-synapse boutons in CA1 pyramidal neurons after learning trace eyeblink conditioning (Geinisman et al. 2000, 2001), CA1 field potentials in response to s. radiatum stimulation were also increased (Power et al. 1997). Acquisition of the learned response was facilitated with cholinergic agents or Ca\(^{2+}\) channel antagonists that reduce the sAHP (Deyo et al. 1989; Kowalska and Disterhoft 1994; Kronforst-Collins et al. 1997; Moyer et al. 1992; Oh et al. 1999; Power et al. 2001; Weiss et al. 2000) and with co-agonists to the glycine site on the NMDA receptors in young and aging animals (Thompson et al. 1992; Thompson and Disterhoft 1997a). Conversely, trace eyeblink conditioning was impaired with antagonists to NMDA-mediated transmission, MK801 and PCP (Thompson and Disterhoft 1997a,b). Together, our data suggest that the increased information throughput occurring during learning may reflect synaptic alterations that are dependent for their development on cellular excitability changes such as a reduction in the sAHP.

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