Cholinergic Modulation Differs between Basal and Apical Dendritic Excitation of Hippocampal CA1 Pyramidal Cells

We hypothesize that endogenous cholinergic modulation of dendritic processing of hippocampal CA1 is layer specific, and it specifically enhances spike output resulting from basal as compared with the apical dendritic excitation. Laminar profiles of evoked field potentials were recorded in the CA1 area of urethane-anesthetized rats using multichannel silicon probes and analyzed as current source density. High-frequency stimulation of the pontis oralis (PnO) attenuated the midapical more than the basal or distal apical dendritic excitatory sink. Population spike (PS) and excitatory sink–PS potentiation resulting from basal dendritic excitation were facilitated, while the PS evoked by apical dendritic stimulation was attenuated by PnO stimulation. Perfusion of cholinergic agonist carbocoll onto hippocampal slices in vitro also attenuated the apical more than the basal dendritic excitatory postsynaptic potentials. Excitatory sink attenuation and PS changes after PnO stimulation were blocked by systemic or local scopolamine and by intracerebroventricular (icv) M1 receptor antagonist pirenzepine but not by icv M2 receptor antagonist AFDX–116 or nicotinic antagonists. However, a hippocampal theta rhythm activated by PnO stimulation was blocked by systemic but not by local scopolamine. We conclude that endogenous acetylcholine mediates a stronger presynaptic inhibition of the midapical than basal and distal apical excitation mainly through M1 receptors.

Keywords: current source density, dendritic excitation, excitatory postsynaptic potential, muscarinic receptors, population spike, presynaptic inhibition

Introduction

Acetylcholine (Ach) modulates synaptic processing in the brain and is considered to be vital in arousal, attention, memory, and other cognitive processing (Deutch 1971; Everitt and Robbins 1997; Hasselmo 1999; Jones 2005; Sarter et al. 2005). In the hippocampus, cholinergic inputs from the medial septal-diagonal band of Broca area (Saper 1984; Lopes da Silva et al. 1990; Amaral and Lavenex 2007) are important for memory function (Givens and Olton 1990; Everitt and Robbins 1997; Chang and Gold 2004).

Through muscarinic and nicotinic receptors, Ach mediates presynaptic inhibition of glutamatergic excitation (Valentin0 and Dingledine 1981; Hasselmo and Schnell 1994; Qian and Dingledine 1981; McQuiston and Madison 1999; Lawrence et al. 2006). Alternatively, Ach can hyperpolarize the membrane by activating a SK-type Ca2+-activated K+ conductance (Gulledge et al. 2007). Nicotinic receptors may mediate presynaptic facilitation and postsynaptic excitation (McKay et al. 2007).

While much is known about Ach modulation of excitation and inhibition in vitro, the effect of endogenous Ach on hippocampal neural processing in vivo remains unclear. The release of Ach in the hippocampus is normally accompanied by a hippocampal theta rhythm (Dudar et al. 1979; Keita et al. 2000), and the highest release of Ach occurs during a theta rhythm that accompanies walking (Dudar et al. 1979; Vanderwolf 1988; Leung 1998), that is, during times of spatial exploration or other active behaviors. The population excitatory postsynaptic potential (pEPSP) and population spike (PS) at the apical dendrites of CA1 (Leung 1980; Leung and Vanderwolf 1980; Cao and Leung 1991) were found to be attenuated during behavioral states that are accompanied by a theta rhythm (to be referred as theta states) as compared with behavioral states that are not accompanied by a theta rhythm (nontheta states). Decrease in the apical pEPSP and PS was also found by applying Ach or by septal stimulation (Rovira, Ben Ari, and Cherubini 1983), during somatosensory stimulation (Herreras, Solís, Herranz, et al. 1988; Herreras, Solís, Muñoz, et al. 1988) or during nociceptive pain induced by injecting formalin to the rat’s hindpaw (Zheng and Khanna 2001; Jiang and Khanna 2004). However, Ach or septal stimulation also increased the fimbria-evoked CA1 PS (Krnjević and Ropert 1982), and the commissurally evoked PS in CA1 was observed to be larger during walking as compared with immobility (Buzsáki et al. 1981).

Based on observation of a lack of the basal dendritic pEPSP during walking as compared with immobility (Leung 1980), we hypothesize that basal and apical dendritic synapses are modulated differently by muscarinic cholinergic inputs to the hippocampus, and more specifically, endogenous Ach enhances basal dendritic excitatory transmission and spiking, in contrast to a attenuation of apical dendritic transmission. The differential effects of endogenously released Ach on various parts of the dendritic tree of the CA1 pyramidal cell were studied by current source density (CSD) analysis.

Materials and Methods

Experimental Studies In Vivo

Eighty Long-Evans rats (220–450 g) were used. A rat was anesthetized with urethane (1.2–1.5 g/kg intraperitoneal [i.p.]). The recording probe was positioned in CA1 area (Fig. 1A) at P3.8–4.2, L2.4–3 with respect to bregma, using the atlas of Paxinos and Watson (1986). Stimulating electrodes were placed in: 1) medial perforant path...
(MPP), at P8, L4.4; 2) stratum oriens (OR) in CA1 or CA3a for basal dendritic excitation in CA1; and 3) stratum radiatum (RAD) in CA1 or CA3b for CA1 apical dendritic excitation. Both OR and RAD stimulating electrodes were lowered vertically from P3.2, L3.3, and adjusted according to electrophysiological criteria (Leung and Shen 1995). In addition, pontis oralis (PnO) stimulation is applied at P7.5, L1, 7--8 mm ventral (V) to skull. Stimulating electrodes are 125-$\mu$m Teflon-insulated wires except at the cut tips. Bilateral guide cannulae were placed over the lateral ventricle at P0.8, L1.4, V2.3. A screw over the cerebellum serves as the anode. Stimulus currents were delivered (with pulse duration of 0.2 ms) through a photo-isolated stimulus isolation unit 6 (Astro-Med/Grass Instrument, West Warwick, RI). Stimulation repetition rate was <0.1 Hz.

