Regulation of the activity of hippocampal stratum oriens interneurons by alpha 7 nicotinic acetylcholine receptors

Amber V. Buhler
Pacific University

Tom V. Dunwiddie

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Abstract
GABAergic interneurons have been shown to be a major target of cholinergic inputs to the hippocampus. Because these interneurons project to pyramidal neurons as well as other interneurons, activation of the cholinergic system is likely to produce a complex modulation of local inhibitory activity. To better understand the role of postsynaptic alpha 7 nicotinic acetylcholine receptors in the hippocampus, we have characterized the effects of nicotinic agents on local interneurons of the rat CA1 stratum oriens in terms of activation, desensitization, and region of axonal termination. Fast application of acetylcholine onto stratum oriens interneurons during whole-cell recordings from hippocampal slices activated the majority of cells tested, and these responses were mediated almost entirely by alpha 7 nicotinic acetylcholine receptors. Anatomical reconstructions showed no clear relationship between the acetylcholine responsivity of interneurons and the regions to which their axons project. Currents mediated by a7 receptors declined markedly during repetitive activation in the theta rhythm range (4-12 Hz) when activated by either pressure application or synaptic release of acetylcholine. However, the decay of alpha 7 receptor-mediated currents was unaffected by treatment with the cholinesterase inhibitor neostigmine (10 nM-10 μM), suggesting that hydrolysis of acetylcholine is not a rate-limiting step in the termination of these responses. From these findings we suggest that nicotinic receptor activity in this region has an extensive and complex impact on local inhibitory circuits that is mediated by activation of several classes of intrinsic GABAergic cells. In addition, desensitization of the a7 nicotinic acetylcholine receptor is likely to contribute to the decay of individual responses to pressure application of agonist, and may also act in a cumulative fashion to impair the ability of these receptors to support repetitive activity during trains of activation. If applicable to alpha 7 receptor responses in vivo, we suggest it may be difficult to enhance these responses for therapeutic purposes with cholinesterase inhibitors.

Keywords
Acetylcholine, alpha bungarotoxin, axons, desensitization, GABA, neurons

Disciplines
Molecular and Cellular Neuroscience | Pharmacy and Pharmaceutical Sciences | Systems Neuroscience

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REGULATION OF THE ACTIVITY OF HIPPOCAMPAL STRATUM ORIENS 
INTERNEURONS BY $\alpha 7$ NICOTINIC ACETYLCHOLINE RECEPTORS

A. V. BUHLER** and T. V. DUNWIDDIEa,b,c

*Department of Pharmacology, University of Colorado Health Sciences Center, P.O. Box C-236, 4200 East 9th Avenue, Denver, CO 80262, USA

bNeuroscience Program, University of Colorado Health Sciences Center, Denver, CO, USA

cDepartment of Veterans Affairs Medical Research Service, Denver, CO, USA

Abstract—GABAergic interneurons have been shown to be a major target of cholinergic inputs to the hippocampus. Because these interneurons project to pyramidal neurons as well as other interneurons, activation of the cholinergic system is likely to produce a complex modulation of local inhibitory activity. To better understand the role of post-synaptic $\alpha 7$ nicotinic acetylcholine receptors in the hippocampus, we have characterized the effects of nicotinic agents on local interneurons of the rat CA1 stratum oriens in terms of activation, desensitization, and region of axonal termination. Fast application of acetylcholine onto stratum oriens interneurons during whole-cell recordings from hippocampal slices activated the majority of cells tested, and these responses were mediated almost entirely by $\alpha 7$ nicotinic acetylcholine receptors. Anatomical reconstructions showed no clear relationship between the acetylcholine responsivity of interneurons and the regions to which their axons project. Currents mediated by $\alpha 7$ receptors declined markedly during repetitive activation in thetheta rhythm range (4-12 Hz) when activated by either pressure application or synaptic release of acetylcholine. However, the decay of $\alpha 7$ receptor-mediated currents was unaffected by treatment with the cholinesterase inhibitor neostigmine (10 nM-10 $\mu$M), suggesting that hydrolysis of acetylcholine is not a rate-limiting step in the termination of these responses.

