**Contribution of \( I_h \) and GABA\(_B\) to Synaptically Induced Afterhyperpolarizations in CA1: A Brake on the NMDA Response**

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**INTRODUCTION**

The Schaffer collateral (SC) and perforant path (PP) inputs arrive at very different locations in the dendrites of CA1 pyramidal neurons. The PP terminates at thin and distal dendrites in stratum lacunosum-moleculare more than 300 \( \mu \)m from the soma. In contrast, the SC arrives more proximally in the stratum radiatum. To understand the computations performed by CA1 (see discussion), it will be important to understand the generation of the response at each input and their mechanisms of interaction.

There has been substantial recent progress in understanding dendritic processing in cortical and hippocampal pyramidal cells. Passive electrotonic decay of signals from active synapses to the spike generating site near the soma potentially weakens the impact of distal synapses (Carnevale and Johnston 1982; Major et al. 1994; Spruston et al. 1993). However, it now appears that many factors can affect synaptic integration that make the situation more complex (Cash and Yuste 1999; Margulis and Tang 1998; Nettleton and Spain 2000). One important mechanism is distance-dependent compensation of the synaptic conductance itself, which serves to equalize the somatic effect (Andrasfalvy and Magee 2001; Magee and Cook 2000).

Second, the synaptic current can interact with the intrinsic voltage-dependent conductances (Andreasen and Lambert 1999; Hoffman et al. 1997; Kepecs and Raghavachari 2002; Magee 1999; Poirazi et al. 2003; Seamans et al. 1997; Stuart and Sakmann 1995). Third, the synaptically induced \( N \)-methyl-\( \beta \)-aspartate (NMDA) conductance is voltage-dependent, and this can lead to supralinear interactions and dendritic spikes (Mel 1993; Pongracz et al. 1992; Schiller and Schiller 2004). Fourth, an important site of integration may not be at the spike generation site in the soma, but rather in the dendrites (Andreasen and Lambert 1998; Benardo et al. 1982; Golding and Spruston 1998; Regehr et al. 1993). For instance, recent work on cortical neurons (Larkum et al. 1999) revealed that interaction between proximal and distal inputs at dendritic integration zones determines whether single spikes or bursts are generated.

Our goal in this study was to characterize the two major inputs to CA1 cells, setting the stage for the analysis of the interactions between the two pathways. Previous local field recording and voltage-clamp analysis showed that the distal PP input to CA1 has a larger NMDA/AMPA ratio than the SC (Otmakhova and Lisman 1999; Otmakhova et al. 2002), suggesting that NMDA channels might contribute strongly to the excitatory postsynaptic potential (EPSP) in the PP. However, such a contribution might be limited if the membrane potential was made negative by a hyperpolarizing component of the synaptic potential. For this reason, it is important to understand the origin of the afterhyperpolarization (AHP) associated with synaptic responses. There has been some previous work on these potentials, but no systematic comparison of the AHPs in the two pathways. We have therefore analyzed the mechanisms(s) of the AHP evoked by single subthreshold synaptic stimuli in the PP and SC and sought to determine their effect on the NMDA component of the excitatory response.

**METHODS**

Transverse hippocampal slices (350 \( \mu \)m thick) were prepared from 17- to 25-day-old Long-Evans rats. Part of the dentate gyrus and the CA3 field was cut from the slices by a single diagonal cut as described previously (Otmakhova and Lisman 1999). Slices were preincubated in an inverse interface chamber for 2–6 h before an experiment (Otmakhova et al. 2000, 2002). For recording, slices were placed on the glass bottom of the recording chamber and superfused with artificial cerebrospinal fluid (ACSF) using a pump with flow rate...
Whole cell patch clamp

Whole cell current-clamp recordings were performed using an Axoclamp-2A amplifier (Axon Instruments, Union City, CA) with a low-pass filter set at 1 kHz. Additional amplification was provided by the DC amplifier/filter LPF-202A (Warner Instruments, Hamden, CT). The patch pipettes had a resistance of 3.5–5.5 MΩ when filled with pipette solution. The pipette solution contained (in mM) 150 K-methylsulphate, 20 KC1, 10 HEPES, 4 MgATP, 0.3 Na3GTP, 0.1 EGTA, and 10 Na-phosphocreatine (pH 7.3; osmolarity, 300 mOsm). In some experiments, QX-314 (5 mM) was added to the pipette solution. A number of experiments were performed with Cs

Although there has been previous work on the mechanism of these AHPs (Dvorak-Carbone and Schuman 1999; Empson and Heinemann 1995; Jones 1995; Nicoll and Alger 1981), they have not been systematically compared at the two inputs. To characterize the AHP, the synaptic responses in the PP and SC inputs were recorded at a somatic potential of 70 mV in the presence of PTX (50 µM) and ± APV (100 µM). Under these conditions, small (1–7 mV) EPSPs in both inputs were followed by an AHP (Fig. 1A). Pooling data from 13 cells, we found that, in both inputs, the area of the AHP correlated with the area of the EPSP (Fig. 1B) and the amplitude of the EPSP (data not shown). The slope of this correlation was larger in the PP (B = −181, R = 0.84, P < 0.001 for the area and B = −182, R = −0.85, P < 0.001 for the amplitude) than in the SC (B = −56, R = 0.77, P < 0.002 for the area and B = −56, R = −0.74, P < 0.003 for the amplitude). Therefore, phenomenologically, the AHP was larger in the PP than in the SC for the same size of somatically recorded EPSP (Fig. 1C).