Silicon recording probes, with 16 recording sites spaced 50 $\mu$m apart on a vertical shank, were provided by the National Institutes of Health Center of Neural Communication Technology, University of Michigan or purchased from NeuroNexus (Ann Arbor, MI). The signals were amplified 200--1000x by preamplifier and amplifier and passed through a high-pass filter with 0.08-Hz corner frequency. Sixteen sample-and-hold circuits held the signals during simultaneous acquisition at 20–25 kHz, via a 16-bit analog-to-digital converter (Townsend et al. 2002) or a RA16PA acquisition System 3 made by Tucker-Davis Technologies (Alachua, FL). Single and average ($N = 4$) sweeps were stored by a custom program. One-dimensional CSD$(z, t)$ as a function of depth $z$ and time $t$ was calculated by a second-order differencing formula in equation 1 below (Freeman and Nicholson 1975; Leung 1990):

$$CSD(z, t) = \sigma [2\Phi(z, t) - \Phi(z + \Delta z, t) - \Phi(z - \Delta z, t)]/[(\Delta z)^2].$$

where $\Phi(z, t)$ is the potential at depth $z$ and time $t$, $\Delta z$ is the spacing (50 $\mu$m) between adjacent electrodes on the 16-channel probe. The simultaneously acquired signals were sufficiently noise free that no spatial smoothing of the CSDs was necessary. The conductivity $\sigma$ was assumed to be constant, and the CSDs were reported in units of V/mm$^2$ without using an actual value of $\sigma$.

In a urethane-anesthetized rat, the typical pattern of spontaneous electroencephalography (EEG) in the hippocampus was an irregular (nontheta) activity with no obvious rhythm (Fig. 1B). Hippocampal theta rhythm ("theta state") may arise spontaneously or more reliably induced by pinching the tail or by stimulation of the brainstem, in particular at the PnO (Fig. 1B; Bland et al. 1994; Heynen and Bilkey 1994; Vertes and Kocsis 1997; Jiang and Khanna 2004). Release of endogenous Ach was shown to accompany a theta rhythm (Dudar et al. 1979; Keita et al. 2000). In all rats, a 100-Hz train stimulation was applied to PnO at 150 $\mu$A (0.2-ms pulses) for 1 s; ~75-$\mu$A intensity was the threshold to induce a detectable theta in most rats. For spectral

Figure 1. (A) Diagram of 16-channel recording electrode in CA1 and stimulating electrodes in CA3. Stimulating electrode was placed in stratum OR and stratum RAD of CA3 to activate excitatory afferents to CA1 basal and apical dendrites, respectively. (B) Recording at CA1 near the hippocampal fissure, without or with high-frequency stimulation [1 s] of the PnO preceding paired-pulse stimulation of CA3 afferents to CA1. (C) Mean logarithmic power spectrum of EEG (arbitrary units) recorded in CA1 stratum RAD shows 7.45 Hz peak theta ($\Theta$) activated by PnO stimulation, which was mostly blocked by scopolamine (5 mg/kg i.p.). (D) Mean and one standard error of the mean of the PnO-activated attenuation (percent of baseline) of the apical dendritic population excitatory postsynaptic sink (E1) and PS (P1) as a function of delay time from the end of the PnO stimulation train ($n = 5$ rats).
analysis of the EEG evoked during PnO stimulation, the stimulus artifacts were reduced by replacing the sampled data near the stimulus artifacts with the average EEG immediately before and after the stimulus. EEG during the last 0.67 s of the 1-s PnO activation was analyzed by Fast Fourier Transform and shown as autpowper spectra with 1.49-Hz resolution (Leung 1985). A single sweep of EEG was considered to have theta power if there was a detectable power peak within the frequency range of 2.98-8.94 Hz. Theta peak frequency was the frequency at the peak power, and the rise of theta (a measure of rhythmicity) was measured as the rise of the peak theta power above the power at 1.49 Hz. Only the most ventral electrode of the silicon probe (near the hippocampal fissure) was used for analyzing theta power. Linear correlations were made between theta characteristics (power, rise, and frequency) and synaptic responses (sink and PS amplitudes) in 20 sweeps in selected rats.

Train stimulation of the medial septum, like PnO stimulation, also released Ach in the hippocampus (Krnjevic et al. 1988). We did not use septal stimulation because: 1) it typically induced desynchronization and not hippocampal theta (Kramis and Vanderwolf 1980) and 2) it directly activated noncholinergic neurons such that the septal effects on evoked PS could only be partially abolished by large doses of muscarinic antagonist scopolamine (Krnjevic and Ropert 1982). PnO stimulation had several advantages as follows: 1) antidromic stimulation of hippocamposeptal afferents was avoided, 2) hippocampal theta of different frequencies could be elicited dependent on PnO stimulation intensity, 3) hippocampal theta elicited by PnO stimulation had amplitude and phase profiles that resembled the physiological theta (Heynen and Bilkey 1994), and 4) PnO neurons typically fired more during walking than immobility in the behaving rat (Vertes and Kocsis 1997).