From these findings we suggest that nicotinic receptor activity in this region has an extensive and complex impact on local inhibitory circuits that is mediated by activation of several classes of intrinsic GABAergic cells. In addition, desensitization of the $\alpha 7$ nicotinic acetylcholine receptor is likely to contribute to the decay of individual responses to pressure application of agonist, and may also act in a cumulative fashion to impair the ability of these receptors to support repetitive activity during trains of activation. If applicable to $\alpha 7$ receptor responses in vivo, we suggest it may be difficult to enhance these responses for therapeutic purposes with cholinesterase inhibitors. ß 2001 IBRO. Published by Elsevier Science Ltd.

Key words: $\alpha$-bungarotoxin, GABA, acetylcholinesterase, theta rhythm, desensitization, synaptic.

Nicotinic acetylcholine receptors (nAChRs) in the hippocampus play important roles in regulating the activity of this brain region, and have been implicated in diverse phenomena such as auditory gating deficits in schizophrenia (Freedman et al., 1994), modulation of attention, learning, and memory (Hindmarch and Sherwood, 1995; Newhouse et al., 1995), and dysfunction in Alzheimer's disease (Guan et al., 2000; Wang et al., 2000). GABAergic inhibitory interneurons of the hippocampus, which are major targets of septal cholinergic fibers (Frotscher and Leranth, 1985; Leranth and Frotscher, 1987), have been shown to be synthetically activated by nicotinic receptors (Frazier et al., 1998a; Alkondon et al., 1998). These interneurons play a central role in the regulation of hippocampal activity, and are involved in the generation and maintenance of behaviorally relevant rhythmic activity states such as theta rhythm (Buckmaster and Soltész, 1996; Buzsáki and Chrobak, 1995; Cobb et al., 1995; Stewart and Fox, 1990), which has been shown to be affected by both nicotinic (Cobb et al., 1999) as well as muscarinic agents (Stewart and Fox, 1990).

Hippocampal interneurons are a highly heterogeneous population, and can be subdivided into many distinct subtypes based on morphological characteristics including cell body location and axonal projections, on neurochemical markers, or on physiological characteristics (Freund and Buzsáki, 1996; Parra et al., 1998) with each subtype likely playing a unique functional role in the hippocampal circuitry. These subtypes can be...
broadly grouped into cells responsible for four distinct types of inhibition: (1) interneurons that activate postsynaptic GABA_A receptors on the somata of pyramidal cells, (2) interneurons that activate postsynaptic GABA_A receptors on the dendrites of pyramidal cells, (3) interneurons that activate postsynaptic GABA_A receptors predominantly on other interneurons (causing disinhibition of pyramidal neurons), and (4) interneurons that activate postsynaptic GABA_A receptors on pyramidal cells. The effect of nicotinic activation in the hippocampus will depend entirely on which of these functional subgroups are activated; therefore, a detailed characterization of the effect of nicotinic activation of specific interneuronal subtypes is crucial to understanding normal hippocampal function, as well as the pathological consequences of alterations of nicotinic systems in the hippocampus.