Mechanisms of AHPs

Earlier work suggested the participation of Ca2+-dependent potassium currents in post-EPSP AHPs in SC (Nicoll and Alger 1981). We tested whether removing various sources of Ca2+ affected the AHP of small subthreshold responses. We found that blockade of NMDA receptors with ± APV (100 µM) did not decrease the AHP (not significant, n = 7; Fig. 2A). Similarly, blockade of T-type and L-type Ca2+ channels (Fig. 2B) with a mixture of 50 µM NiCl2 and 50 µM nifedipine did not substantially block the SC or PP AHP (P > 0.4, n = 4; Fig. 2B). One Ca2+-dependent potassium current implicated in the SC AHP is the Ca2+-dependent slow I_AHP potassium current (Nicoll and Alger 1981). This current is reliably inhibited by cAMP-dependent protein kinase A (PKA) (Madison and Nicoll 1986). We found that forskolin (50 µM), an activator of

Statistical analysis

For whole cell patch-clamp experiments, 5–10 evoked responses were averaged for each cell. For the measurements of response area, we used an absolute sum of recorded voltage over time starting immediately after the stimulus artifact and until the end of the recorded trace (700 ms). The peak amplitude (A_peak) was determined in a 3-ms window centered at the peak of the synaptic response. The area or peak voltage of a synaptic response was calculated relative to the average value of the baseline in a 50-ms window before the stimulus. Means and SE were calculated. Two-tailed t-test with α < 0.05 was used for statistical analysis (Microsoft Excel). We did not correct for junction potential. For trace illustrations, the data in each cell were first scaled by the peak EPSP amplitude and then were averaged between cells. For field EPSP (fEPSP) analysis, responses were collected and averaged in 1-min periods. fEPSP and fiber volley amplitudes were calculated (mV). Data were normalized relative to baseline. The effect of drug was estimated in each slice relative to baseline and analyzed for the whole experimental series using two-tailed paired t-test for means (Microsoft Excel, α < 0.05).

Chemicals

Most of the chemicals for intracellular solutions and ACSF were purchased from Sigma. ZD7288, 2-hydroxy-saclofen, and SKF97541 were bought from Tocris; forskolin was purchased from Alomone Laboratories.

RESULTS

A prominent feature of the synaptic response under current-clamp conditions was a substantial AHP following the EPSP. This was observed in both PP and SC inputs (Fig. 1A). Although there has been previous work on the mechanism of these AHPs (Dvorak-Carbone and Schuman 1999; Empson and Heinemann 1995; Jones 1995; Nicoll and Alger 1981), they have not been systematically compared at the two inputs. To characterize the AHP, the synaptic responses in the PP and SC inputs were recorded at a somatic potential of −70 mV in the presence of PTX (50 µM) and ± APV (100 µM). Under these conditions, small (1–7 mV) EPSPs in both inputs were followed by an AHP (Fig. 1A). Pooling data from 13 cells, we found that, in both inputs, the area of the AHP correlated with the area of the EPSP (Fig. 1B) and the amplitude of the EPSP (data not shown). The slope of this correlation was larger in the PP (B = −181, R = 0.84, P < 0.001 for the area and B = −182, R = −0.85, P < 0.001 for the amplitude) than in the SC (B = −56, R = 0.77, P < 0.002 for the area and B = −56, R = −0.74, P < 0.003 for the amplitude). Therefore, phenomenologically, the AHP was larger in the PP than in the SC for the same size of somatically recorded EPSP (Fig. 1C).

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adenylyl cyclase (and indirectly PKA), did not inhibit the AHP in either the PP or SC \( (P > 0.25, n = 5; \text{Fig. 2C}) \). Taken together, these results provide no indication that the AHP is due to \( \text{Ca}^{2+} \)-activated potassium channels.

The experiments described above (Figs. 1 and 2) were done with the \( \text{GABA}_A \) conductance blocked, and it was of interest to see how responses would differ when \( \text{GABA}_A \) channels were functional. Figure 3A shows that, at \(-70 \text{ mV}\), the \( \text{GABA}_A \) chloride channel blocker PTX (50 \text{ mM}) neither affected the SC nor the PP AHPs \( (P > 0.6 \text{ and } P > 0.25, \text{respectively; } n = 5) \). This indicates that the AHP does not depend on \( \text{GABA}_A \) inhibition. PTX decreased the amplitude and the area of both the SC and PP EPSPs; the implication of this finding will be discussed elsewhere.