After establishing a stable baseline response, cholinergic drugs were administered. Nonspecific muscarinic antagonist scopolamine hydrochloride was given at 5 mg/kg i.p., a dose previously shown to be effective (Leung 1985; Leung et al. 2003). Other drugs were given through an intracerebroventricular (icv) cannula on the same side of the cerebrospinal fluid (aCSF) at 30-32°C, saturated with 95% O2-5% CO2. This consisted of NaCl 124 mM, KCl 5 mM, NaH2PO4.H2O 1.25 mM, MgSO4.7H2O 2 mM, CaCl2.6H2O 2 mM, NaHCO3 26 mM, and glucose10 mM. Extracellular recordings were made using microelectrodes filled with 2 M KCl and K+ concentrations of 80–200 M, with a 60 mV spike height were recorded. The aCSF consisted of NaCl 124 mM, KCl 5 mM, NaH2PO4.H2O 1.25 mM, MgSO4.7H2O 2 mM, CaCl2.6H2O 2 mM, NaHCO3 26 mM, and glucose10 mM. Extracellular recordings were made using microelectrodes filled with aCSF of resistance 6–10 MΩ. In most experiments, pEPSP was recorded in either RAD or OR in CA1, and the afferents were stimulated in the same layer by a concentric stimulation electrode, <0.5 mm away. In some experiments, 2 field recordings were made at the dendritic and somatic layer, following stimulation of either RAD or OR. Alternatively, extracellular recording was fixed at one layer, and alternative RAD or OR stimulation were delivered. Intracellular recordings were made using micropipettes filled with 2 M K+ acetate of 80–200 MΩ resistance. Impalements were made at the CA1 cell layer, and only neurons with >60 mV spike height were recorded. Intracellular and extracellular recordings were made in the same cell/slice during baseline and for 10–15 min after perfusion of carbamylcholine (carbachol [CCH]) at a dose of 10–3 to 10–5 M, followed by washout of CCH. The rising slope of the intracellular EPSP (iEPSP) was measured without compensating for the depolarization.
induced by CCH. The CCH-induced depolarization varied from an average of 3 mV after $10^{-4}$ and $10^{-5}$ M CCH and 9 mV after $10^{-3}$ M CCH (Yim and Leung 1988). Neurons with a depolarizing afterpotential were identified as pyramidal cells (Liu and Leung 2003).

**Results**

**The Effect of PnO Activation on Hippocampal EEG and Apical Excitation of CA1**

In urethane-anaesthetized rats, high-frequency stimulation of the PnO activated a hippocampal theta rhythm during and immediately after the stimulation (Fig. 1B). PnO stimulation will be referred to as PnO activation or simply as activation. The EEG during the last 0.67 s of the 1-s PnO activation was analyzed by power spectra. PnO activation induced increased theta power with no significant change in gamma (30-100 Hz) power (Fig. 1C). The peak theta frequency was between 2.9 and 7.5 Hz, averaging 5.08 ± 0.22 Hz ($n = 23$ rats). The depth profile of the theta rhythm showed an amplitude peak at the apical dendrites of CA1, and ~180° phase reversal was found across the CA1 cell layer (not shown). After 5 mg/kg i.p. scopolamine, the PnO-activated theta power peak was reduced by more than 50% in all rats and abolished in 4 of 7 rats. The effect of PnO activation on subsequent synaptic responses, evoked by stimulation of CA3, was approximately uniform from 30- to 1200-ms delay latency after the end of the PnO train (Fig. 1B). During the latter period, the attenuation of the apical dendritic evoked PS was nearly constant (Fig. 1D). The delay of CA3 stimulation from the end of the PnO train was 30 ms for the following experiments.

Stimulation of the apical dendritic afferents to CA1 resulted in an excitatory synaptic sink at the apical dendrites (50 to 250-μm depth in Fig. 2B1) accompanied by a source in the cell layer and distal dendrites (Fig. 2B1, C2). The apical excitatory sink and its associated source were smaller with PnO activation than without (Fig. 2C2). At a stimulus intensity that evoked a PS amplitude of 50-75% of the maximum ("high" stimulus intensity), the activation ratio of the total excitatory sink summed at all apical dendritic layers (activation ratio = total sink with PnO stimulation/total sink without PnO stimulation)

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**Figure 2.** (A) Average evoked potentials (AEPs) at different depths following low-intensity single-pulse stimulation of the apical dendritic afferents to CA1. (B1) CSD profile of low-intensity stimulation (B1) and high-intensity stimulation shows an apical dendritic excitatory sink at depth 50-250 μm, accompanied by a source in stratum pyramidale (0-μm depth) and distal layers (>350 μm). The apical sink was attenuated by prior points orals (PnO) stimulation. (C) Spatial profile of AEPs (C1) and CSDs (C2) at the time instant of vertical dotted line in A and B1. (D–E) Plot of apical excitatory sink E1 (D) and PS P1 (D) versus stimulation intensity shows decreased response after PnO stimulation (dotted traces). (F) Plot of P1 versus E1 shows a decreased PS at a fixed E1 after PnO stimulation.
was 0.84 ± 0.02 ($n = 17$); a similar activation ratio was also found for the average evoked potential negative peak of slope at the apical dendrites (Fig. 2A,C,F). P1 evoked by high-intensity CA3 stimulation was also attenuated by PnO activation (Fig. 2B2), giving a P1 activation ratio of 0.52 ± 0.04 ($n = 17$) that showed significant PS attenuation in every rat.