One particular subtype of hippocampal nAChR that has been the subject of considerable interest is that containing the α7 subunit. Several studies have characterized the pharmacology and kinetics of α7 nAChR-mediated responses in interneurons in the stratum radiatum subregion of CA1, either in response to exogenously applied acetylcholine (ACh; Frazier et al., 1998b; Alkondon et al., 1999; Jones and Yakel, 1997), or to stimulation of endogenous cholinergic fibers (Frazier et al., 1998a; Alkondon et al., 1998). However, it is still not clear if there are anatomically distinct interneuronal subtypes that are not activated by nAChRs. Although most studies have focused exclusively on interneurons with cell bodies in the stratum radiatum, McQuiston and Madison (1999) reported nicotinic responses in interneurons in all major subregions of the CA1. This study categorized interneurons by examining axonal projection in addition to cell body location, and suggested that ACh-responsive interneurons project almost exclusively to dendritic regions of CA1, whereas ACh-insensitive interneurons project primarily to the cell body regions (McQuiston and Madison, 1999). As these two anatomical subtypes of interneuron are believed to produce functionally distinct forms of inhibition (see Discussion), a difference in nicotinic sensitivity would suggest a very specific role for the α7 nAChR in inhibition in this region. In addition, McQuiston and Madison showed that a large fraction (63%) of stratum oriens interneurons exhibited both fast (α7) and slow (non-α7) nicotinic responses whereas stratum radiatum interneurons only exhibited fast α7 nAChR-mediated currents (McQuiston and Madison, 1999; Frazier et al., 1998b). Therefore, a major goal of these studies was to identify the types of nAChR currents seen in stratum oriens interneurons, and to test the relationship between ACh responsibility and region of axonal projection.

It is also unclear what factors limit α7 nAChR responses in the hippocampus. A notable aspect of α7 nAChRs is their rapid desensitization, which could have substantial impact on their response to repetitive activity of their presynaptic afferents, such as during theta activity in the hippocampus. Septal neurons, including the cholinergic neurons, fire in vivo at theta frequencies (4–12 Hz; Brazhnik and Fox, 1997; Stewart and Fox, 1990), but whether synaptic α7 nAChRs desensitize at these frequencies of stimulation is unknown. Another factor that could significantly influence transmission at these synapses is the rate at which synthetically released ACh is degraded by acetylcholinesterase (Cooper et al., 1991). Although acetylcholinesterase is quite important in limiting ACh action at the neuromuscular junction (Taylor, 1985), its role at α7 synapses, where choline may also be an agonist (Mike et al., 2000) and the receptor is subject to such rapid desensitization (Couturier et al., 1990), is less clear. Therefore, the second major goal of this study was to determine whether inhibition of ACh breakdown could enhance α7 nAChR-mediated responses, or whether rapid desensitization of this receptor limits any prolongation of these currents. The third major goal of this work was to determine whether receptor desensitization or inactivation may limit the contribution of α7 nAChRs to any nicotinic component of hippocampal theta rhythm.