In previous work (Otmakhova et al. 2002), we did not observe an AHP under current-clamp conditions using a K\(^+\)-based intracellular solution containing OX-314. We therefore examined more systematically the effects of QX-314 (5 mM). Figure 3B shows that the AHP was suppressed in both pathways \( (n = 7) \). QX-314 blocks the sodium-, some calcium-, G-protein-coupled potassium-, and hyperpolarization-activated cation current \( I_h \) (Connors and Prince 1982; Lambert and

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**FIG. 1.** Afterhyperpolarizations (AHPs) induced by perforant path (PP) and Schaffer collateral (SC) stimulation. These are measured at the soma rather than the site of generation and so should be considered phenomenological descriptions. A: examples of the SC and PP synaptic responses in artificial cerebrospinal fluid (ACSF) containing picrotoxin (PTX) and APV. Each trace is an average of 5 sweeps. Scale bars are on the right. B: area of AHP in both inputs correlates with the area of the excitatory postsynaptic potential (EPSP; pooled from 13 cells). Slope of this correlation in the SC is smaller than in PP. Inset: the same holds true for a single cell when EPSP size is changed by changing the power of stimulation. Axes titles are the same as in main figure. C: averaged data \( (n = 13) \) shows that the AHP area and AHP/EPSP area ratio in the PP is significantly larger than in the SC input. Significance: **\( P < 0.01 \), ***\( P < 0.001 \).
Wilson 1993; Perkins and Wong 1995; Talbot and Sayer 1996). Of these, the \( I_h \) current was a potentially important contributor to the AHP (Pape 1996), and so we sought to further test its role.

To examine the contribution of \( I_h \), we used the selective channel blocker, ZD7288 (30 \( \mu \)M). The concentration used is higher than the half-maximal inhibitory concentration in CA1 (10 \( \mu \)M) (Gasparini and DiFrancesco 1997) and other structures (0.2–6 \( \mu \)M) (Larkman and Kelly 2001; Satoh and Yamada 2000), but is below that required for complete block (50 \( \mu \)M; Gasparini and DiFrancesco 1997). We avoided higher concentrations because they suppressed excitatory postsynaptic currents (EPSCs) (Otmakhova et al. 2002), suggesting the possibility of nonspecific action (Chevaleyre and Castillo 2002). Application of ZD7288 strongly hyperpolarized the somatic membrane potential \((-14.2 \pm 1.5 \text{ mV after 10 min})\). We used steady current injection to return the somatic membrane potential to \(-70 \text{ mV} \) and observed the effects on the synaptic response. In the SC, ZD7288 essentially eliminated the AHP altogether, whereas in the PP, the AHP was only

**FIG. 2.** \( \text{Ca}^{2+} \)-dependent potassium currents do not participate in AHP generation. *A*: blockade of \( \text{Ca}^{2+} \) influx through the \( N \)-methyl-D-aspartate (NMDA) channels by APV does not inhibit AHP. Average of 7 cells in PTX. Here and in further traces, responses in both inputs were scaled by the peak amplitude before averaging. This was possible because the AHP/EPSP area ratio does not depend on the amplitude of the EPSP as follows from the linear correlation on Fig. 1B. The power of stimulation was always adjusted such as to evoke an EPSP in the range where this correlation was observed. Bar graphs here and elsewhere show raw data averages. Significance: * \( P < 0.05 \). *B*: blockade of \( \text{Ca}^{2+} \) influx through voltage-dependent \( \text{Ca}^{2+} \) channels does not block the AHPs. Average of 4 cells in PTX and APV. *C*: forskolin (50 \( \mu \)M for 20 min), an inhibitor of the \( \text{Ca}^{2+} \)-dependent slow \( I_{\text{AHP}} \), does not affect the AHPs. Average of 5 cells normalized in PTX and APV.
partially reduced (by 49%, $P < 0.01$, $n = 6$), but not eliminated (Fig. 3).

To understand the component of the PP AHP that was not attributable to $I_h$ (Fig. 3C), we first asked whether this component required the EPSP (depolarization). Eliminating the EPSP with NBQX (10 μM) blocked the AHP in the SC ($n = 5$, Fig. 4A), but in the PP, a substantial AHP remained ($n = 4$, Fig. 4B1). We conclude that a component of PP AHP is EPSP-independent. This component was blocked by the GABA<sub>B</sub> blocker, 2-hydroxy-salolfen (100–200 μM, $n = 4$, Fig. 4B2).