For the specific example shown, input–output plots of E1 (first-pulse excitatory sink slope; Fig. 2D) or P1 (first evoked PS, * in Fig. 2B2) versus CA3 stimulation intensity show smaller amplitudes with, as compared with without, PnO activation.

Group data confirmed the latter results. The total apical dendritic sink evaluated in a group of 9 rats clearly showed a significant decrease with PnO activation as compared with without activation (Fig. 3A1) across a wide range of stimulus intensities (see Materials and Methods for procedures). The excitatory sink (E1) activation ratio was not significantly different at different stimulus intensities, including that below the PS threshold ($n = 9$ rats). Similarly, the normalized P1 amplitude (normalized by the highest P1 amplitude in each rat) was significantly smaller with PnO activation as compared with without activation (Fig. 3A2). In contrast, when the normalized P1 versus normalized total sink was plotted, there was no group difference of normalized P1 at a particular category of normalized E1 (Fig. 3A3). An increase in P1 at low

**Figure 3.** Group input–output graphs of excitatory sink (E1) and PS amplitude (P1) evoked by the first pulse applied to evoke (A) an apical dendritic sink in 9 rats or (B) a basal dendritic sink in 7 rats. E1 in this figure refers to the total sink at either the apical or basal dendrites (i.e., summed across all depths showing a sink). Normalized E1 is the total sink divided by the maximal total sink for each rat. Normalized P1 is the PS amplitude divided by the maximal PS amplitude (across all conditions) of each rat. Two-factor ANOVA was run for each graph, and * indicates a significant post hoc Newman–Keuls test ($P < 0.05$). Significant group effects were found for $A1$ ($F_{1,8} = 11.8$, $P < 0.01$), $A2$ ($F_{1,8} = 13.3$, $P < 0.01$), and $B3$ ($F_{1,6} = 13.6$, $P < 0.02$). Significant group × intensity interaction was found for $A1$ ($F_{10,80} = 5.23$, $P < 0.001$), $B7$ ($F_{7,42} = 2.99$, $P < 0.02$), and $B2$ ($F_{5,30} = 3.83$, $P < 0.01$).

**The Effect of PnO Activation on Basal Dendritic Excitation of CA1**

Basal dendritic excitation of CA1 showed a different modulation by PnO activation. Basal dendritic excitation was characterized by a negative potential and an excitatory sink at the basal dendrites, accompanied by a positive potential and a source at the cell body layer (Fig. 4A,B1,C2). At a high-intensity stimulus (that evoked 50–75% of the maximal PS), a small but significant attenuation of the total sink integrated over the basal dendritic layers was found with PnO activation as compared with without activation (Figs 3B1 and 4B2). The activation ratio of the total basal dendritic sink (E1) was 0.95 ± 0.01 ($n = 19$), which was significantly less than the activation ratio of the total apical excitatory sink ($0.84 ± 0.02$, $n = 17$).

While basal dendritic E1 attenuation by PnO activation was less consistent at low intensity that did not evoke a PS, there was no significant difference in the E1 activation ratio below and above the PS threshold ($n = 9$ rats). In contrast to apical excitation, the P1 evoked by basal dendritic excitation was reliably enhanced by activation, as shown for the specific example (* in Fig. 4B2) and in the group data at mid to high normalized stimulus intensity (Fig. 3B2). The basal dendritic P1 activation ratio at high-intensity stimulation was $1.5 ± 0.12$ ($n = 10$ rats). An excitatory sink–PS potentiation (E-S potentiation) was found with activation as compared with without activation, as revealed by the enhanced P1 at various levels of E1 in the group data (Fig. 3B3) and in the specific example (Fig. 4F).

**The Effect of PnO Activation on Direct and Polysynaptic Transmission of the MPP in the Hippocampus**

The entorhinal cortex projects directly to the distal apical dendrites of CA1 through the perforant path. In urethane-anesthetized rats, MPP stimulation evoked a sink at the CA1 distal dendrites (SLM; Leung et al. 1995), with an onset latency of 2.5 ms and a peak at 5.2 ms (inset of Fig. 5A). The distal sink was accompanied by current sources at stratum RAD (150–250 µm in Fig. 5A). The SLM sink showed paired-pulse facilitation (Leung et al. 1995) as indicated by the larger second-pulse response (in Fig. 5A, right column) as compared with the first-pulse response at an interpulse interval of 80 ms (Fig. 5A, left column). The distal dendritic sink and its associated source were not significantly changed by activation (Fig. 5A,B). The sink slope with activation was not significantly different from that without activation (activation ratio: 0.97 ± 0.03 [$n = 8$]; $P > 0.3$, paired Wilcoxon; Table 1).