**EXPERIMENTAL PROCEDURES**

**Whole-cell recording**

Housing and treatment of all animals were in accordance with protocols approved by the University of Colorado Animal Care and Use Committee, and were designed to minimize any animal suffering as well as the number of animals used in this study. Unanesthetized young (18–27 day old) male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA, USA) were decapitated and 300 μM thick coronal slices were made of their hippocampi using a Vibratome (Pelco, Tred Pella, Redding, CA, USA). Slices were incubated either at room temperature or 32°C in oxygenated artificial cerebral spinal fluid consisting of (in mM): 124 NaCl, 3.3 KCl, 2.5 CaCl_2, 1.2 KH_2PO_4, and 25.9 NaHCO_3 for at least 1 h before recordings were performed. Recordings were made at room temperature while superfusing tissue with oxygenated artificial cerebral spinal fluid at a rate of 2 ml/min. A Flaming/Brown electrode puller (Sutter Instrument, Novato, CA, USA) was used to pull 6–9 MΩ whole-cell patch pipettes and 2 μm inner diameter drug application pipettes. Recordings were made with a potassium glutocnate recording solution containing (in mM): 130 K-glucuronate, 1 EGTA, 2 MgCl_2, 0.1–0.5 CaCl_2, 2.54 disodium ATP, and 10 HEPES (free acid) adjusted to pH 7.3 with KOH with 0.5% bicytin added in some cases. Hippocampal interneurons were visualized with an upright microscope utilizing differential interference contrast (DIC) optics. Interneurons were identified visually by their location outside of the pyramidal neuron layer, and electrophysiologically by their resting membrane potential, short spike duration, and short interspike interval (Frazier et al., 1998b). In some cases anatomical reconstruction of bicytin-filled cells enabled further categorization of the type of interneuron from which recordings were made. Evoked synaptic responses were obtained by stimulation of the stratum oriens layer through a twisted bipolar stimulating electrode made from 0.0026-inch Formvar-coated nichrome wire. Agonist application was achieved by pressure application of 600 μM–1 mM ACh through a drug application pipette with a Picospritzer (General Valve, Fairfield, NJ, USA) at pressures of 10 psi and durations of 3–10 ms directly (within 10 μm) onto the cell body of the interneuron. When a KCl solution was applied to cells in an identical fashion, inward currents were elicited with a 10-90% rise time of 10.5 ms, and a time constant of decay of 69 ms. Because of the effects of acetylcholinesterase, the period during which receptors are exposed to ACh would be expected to be even shorter. Repeated responses to pressure application of KCl at short...
interpulse intervals (80 ms or greater) were of nearly identical amplitude, indicating that the pressure application system ejected similar amounts of solution even with short cycle times. Evoked action potentials and voltage clamp currents were recorded with an AxoClamp 2A amplifier (Axon Instruments, Foster City, CA, USA). Bath application of other drugs was achieved by addition of these agents to the perfusion medium through syringe pumps (Razel Scientific Instruments, Stamford, CT, USA). Responses were acquired on a microcomputer using NeuroPro software (RC Electronics, Stampfort, PA, USA) or in MiniAnalysis (Synaptosoft, NJ, USA). After-hyperpolarizations were measured as described and illustrated in Lacaille and Williams (1990): the amplitude of the fast after-hyperpolarization, depolarizing after-potential, and medium after-hyperpolarization were measured following a single spontaneous action potential, while the amplitude of the slow after-hyperpolarization was measured following a train of action potentials stimulated by a 500 pA/50 ms depolarizing pulse. Firing frequency was measured during a 500 pA/250 ms depolarizing pulse and expressed as spikes per 50 ms rather than frequency so that cells that spiked only once during this period of time could be included. Spike frequency adaptation (accommodation) was measured as a ratio of the number of spikes during the last 50 ms to the first 50 ms during a 500 pA/250 ms depolarizing pulse, and sag ratio was measured as the ratio of the amplitude of the response to hyperpolarization at the peak (at about 75 ms) to the end (at 225 ms) of a 500 pA/250 ms hyperpolarizing pulse in current clamp mode (Lacaille and Williams, 1990). Bi-exponential decay curves were fit with the equation $I(t) = I_{a1} \exp(-t/t_{a1}) + I_{a2} \exp(-t/t_{a2})$. Mean values are reported ± S.E.M. Statistical tests performed included Student’s t-test, analysis of variance (ANOVA), and when appropriate for post-hoc analysis, the Tukey-Kramer multiple comparisons test. All tests were deemed significant if $P < 0.05$.

Anatomical reconstruction

During whole-cell recording cells were passively filled with 0.5% biocytin through the recording pipette for between 40 min and 2 h. Tissue was then immediately placed in chilled 4% paraformaldehyde and stored until later processing. After 24 h rinse in Tris-buffered saline (TBS), tissue was incubated for 1 h in TBS-X (TBS and 0.5% Triton X-100) and 0.1% streptavidin-cy3 (Jackson Laboratories, Westgrove, PA, USA). After one 15 min rinse in TBS, slices were mounted using Vectashield (Vector Laboratories, Burlington, CA, USA) fade-resistant medium and visualized with a Nikon PCM 2000 confocal microscope equipped with epifluorescence. Images were acquired and stored utilizing a confocal imaging program Simple PCI (Compix, PA, USA).