**FIG. 3.** $I_h$ current is responsible for the SC AHP and is a component of the PP AHP. A: GABA<sub>A</sub> current does not contribute to the AHP; GABA<sub>A</sub> channel blocker, PTX, does not affect the SC and PP AHPs. Average of 5 cells in control. Significance: *$P < 0.05$, **$P < 0.01$. B: intracellular QX-314 (5 mM) suppresses both the SC and PP AHPs. Average of 7 cells 5 and 35 min after breaking the membrane seal. C: SC AHP is produced by the $I_h$ current because it is completely blocked by the $I_h$ channel blocker, ZD7288 (30 μM). PP AHP is only partially inhibited by ZD7288. Average of 6 cells in PTX + APV.
Thus the AHP in the PP has a GABA_B component in addition to that generated by I_h. Additional evidence of the dual character of the PP AHP is that 2-hydroxy-saclofen (200 µM) could not completely suppress it (Fig. 4D). The GABA_B and I_h components of the AHP have very similar (slow) kinetics, and there is no concern about differential electrotonic attenuation in making the relative estimate. The ZD7288 experiment (Fig. 3C) showed that GABA_B contributed 53% to the PP and 11% to the SC total AHP area. The EPSP blockade experiments (Fig. 4, A and B) suggested 81% contribution in the PP and

FIG. 4. A postsynaptic GABA_B-dependent inhibition produces the depolarization-independent part of the PP AHP, but is negligible in the SC. A: there was almost no AHP in SC when 2- to 4-mV EPSP were blocked. Average of 5 cells in PTX and APV.

B1: a substantial part of the PP AHP was still present after the blockade of EPSP. Average of 4 cells in PTX and APV. Significance: *P < 0.05, **P < 0.01, ***P < 0.001. B2: continuation: the GABA_B blocker, 2-hydroxy-saclofen (saclofen), blocked the EPSP-independent component of the AHP. C: a postsynaptic GABA_B-dependent AHP could only be observed after the blockade of the EPSP in SC and increase of the power of stimulation 6–10 times, especially if combined with somatic depolarization via patch electrode. A single cell example, each trace is an average of 5 sweeps. D: the GABA_B blocker, 2-hydroxy-saclofen, inhibits but does not block the PP AHP. Average of 5 cells in PTX + APV. E and F: combination of GABA_B and I_h blocker completely eliminates the SC and PP AHP. Average of 5 cells in PTX + APV.
14% in the SC AHP. Both approaches suggest much higher GABA B participation in the PP AHP. We further found that the blockade of both GABA A and I h current by the mixture of ZD7288 (30 μM) and 2-hydroxy-saclofen (100 μM) completely eliminated the AHP in both inputs (n = 5, P < 0.001; Fig. 4, E and F). We conclude that I h is almost entirely responsible for the SC AHP, with very little GABA B contribution, but both I h and feed-forward monosynaptic GABA B inhibition contribute to the PP AHP.

Resolving issues related to GABA B

The small size of the postsynaptic GABA B component in the SC is surprising because of previous reports of such a component (Isaacson et al. 1993; Pham et al. 1998; Solis and Nicoll 1992a,b). To test the possibility that this discrepancy might be simply quantitative, we varied the power of SC stimulation (after blockade of EPSP to avoid spikes). We found that increasing the power of stimulation 6- to 10-fold produced a GABAB component. This component increased (approximately fourfold) when the soma was depolarized to –50 mV (n = 4, Fig. 4C), as expected for a GABA B response. Therefore a postsynaptic GABA B component is present in the SC but is negligible when stimulation evokes only a small (2–5 mV) EPSP. Further quantification of this issue will be dealt with in the final part of RESULTS.

Other previous work has suggested that GABA B is important in the SC, but not in the PP, input (Ault and Nadler 1982; Colbert and Levy 1992). These conclusions were based on the finding that GABA B agonist suppressed the amplitude of fEPSPs in the SC, but not in the PP. However, the localization of these effects was not determined in these studies. Thus one resolution might be that these effects in the SC are due to presynaptic action. The experiments described here were undertaken to test this possibility.

To analyze the input differences in GABA B action at the presynaptic terminal of excitatory synapses, we measured the effect of the GABA B agonists on paired-pulse facilitation (PPF) of the fEPSPs, a standard measure of presynaptic function. The inputs were stimulated every 20 s with pairs of stimuli 50-ms apart. PPF was calculated as the ratio of the second to first fEPSP amplitude. After 15 min of stable baseline, we bath-applied the selective GABA B agonist SKF97541 (1 μM) for 10 min. This decreased the SC fEPSP amplitude by 86% (P < 0.001, n = 6; Fig. 5B) and produced a simultaneous increase of PPF, as expected of presynaptic action (Fig. 5A). Both effects were reversible. The effect on the PP fEPSP and PPF was not significant. This suggests that presynaptic GABA B control is present in the SC but is small or absent in the PP.