The MPP provided major excitation at the middle molecular layer (MML) of the dentate gyrus (DG). The MPP to MML sink and the corresponding outer molecular layer source were not significantly affected by PnO activation (Fig. 5A). Similarly, the amplitude of the MPP-evoked PS at the granule cell layer (# in inset of Fig. 5B) was not consistently changed by PnO activation. However, the MPP-evoked polysynaptic sink in CA1 stratum RAD (* and ** after the first and second pulse, respectively, in Fig. 5A) and its associated source were greatly reduced by PnO activation. The polysynaptic, long latency (12–15-ms latency) stratum RAD sink in CA1 evoked by the first MPP stimulus pulse (* in Fig. 5) was decreased to 40 ± 10%
(n = 5) of the baseline slope during activation, while that evoked by the second MPP stimulus pulse (** in Fig. 5) was 67 ± 10% (n = 5) of the respective baseline slope.

**The Effect of Cholinergic Antagonists on PnO Modulation**

PnO activation effect was assessed by the activated ratio of E1 (or P1) with PnO activation to that without activation before and after drug injection. In rats given no drug other than those required for anesthesia, the activated ratio for basal and apical E1 (at intensity that evoked 50–75% of the maximal P1) were ~0.95 and ~0.84, respectively (Table 1). Activated ratio for basal and apical P1 were ~1.5 and ~0.53, respectively (Table 1), that is, basal P1 was increased while apical P1 was decreased by PnO activation.

Whether the effects of PnO activation were mediated by cholinergic receptors were tested by systemic administration of muscarinic receptor antagonists. The nonspecific muscarinic antagonist scopolamine (5 mg/kg i.p.) practically abolished the effects of activation on E1 and P1 following both apical and basal excitation. Scopolamine i.p. significantly increased the activation ratio of E1 and P1 for the evoked apical response in CA1 (Table 1). Similarly, scopolamine abolished the effect of activation on the basal dendritic response (Table 1A). The “increase” of the basal P1 and the “decrease” in the basal E1 (Table 1) during activation were both abolished by systemic scopolamine. Similarly, E-S potentiation after PnO activation (Fig. 3B3) was greatly reduced or abolished by systemic scopolamine (2 rats; data not shown).

The specific M1 receptor antagonist pirenzepine (40-μg icv), like systemic scopolamine, significantly reduced the effect of activation on apical E1 and basal E1 (Table 1B). Pirenzepine also significantly attenuated the decrease of apical P1 during activation (Table 1B) but only showed a trend in attenuating basal P1 increase during activation (Table 1B).

The M2 receptor antagonist AFDX-116 (21-μg icv) had no significant effect on the attenuation of E1 at the apical or basal synapses during activation (Table 1C). AFDX-116 icv also did not significantly change the magnitude of the excitatory sink E1 at either apical or basal dendritic synapses. However, icv AFDX-116 had a significant effect in “increasing” apical P1 and “decreasing” basal P1 without activation (both effects, P < 0.05, CA1 (Table 1)).
micropipette (Materials and Methods), and the effects of PnO activation were studied. At the apical synapse, the activation ratio of E1 before local application of scopolamine was 0.85 ± 0.01 (n = 5) that increased to 0.98 ± 0.02 (n = 5) after local scopolamine (P < 0.05, paired Wilcoxon). The activation ratio of P1 increased from 0.72 ± 0.10 (n = 5) to 1.13 ± 0.03 (n = 5) after local scopolamine (P < 0.05, paired Wilcoxon). After local scopolamine, PnO activation, as compared with no activation, increased the apical P1 (arrow in Fig. 6A), whereas PnO activation decreased apical P1 during baseline (Fig. 6A). Similarly, the E1 activation ratio at the basal dendritic synapse was increased from 0.94 ± 0.06 to 1.02 ± 0.09 (n = 3) after local scopolamine (P < 0.05, paired t-test, n = 3). After local scopolamine, the P1 activation ratio for the basal synapse was above unity (Fig. 6B). Basal P1 activation was not significantly changed before and after local scopolamine (1.62 ± 0.51 and 1.55 ± 0.21, respectively, both n = 3). In addition, the frequency and power of the theta rhythm during PnO activation were not significantly changed by local scopolamine (Fig. 6C).

**Relation between PnO-Activated Theta Rhythm and CA1 Synaptic Activation**

The result of locally applied scopolamine suggests that theta rhythm was controlled independently of the attenuation of apical P1. The relation between PnO-activated theta (frequency, power, or rise of the theta peak) and the magnitude of the apical P1 (or E1) in single sweeps was further studied by correlation in a large number of sweeps (Fig. 1B); the PnO stimulus intensity was fixed at 150 μA. There was no significant correlation (average correlation coefficient <0.02) between
any of the theta characteristics (frequency, power, or rise) with apical P1 (or E1) magnitude in 4 rats. In other rats ($n > 5$), PnO activation induced no detectable hippocampal theta rhythm, but apical P1 attenuation with activation was still observed. Similarly, there was no significant correlation between basal P1 or E1 magnitude and the PnO-activated theta frequency, power, or rise ($n = 4$ rats). When theta power and frequency varied greatly with different PnO stimulus intensities (25–300 µA), no consistent correlation between theta characteristics (frequency, power, or rise) and apical P1 magnitude was found in 4 rats.

**CCH-Attenuated Basal Dendritic EPSP Less Than Apical Dendritic EPSP In Vitro**

In order to further elucidate whether the same concentration of Ach acted directly in the hippocampus to alter basal and apical dendritic synaptic transmission, cholinergic agonist CCH ($10^{-5}$ to $10^{-3}$ M) was directly applied onto hippocampal slices in vitro. During baseline, stratum RAD stimulation evoked an iEPSP in CA1 pyramidal cells with an initial rising slope of $>2$ mV/ms at 1.5–2 $\times$ threshold intensity (Fig. 7A1; 30- and 40-µA traces, respectively), and the evoked spike was suppressed or delayed after CCH. However, CCH doses of $10^{-4}$ and $10^{-3}$ M attenuated the iEPSP significantly more at the apical than basal dendritic synapse (2-way ANOVA main effect: $F_{1,14} = 21.8$, $P < 0.001$, with a nonsignificant dose effect; Fig. 7C). Spiking suppression or delay was found for both RAD- and OR-evoked responses (Fig. 7A,B).

Evoked field potentials (pEPSPs) also showed a weaker attenuation by CCH on the OR-evoked response (Fig. 7B2) than the RAD-evoked response (Fig. 7A2). Group data of the attenuation of the OR-evoked and RAD-evoked pEPSPs were significantly different at both $10^{-5}$ and $10^{-4}$ M doses of CCH (2-way ANOVA main effect: $F_{1,13} = 15.5$, $P < 0.002$; dose effect: $F_{1,13} = 7.12$, $P < 0.02$; Fig. 7C). Evoked field responses derived from the same slice, following OR and RAD stimuli, were available before and after CCH perfusion. In 9 slices, $10^{-4}$ M dose of CCH induced a larger attenuation of the RAD-evoked field EPSP response than the OR-evoked field EPSP response (stimuli at 2 $\times$ threshold intensity), recorded either at OR ($n = 3$) or RAD ($n = 6$).

Individual slices or cells may demonstrate an increase in spike probability following OR stimulation (Fig. 7B2). However,
Cholinergic Control of Hippocampal Neural Processing

In CA1 area of urethane-anesthetized rat, PnO activation of the hippocampus resulted in a 4–7.5 Hz hippocampal theta rhythm, accompanied by a decrease in apical dendritic excitatory sink and PS but with an increase in basal dendritic PS and a small attenuation of the basal excitatory sink (Table 1). Administration of scopolamine locally in the hippocampus eliminated the activation-induced attenuation of the excitatory sink but did not block the facilitation of the basal PS during PnO activation. The PnO-activated hippocampal theta rhythm was also not blocked by scopolamine administration into hippocampal CA1 area. However, hippocampal theta was blocked by systemic scopolamine. Scopolamine administration to sites outside of the hippocampus, like the medial septum (Stewart and Fox 1989; Givens and Olton 1990) and the posterior hypothalamus (Blond et al. 1994), was shown to block hippocampal theta rhythm. While we have referred to PnO-activated state as the theta state, the PnO-activated synaptic effects are independent of the theta rhythm. Local scopolamine administration into the hippocampus attenuated the effects of PnO activation on excitatory sink attenuation but not theta rhythm generation. The magnitude of apical or basal P1 (or E1) was not correlated with theta power or frequency induced by a fixed PnO stimulus intensity. Jiang and Khanna (2004) had reported other independent properties of apical P1 attenuation and the theta rhythm under urethane anesthesia.

PnO activation in a urethane-anesthetized rat reproduced many features of hippocampal activation in the behaving rat, including a cholinergically mediated hippocampal theta rhythm and a decrease in the apical dendritic pEPSP and PS in CA1 (see Introduction). The report here shows definitively that the basal dendritic pEPSP and excitatory sink in CA1 were less strongly modulated by activation in the urethane-anesthetized rat, in part because of a lack of cholinergic presynaptic inhibition. The present results also resemble those of a reduced pEPSP and enhanced PS following septal stimulation in the urethane-anesthetized rats (Krnjević and Ropot 1982; Rovira, Ben Ari, and Cherubini 1983; Rovira, Ben Ari, Cherubini, Krnjevic, and Ropot 1983). On the other hand, icv physostigmine was reported to increase both basal and apical PSs without affecting the pEPSP (Ovsepian 2008), different from the effect of endogenous release of Ach by PnO or medial septal stimulation.

Presynaptic Inhibition of Excitatory Synaptic Transmission

This study provides original data that the 2 different subfields—stratum OR and stratum RAD—that receive from the Schaffer collaterals are modulated differently by endogenous Ach (Fig. 8). As a consequence, in vivo spiking was attenuated at the apical synapses but enhanced at the basal synapses. In addition, the distal dendritic or SLM excitatory sink was not modulated by endogenous release of Ach (Fig. 8). A decrease in a dendritic excitatory sink following PnO activation is interpreted to result from a decrease in presynaptic release of glutamate, mediated by a decreased presynaptic Ca2+ influx (Qian and Saggau 1997). The differential cholinergic inhibition of dendritic synapses in hippocampus is only partly predicted by the innervation pattern of cholinergic fibers (Aznavour et al. 2002). The relative lack of cholinergic immunoreactive fibers in the SLM (Aznavour et al. 2002) suggests a lack of cholinergic modulation of distal dendritic excitation. However, cholinergic immunoreactive fibers were of higher density in stratum OR than in stratum RAD (Aznavour et al. 2002), not corresponding

Discussion

as a group, no differential modulation of OR- and RAD-evoked spiking (single cell or PS) by CCH was found.