As a further test of the presynaptic effect in SC, we studied the effect of GABA B agonist on the EPSPs recorded by whole cell methods. Application of GABA B agonist, SKF97541 (1 μM), caused a substantial hyperpolarization (–6 ± 0.3 mV, P < 0.001, n = 6). To eliminate this postsynaptic effect, we used QX-314 (Lambert and Wilson 1993) delivered through the patch pipette. The efficacy of this drug was shown by the fact that it hyperpolarized the membrane by 6.3 mV (P < 0.01) and completely blocked AHPs (after 40 min). Under these conditions, any effect of GABA B agonist should
be presynaptic. We found that SKF97541 reversibly suppressed the EPSPs (measured at -70 mV) by 85% (n = 6, Fig. 5C), confirming a presynaptic site of GABA_B action in the SC. The same agonist application produced a smaller suppression of the EPSP in the PP (49%, P < 0.01). This suppression did not recover even after 40 min of washout (36% less than control, P < 0.01, Fig. 5C). The lack of reversibility makes it difficult to interpret. In any case, if there is a presynaptic GABA_B effect in the PP, it is small compared with the SC, in agreement with the results described in the previous paragraph.

Control of the EPSP

Our manipulations of dendritic conductances provided information about how these conductances affected the EPSP, which we summarize here. Calcium channel blockers (NiCl_2 and nifedipine) significantly increased the area of the EPSP (by 57% in the SC and by 48% in the PP input, P < 0.05, n = 4; Fig. 2B), suggesting that some Ca^{2+}-dependent potassium channels normally suppress the EPSP. One candidate could be the large-conductance Ca^{2+}-activated potassium current (Poo-las and Johnston 1999). However, this suggestion requires further testing because some calcium channel inhibitors (including nifedipine) also directly inhibit the A-current (Mathie et al. 1998). Therefore the increase in EPSP amplitude by NiCl_2 and nifedipine could be due to the inhibition of A-current, as was shown before in CA1 pyramidal cells (Hoffman et al. 1997). The intracellular perfusion of QX-314 (that blocked both I_h and GABA_B channels) significantly increased EPSP amplitude (by 30%, P < 0.01, n = 7), area (by 80% in SC and 120% in PP, P < 0.01; Fig. 3B), and EPSP decay time (P < 0.05), but this result is difficult to interpret given the complexity of QX-314 action.

It was of interest to ask whether the particular ion channels responsible for AHPs contribute to the shaping of EPSP. The I_h blocker ZD7288 significantly increased the EPSP area in both the SC (by 105%, P < 0.001) and the PP (by 150%, P < 0.01, n = 6; Fig. 3C). The peak amplitude increased in the SC by 37% and in the PP by 49% (P < 0.01). Furthermore, ZD7288 increased the AMPA EPSP decay in SC from 63.4 ± 3.7% to 86.3 ± 4.7 ms (P < 0.01). In the PP, the increase was even stronger—from 69.6 ± 4 to 111.6 ± 7.2 ms (P < 0.001). This suggests that I_h current determines the shape and duration of even single excitatory responses. The results in Fig. 4B1 suggest that GABA_B in the PP also has a fast enough kinetics to affect the late part of the EPSP. This is shown more directly in Fig. 4D1 in the presence of PTX and APV, the GABA_B blocker increased the PP EPSP amplitude (by 18%, P < 0.01, n = 8) and area (by 46%, P < 0.01). This effect was significant, although substantially weaker than the effect of the I_h blocker. The APV effect of the postsynaptic action, since presynaptic action in the PP was not prominent in previous experiments, and in iEPSP experiments, the GABA_B blocker did not affect either SC or PP iEPSP (data not shown). Taken together, these data indicate that the I_h and GABA_B conductances that cause the AHP act sufficiently quickly to affect the EPSP.

Effect of the AHP on the PP NMDA-mediated EPSP

We next investigated the role of the AHP in modulating the NMDA component of the EPSP. To test whether I_h and GABA_B exert this function, we inhibited these conductances and asked whether this would reveal an NMDA component of the EPSP. The results are presented in Fig. 6. It can be seen that, with K^+-based intracellular solution, there is no significant NMDA component in the PP and SC EPSPs near resting potential (not significant, n = 7) for small EPSPs. In the next experiment, we first measured EPSPs in ACSF with PTX (50 μM; Fig. 6B; n = 6). Then the I_h blocker (ZD7288, 30 μM) and the GABA_B blocker (2-hydroxyaselenofen, 100 μM) were added. This completely blocked the AHP and increased the EPSP in both inputs. In the SC, the increase in EPSP amplitude was not significant, but the area increased by 88 ± 14% (P < 0.001). In the PP, the EPSP amplitude was increased by 49 ± 8% (P < 0.001) and area grew more than three times (by 258 ± 32%, P < 0.003). Under these conditions, blocking NMDA channels with ±APV (100 μM) decreased the PP EPSP amplitude by 30 ± 3% and area by 34 ± 5% (P < 0.001). These results confirm that I_h and GABA_B contribute strongly to a “braking” action on the NMDA component of the EPSP in the PP. As another way of examining the same issue, we directly compared the effects of APV in control (n = 7) and after the blockade of I_h and GABA_B currents (n = 6). In the SC, the difference in APV effects did not reach significance (P > 0.2). In the PP, however, the APV effect after the blockade of I_h and GABA_B currents was significantly larger than in control (P < 0.01 for amplitude and P < 0.05 for the area changes in PP EPSP).