Figure 7. CCH perfusion in vitro decreased apical dendritic EPSPs more than basal dendritic EPSPs of CA1 pyramidal cells. (A) Intracellular recording in the soma of a CA1 pyramidal cell (column 1; threshold intensity: 20 μA) and extracellular field recording at the CA1 stratum (str.) RAD (column 2; slice threshold intensity: 15 μA) following apical dendritic (str. RAD) stimulation in a hippocampal slice in vitro. At 10–12 min after CCH perfusion, a large decrease in the rising slope (arrow) of the iEPSPs (column 1) and population EPSPs (column 2) was observed. CCH suppressed single-spike firing at 30-μA stimulation and reduced spike doublet evoked by 80-μA stimulation to a single spike (abolished the second slow spike). (B) Intracellular and field recordings following basal dendritic (str. OR) stimulation before (baseline) and after CCH. CCH slightly reduced the rise of the iEPSP (B1) after 30-μA stimulation but did not decrease the rise of the average EPSP (W = 4 sweeps) after 40-μA stimulation (Inset shows vertically expanded trace). (B2) Field EPSP evoked by str. OR stimulation recorded at str. RAD shows a positive phase that changed little with CCH and the emergence of a PS (*). (C) Summary of dose-response (percent baseline) of the intracellular (‘cell’) and extracellular (field) EPSP to various doses of CCH perfused for 10–12 min. Mean and standard error of the mean are shown with number of cells/slices.
to the higher sensitivity of the apical as compared with basal dendritic sink to cholinergic modulation.

Direct perfusion of the hippocampal slice in vitro also gave the result that the EPSP, extracellulary or intracellularly recorded, was more attenuated by CCH at the apical as compared with the basal dendritic synapses. It has long been known that the apical EPSPs in CA1 neurons in vitro were attenuated by CCH/Ach perfusion (Hounsgaard 1978; Valentino and Dingledine 1979; Fernández de Sevilla and Buño 2003; Kremin et al. 2006) but comparison with the basal EPSPs has not been done previously. The CCH-induced decrease in EPSPs in vitro was not accompanied by a decrease of the presynaptic volley and was not compensated completely by restoration of the resting membrane potential (Valentino and Dingledine 1979; Yim and Leung 1988). In the present study, the slope measurement of the intracellular and extracellular EPSP (E1) at <3 ms from the onset minimized the contribution of the evoked inhibitory postsynaptic conductance change (Poon et al. 2006). The attenuation of E1 in vivo was found over a large range of stimulus intensities (Figs 2D and 4D). In accordance with previous studies, the attenuation of EPSP slope by CCH in vitro was interpreted as a presynaptic inhibition effect, and in this case, differentially larger at the apical as compared with the basal excitatory synapses. Post-synaptic membrane changes (such as depolarization) may contribute a minor share to the EPSP attenuation by CCH, although there is no evidence that depolarization can mediate differential effects at basal versus apical postsynaptic membrane. The in vitro data suggest that the same concentration of CCH, and by inference Ach in vivo, may presynaptically modulate the apical and basal excitatory synapses differently.

Presynaptic cholinergic inhibition of glutamatergic synapses in CA1 was not found for the MPP synapses at the distal apical dendrites in vivo, extending the results of CCH perfusion in vitro (Hasselman and Schnell 1994). Direct stimulation of the MPP arising from the entorhinal cortex in vivo, as reported here, avoided the likelihood that other fibers were stimulated, such as those arising from nucleus reuniens that traveled in the SLM (Dolleman-Van der Weel et al. 1997) together with the entorhinal cortical afferents. Endogenous release of Ach in vivo also avoided the use of nonphysiological activation by CCH in vitro. Both synaptic and nonsynaptic cholinergic receptors will be activated by CCH for a long duration of time in vitro as compared with physiologically released Ach in vivo. In contrast to the lack of PnO modulation of the direct MPP to CA1 synapses in SLM, the polysynaptic MPP to CA1 response at the midapical dendrites was greatly attenuated by PnO activation (Fig. 54), and this attenuation was blocked by 5 mg/kg i.p. scopolamine (data not shown). A similar attenuation of the polysynaptic MPP to CA1 response at the midapical dendrites during walking as compared with immobility was shown in the behaving rat (Buzsáki et al. 1983). Cholinergic presynaptic inhibition of the proximal apical (CA3 to CA1) synapses in CA1 is a major reason for the attenuation of the polysynaptic MPP to CA1 response during activation. PnO activation also attenuated the DG to CA3 (mossy fiber) PS output (data not shown) but not the PS output of the DG neurons during MPP stimulation (Fig. 5B).

**Presynaptic Control and Excitatory Sink-PS (E-S) Coupling**

At the basal dendritic synapse, PnO activation enhanced the first-pulse PS (P1) while it slightly decreased the excitatory synaptic current (E1). Thus, basal dendritic E-S coupling, or P1 at a fixed E1 amplitude, was enhanced by PnO activation (Fig. 3B3). No significant change in E-S coupling was found at the apical dendrites in a group of rats (Fig. 3A3).

Basal dendritic E-S coupling was attenuated by systemic and local scopolamine but not by icv AFDX-116, suggesting that M1 but not M2 receptors are critically responsible for the E-S potentiation during PnO activation. Cholinergic depolarization of the pyramidal cells by blockade of different K+ currents, I_k, enhancement of cation channels (Dutar and Nicoll 1988; Fisahn et al. 2002), and decrease of inhibition (Yim and Leung 1988; Behrends and ten Bruggencate 1993) would tend to increase spiking. However, no enhancement of spiking was found with CCH perfusion in vitro (Fig. 7), which was also found to suppress depolarizing current induced spiking at a fixed membrane potential (Yim and Leung 1988). We suggest that a noncholinergic mechanism in the hippocampus in vivo may be involved in the facilitation of a PS (at a fixed stimulus intensity) by PnO activation, and the latter was still observed during hippocampal administration of scopolamine, whether following apical or basal excitation. Nicotinic antagonists icv was also without effect on spike facilitation during PnO activation. A likely mechanism is that PnO stimulation activated septohippocampal GABAergic neurons that inhibit the hippocampal inhibitory interneurons and provide disinhibition of pyramidal cells (Freund and Buzsáki 1996; Toth et al. 1997). We also found that the PS facilitation effect of PnO activation was blocked by systemic scopolamine or icv pirenzepine (Tables 1 and 2), suggesting that systemic muscarinic antagonist blocked PS facilitation, and one possible site was to block the muscarinic receptors on septohippocampal GABAergic neurons (Wu et al. 2000).

We reported that AFDX-116 icv resulted in an increase in the apical P1 but a decrease in the basal P1 (during no-activation), without a significant change in excitatory sinks. The latter results suggest differential control of the tonic state of evoked spiking by M2 receptors. The basal PS originated near the basal...
Muscarinic Receptors Mediating PnO Activation Effects

Presynaptic inhibition of glutamate release in CA1 depends critically on M1 receptors (Tables 1 and 2), as indicated by the effect of scopolamine, local or systemic, and the specific M1 receptor antagonist pirenzepine icv, at both basal and proximal apical dendrites (Fig. 8). The involvement of M1 receptors in presynaptic glutamatergic transmission is in agreement with previous in vitro results using pirenzepine (Sheridan and Sutor 1990) or M1 knockout mice (Kremin et al. 2006). M1 receptors were also implicated in long-term synaptic plasticity (Marino et al. 1998; Shinoe et al. 2005; Doralp and Leung 2008). The lack of hippocampal M1 presynaptic receptors and M1 receptor modulation of synaptic plasticity may be responsible for the cognitive deficits and hyperactivity in M1 knockout mice (Miyakawa et al. 2001; Anagnostaras et al. 2003).

M2 receptors were inferred to be not critical for presynaptic inhibition of glutamatergic synapse, since M2 antagonists AFDX-116 and methochramine increased apical pEPSP slope and presumably apical P1.

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M2 receptors were inferred to be not critical for presynaptic inhibition of glutamatergic synapse, since M2 antagonists AFDX-116 and methochramine did not significantly block the PnO-activated attenuation of the excitatory sink (Tables 1 and 2). M2 receptor antagonist AFDX-116 was effective in modulating the tonic spiking probability at basal and apical synapses, and it also blocked the recurrent inhibition mediated by CA1 pyramidal cell firing and increased P2 amplitude at a given P1 amplitude (data not shown; see similar data of Steffensen et al. 2006). Presynaptic inhibition of GABAergic terminals has been proposed to involve M2 receptors (Levey et al. 1995; Hájos et al. 1998; Fig. 8). No nicotinic effect on synaptic transmission could be demonstrated in the present study.

Based on studies of the neocortex and piriform cortex, Hasselmo (1999), Sarter et al. (2005) proposed that transmission of association fibers rather than extrinsic fibers are modulated by subcortical cholinergic inputs. Here, we showed that not all association fibers in the hippocampus are the same, as underscored by the relative lack of cholinergic attenuation of the basal as compared with the apical dendritic excitatory synapses in CA1.

Neural Processing Is Different between Apical and Basal Dendritic Synapses in CA1

It is known that different sets of CA3 neurons synapse on basal and apical synapses in CA1 (Ishizuka et al. 1990; Li et al. 1993).

The existence of basal and apical synaptic transmission with different properties has been suggested to provide a higher sensitivity and diversity for synaptic plasticity in CA1 (Leung and Shen 1995). In addition, the present study suggests the significance of differential dendritic processing in CA1 area during different behaviors, namely theta versus nontheta states. At the midapical (Schaffer collateral) synapses, single-pulse apical spike transmission is high during a nontheta state (without PnO activation), and CA1 pyramidal cell firing is determined by relatively strongly activated synapses. In contrast, during a theta state (PnO activation), single-pulse apical spike transmission is relatively weak, and temporal integration of many apical afferents onto a CA1 single neuron will decide the spike output.

Transmission at the CA1 basal dendrites appears to follow another agenda in being stronger during a theta state than a nontheta state. A corollary of enhancing basal dendritic spiking is facilitation of basal dendritic long-term potentiation (LTP) during a theta state (Leung et al. 2003; Owsepiyan et al. 2004; Doralp and Leung 2008). Low-threshold LTP at the basal dendrites (Leung et al., 1992; Arai et al. 1994; Leung and Shen 1995) may serve a special function in the plasticity of place cells (Muller et al. 1996; Dragoi et al. 2003; Brazhnik et al. 2003, 2004) or in state-dependent spatial memory (Buzsáki 1989). Spatial exploration, a theta-state behavior, is important for the establishment of place fields and spatial memory, and basal dendritic LTP is suggested to provide a key component during the active exploration. In contrast, the higher threshold apical dendritic LTP may serve to provide an off-line consolidation during sleep or other nontheta behaviors (Buzsáki 1989).