The question remained whether potassium currents other than GABA_B may also be important in braking the NMDA response. In this case, the inhibition of potassium currents (without the blockade of I_h) would be sufficient to reveal the NMDA component of EPSP. Our next experiments were designed to answer this question. First, we checked whether the blockade of the majority of potassium currents with Cs^+ -based intracellular solution would increase the NMDA component in PP or SC EPSP. Although Cs has several actions (see next paragraph), it did not relieve the brake on the NMDA current as judged by the fact that there was still little effect of APV on the EPSP (not significant, n = 4; Fig. 6C). However, the additional blockade of I_h channels with ZD7288 (30 μM) substantially slowed the decay time in both inputs (to 60.5 ± 5.5 ms in SC and to 84 ± 5.8 ms in PP, P < 0.01). ZD7288 also increased the PP EPSP amplitude (by 23 ± 3%, P = 0.01, and area by 136 ± 24%, P < 0.04, n = 5; Fig. 6D1); the SC synaptic response did not change significantly, but the area increased by 24 ± 7.8% (P < 0.1). Under these conditions, the NMDA component became evident (Fig. 6D2); in the SC, the application of ±APV (100 μM) decreased the EPSP area by 33 ± 4% (P < 0.02). In the PP, the amplitude was decreased by 23 ± 3% and area by 33 ± 3% (P < 0.01, n = 5). As in previous experiments, the direct comparison of APV effects in control (n = 4) and after the blockade of I_h and GABA_B (n = 5) showed no significant difference in the SC (P > 0.25). In the PP, however, APV affected the EPSP area after the blockade of I_h and GABA_B currents, and this was significantly stronger (P < 0.01) than in control. Therefore, even with majority of potassium channels blocked by intracellular Cs^+, the NMDA component of PP EPSP is still strongly suppressed by the I_h current. We conclude that blockade of potassium channels alone is not sufficient to release the brake.

FIG. 6. A large NMDA component in PP EPSP becomes evident after the blockade of \( I_h \) and GABA\(_B\) currents. Significance: \* \( P < 0.05 \), \** \( P < 0.01 \), \*** \( P < 0.001 \). A: NMDA-dependent component in small EPSPs is virtually absent near resting potential in the experiments with K\(^+\)-based intracellular solution. Average of 7 cells in PTX. \( A \) represents the data already shown in Fig. 2A and is placed here to simplify comparison. B1: PP and SC EPSP area is strongly increased by the mixture of \( I_h \) and GABA\(_B\) blockers, ZD7288 and 2-hydroxy-saclofen. Average of 6 cells in PTX. B2: continuation: in the PP, the NMDA blocker, ±APV, substantially decreased the EPSP area after the blockade of \( I_h \) and GABA\(_B\) currents. C: in experiments with Cs\(^+\)-based intracellular solution (potassium channels blocked), the NMDA-dependent component is still absent near resting potential. Importantly, the AHPs in both inputs are strongly suppressed by the blockade of potassium currents. Average of 4 cells in PTX. D1: \( I_h \) blocker, ZD7288, strongly increased the PP EPSP area, suggesting that intracellular Cs\(^+\) does not block the \( I_h \) channels. Average of 5 cells in PTX. D2: continuation: in the PP, the NMDA blocker, ±APV, substantially decreased the EPSP area after the additional blockade of \( I_h \) current.
Using intracellular Cs\(^+\) allowed us to make an observation relevant to the role of potassium current in generation of \(I\_h\)-dependent AHPs. We found that intracellular Cs\(^+\) strongly inhibited AHPs in both inputs; the AHP/EPSP area ratio in SC decreased to 0.04 ± 0.02 (compared with 0.69 ± 0.09 in control); in the PP, it decreased to 0.45 ± 0.08 (Fig. 6C). However, the intracellular Cs\(^+\) did not block the \(I\_h\) current, since the application of ZD7288 still increased the EPSP areas in both inputs. This suggests the importance of potassium currents in generation of \(I\_h\)-dependent AHP (see discussion).

**Is the GABA\(_B\) IPSP smaller in the SC than in the PP?**

Although it is clear that the relative contribution of GABA\(_B\) to the AHP is much larger in the PP than in the SC, there is a potential complication in assuming that the absolute size (for a comparable local EPSP) is smaller. This complication arises because it is likely that the EPSP, because of its fast kinetics, is attenuated more than the slower AHP. This makes it possible that the locally generated EPSP at the distant PP synapse was actually much larger than appears at the soma. Could such differential attenuation account for the apparent low GABA\(_B\) component in the SC for stimuli adjusted to have equal EPSPs in the soma? To address this question, we took advantage of results showing that EPSP area is much less sensitive to electrotonic attenuation that the EPSP peak (Carnevale and Johnston 1982; Spruston et al. 1993). To determine EPSP area, it was important to eliminate other currents affecting the waveform. Thus the measurements were made under conditions where the AHP was blocked. We determined the EPSP area increase coefficient due to the blockade of \(I\_h\) and GABA\(_B\) currents (= EPSP\(_{ZD}\) + \(\Delta\)EPSP\(_{\text{Control}}\)). We had two variants of such experiments. In the first, the NMDA channels were blocked (Fig. 4, E and F); in these, the EPSP was increased approximately equally in the two inputs (2.72 ± 0.22 times in PP and 3.05 ± 0.74 times in SC, \(P > 0.7\)). In the second set of experiments, the NMDA channels were not blocked (Fig. 6B1). In this experiment, EPSP area in PP increased 3.58 ± 0.35 times, which was significantly higher than 1.87 ± 0.14 times in SC. Therefore we used these latter “worst case” multipliers (3.6 and 1.9, respectively; i.e., a factor of ~2 difference) to estimate the corresponding size of GABA\(_B\) component of AHP area in SC. Multiplying the SC AHP area after EPSP blockade (Fig. 4A) by 2 gives a value (34 × 2 = 68 mV · ms). This corrected value is nevertheless approximately six times less that the PP GABA\(_B\) AHP under the same conditions (~430 mV · ms, Fig. 4B1). We can therefore conclude that GABA\(_B\) component is smaller in the SC than the PP, even for comparable local EPSPs.

**DISCUSSION**

We observed an AHP in both the SC and PP in response to single synaptic stimuli. Our main finding is that these AHPs are generated somewhat differently in the two pathways; \(I\_h\) contributes in both pathways, but GABA\(_B\) contributes strongly (>50%) to the PP AHP, while making <15% contribution to the SC AHP. Ca\(^{2+}\)-activated potassium currents and GABA\(_A\) mechanisms make little or no contribution to the AHP. The differential postsynaptic role of GABA\(_B\) in the PP and SC is complemented by a differential presynaptic role, which is much larger in the SC than the PP. Thus both pathways are subject to GABA\(_B\) regulation of excitation, but have a different locus of action. As will be discussed later, a postsynaptic contribution can affect NMDA currents. Since these are particularly strong in the PP, they can be effectively braked by postsynaptic GABA\(_B\).

These conclusions about GABA\(_B\) are consistent with histochemical data. The highest density of GABA\(_B\) receptors in CA1 was found in s. lacunosum-moleculare (Sloviter et al. 1999). This layer also has four times higher GIRK1 protein (part of the channel controlled by GABA\(_B\)) compared with s. radiatum (Miyashita and Kubo 1997). More direct data on subcellular localization of GABA\(_B\) proteins has recently become available (Kulik et al. 2003). This work showed that, in s. radiatum, the GABA\(_B\) receptors were positioned at presynaptic sites of excitatory synapses (although postsynaptic GABA\(_B\) was also observed), whereas in s. lacunosum-moleculare, GABA\(_B\) receptors were predominantly postsynaptic. Interestingly, two-thirds of postsynaptic GABA\(_B\) receptors were positioned in spines close to excitatory synapses (from inside the postsynaptic density to 240 nm from its edge) (Kulik et al. 2003). The preferential localization of postsynaptic GABA\(_B\) receptors at excitatory synapses in s. lacunosum-moleculare suggests a precise control of the PP excitatory synapses by the GABA\(_B\) inhibition.

**\(I\_h\)-dependent effects on the EPSP**

We found that, in the SC and PP, the blockade of \(I\_h\) conductance increases the amplitude of the small EPSP evoked by single stimuli. Previous work (Magee 1998) showed the increase of simulated EPSPs (sEPSP; EPSP-shaped current injections via the patch electrode) in dendrites of s. radiatum after the blockade of \(I\_h\) current with extracellular Cs\(^+\). Similar effects on the sEPSP were later observed in the neocortex using a more \(I\_h\)-selective blocker, ZD7288 (Williams and Stuart 2000). Especially strong effects of \(I\_h\) were described for the summation of multiple sEPSPs (Magee 1999; Williams and Stuart 2000) and the waveform evoked by direct glutamate application (Takigawa and Alzheimer 2003).

**Role of potassium currents in \(I\_h\)-dependent AHP**

We found that AHP was suppressed by intracellular Cs\(^+\) in both inputs (Fig. 6C). This cannot be attributed to the blockade of the \(I\_h\) current by intracellular Cs\(^+\) because, under the same conditions, EPSP was still increased by the \(I\_h\) blocker, ZD7288. We conclude that potassium currents are essential for \(I\_h\)-dependent AHP. To understand this phenomenon, consider the mechanisms supporting resting membrane potential. A substantial part of dendritic \(I\_h\) channels is active near resting potential and inactivates on synaptic depolarization (Magee 1998). It was suggested that this \(I\_h\) current is a major contributor to the mechanism maintaining the resting potential together with opposing hyperpolarizing potassium currents (Lupica et al. 2001; Pape 1996). Consistent with this idea, in our experiments, \(I\_h\) inhibitor (ZD7288) hyperpolarized somatic potential by ~14 mV, evidently by eliminating the \(I\_h\) opposition to potassium currents. This opposition between \(I\_h\) and potassium currents is probably responsible for \(I\_h\)-dependent AHP. The EPSP, by blocking the \(I\_h\) channels, increases the
input resistance of the cell. The membrane becomes relative more permeable for K\(^+\) compared with Na\(^+\) ions. This leads to a hyperpolarizing potential produced by exiting potassium currents that overshoots the resting potential (baseline) in a form of an AHP (Bal and McCormick 1997; Pape 1996). It is now easy to understand why blockade of potassium currents strongly suppressed the \(I_h\)-dependent AHP. We did not analyze in detail which potassium currents contribute to AHP in opposition to \(I_h\) current. Other studies in other cell types suggest the participation of leak current, pump-current, delayed rectifier, A-current, Ca\(^{2+}\)-dependent, and G-protein–coupled potassium currents in supporting the resting membrane potential or causing membrane oscillations in opposition to \(I_h\) current (Adam et al. 2001; Chapman and Lacaille 1999; Funahashi et al. 2002; MacLean et al. 2003; Pape 1996; Savic et al. 2001; Sekirnjak and du Lac 2002; Siros et al. 2002).

**AHP as a brake on NMDA current**

There has been substantial interest in the possibility that the voltage-dependence of the NMDA-mediated currents could generate regenerative synaptic potentials (i.e., NMDA-spikes/plateaus) (Schiller and Schiller 2001), and the utility of such regenerative potentials has been explored in theoretical models (Kepes and Raghavachari 2002; Koulakov et al. 2002; Lisman et al. 1998; Poirazi et al. 2003). The large NMDA component in the PP might suggest this as a potential site of NMDA spike generation; however, we find that the NMDA response in the PP is strongly braked by the \(I_h\) and GABA\(_B\)-dependent AHPs. Further investigation might be required to determine whether the interplay of GABA\(_B\) and \(I_h\) channel contribution to the NMDA braking mechanisms would be exactly the same in vivo. The kinetics of these conductances might be less similar at higher temperatures. Also, there might be timing differences in initiating of excitatory and inhibitory synaptic responses in s. lacunosum-moleculare, which we could not imitate with the local electrical stimulation in the slice preparation.

An important remaining question is whether the braking mechanism we have described can somehow be circumvented or modulated, allowing regenerative NMDA potentials. The GABA\(_B\) brake could perhaps be overcome if signals arriving from the two pathways are simultaneous and large enough to summate to a threshold for an NMDA spike before the GABA\(_B\) conductances have time to develop (within \(\sim 20–50\) ms of the arrival of a stimulus to the PP, see Fig. 4B). The faster acting \(I_h\) might still serve as a brake, but this conductance is presumably saturable by large depolarizations. A second important possibility is that modulatory mechanisms (Hoffman and Johnston 1998; Pape 1996; Pedarzani and Storm 1995; Worley et al. 1987) may exist that turn off the brake. Of particular note is the possibility that cholinergic modulation, a modulation that enhances synaptic plasticity (Huerta and Lisman 1993, 1996), acts to suppress the GABA\(_B\) conductance in CA1 (Behrends and ten Bruggencate 1993; Patil and Hasselmo 1999; Patil and Alger 1992; Worley et al. 1987).

Understanding the interactions of the PP and SC and the role of NMDA channels in these interactions will be important in elucidating the function of CA1. The hippocampus is involved in the storage of episodic memory (Burgess et al. 2001, 2002; Eichenbaum 2001; Knierim 2003; Suzuki 2003) and contributes to the detection of novelty (Vinogradova 1984). The CA1 region has been suggested to be the site of novelty detection (Abbott and Blum 1996; Hasselmo and Schnell 1994; Levy 1989; Lisman 1999; Lisman and Otmakhova 2001) because it receives two types of inputs relevant to this computation. One of these is the Schaffer collateral input from CA3 that carries predictions based on memory recall. This information may be compared with sensory information arriving from the entorhinal cortex through the perforant path (Abbott and Blum 1996; Hasselmo and Schnell 1994; Levy 1989; Lisman 1999; Lisman and Otmakhova 2001). The work presented here shows that the interactions of these two pathways will involve not only the AMPA currents, but also interplay of \(I_h\), GABA\(_B\), and NMDA currents.

**REFERENCES**


Sloviter RS, Ali-Akbarian L, Elliott RC, Bowery BJ, and Bowery NG. Localization of GABA(B) (R1) receptors in the rat hippocampus by immunocytochemistry and high resolution autoradiography, with specific reference to its localization in identified hippocampal interneuron subpopulations. *Neuropharmacology* 38: 1707–1721, 1999.


Williams SR and Stuart GJ. Site independence of EPSP time course is mediated by dendritic I(h) in neocortical pyramidal neurons. *J Neurophysiol* 83: 3177–3182, 2000.