Drugs

Chemicals were obtained from the following sources: ACh, methyllycaconitine (MLA), α-bungarotoxin (BTX), nicotine, mecamylamine (MEC), dihydro-β-erythroidine (DHβE), choline, neostigmine, atropine, 1αH, 3α, 5αH-tropan-3-yl-3,5-dichlorobenzoate (MDL 72222), bicuculline methiodide (BMI), 6,7-dinitroquinoxaline-2,3(1H,4H)-dione (DNQX), suramin, n,(-)-2-amino-5-phosphonovaleric acid (APV), and biocytin were obtained from either Sigma (St. Louis, MO, USA) or Research Biochemicals (Natick, MA, USA).

RESULTS

Stratum oriens interneurons respond to ACh application with nAChR-mediated responses

Brief pressure application of ACh to the cell bodies of visually identified interneurons in stratum oriens elicited fast inward currents in 49 out of 61 cells tested under voltage clamp conditions. These responses were generally of large amplitude ($-243 ± 30$ pA; $n = 49$), decayed rapidly (Fig. 1A–D), and were uniformly blocked by bath

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**Fig. 1. Nicotinic currents evoked by pressure application of ACh.** (A–D) Averaged responses (six to eight sweeps) from four cells showing fast (α7) currents evoked by local application of 1 mM ACh. Scale is 40 pA by 100 ms for all. Arrows indicate time of ACh application. (E) Averaged responses (four to six sweeps) from a single cell of fast α7 nAChR-mediated currents evoked by pressure application of 1 mM ACh during a control period, during bath application of 150 nM DHβE, and after addition of 75 nM MLA. (F) Averaged responses (three to four sweeps) from a single cell illustrating a mixed fast α7 and slow non-α7 nAChR current evoked by pressure application of 1 mM ACh during a control period, during bath application of 1 μM MEC, and after addition of 20 nM MLA. Scale is 20 pA by 100 ms for E and F. Currents shown in A–D decayed with bi-exponential kinetics with $\tau_1$ ranging from 13 to 30 ms, and $\tau_2$ ranging from 170 to 250 ms.
application of the \( \alpha_7 \) nAChR selective antagonists MLA (75 nM; 95 ± 3% block; \( n = 9 \)) or BTX (100 nM; 88 ± 5% block; \( n = 5 \)). Antagonists selective for non-\( \alpha_7 \) nAChRs did not significantly inhibit these fast ACh responses; neither 150 nM DHβE (99 ± 1% of control; \( n = 3 \)) nor 1 \( \mu \)M MEC (108 ± 1% of control; \( n = 3 \)) significantly inhibited these fast ACh responses, although in every case the same responses were subsequently blocked by MLA (\( n = 4 \)) or BTX (\( n = 2 \); e.g. Fig. 1E). This pharmacological profile is consistent with that expected for nicotinic receptors containing an \( \alpha_7 \) subunit (Albuquerque et al., 1995). An additional, smaller (≈18 pA), more slowly decaying response was seen in 1/61 cells (Fig. 1F), similar to non-\( \alpha_7 \) nAChR nicotinic currents previously reported (Jones and Yakel, 1997; Alkondon and Albuquerque, 1993; McQuiston and Madison, 1999; Alkondon et al., 1999). This slow, MLA-insensitive response was inhibited by 80% by 1 \( \mu \)M MEC (Fig. 1F), suggesting that this response was mediated by a nicotinic receptor not containing the \( \alpha_7 \) subunit.

As has been demonstrated by Alkondon et al. (1997), \( \alpha_7 \) currents evoked by ACh were inhibited markedly by superfusion with 200 \( \mu \)M choline (99 ± 1% block, \( n = 4 \)), which acts as a desensitizing agonist. As we have reported previously for stratum radiatum interneurons, bath superfusion with low concentrations (1 \( \mu \)M) of nicotine also markedly inhibited fast responses to direct application of ACh (70 ± 8% block, \( n = 4 \)).

The ability of these fast \( \alpha_7 \) nAChR-mediated currents to induce firing was determined under current clamp conditions. In 21/21 ACh-responsive cells tested, action potentials were evoked by a brief application of ACh, and in 8/8 of these cells tested, 75 nM MLA or 100 nM BTX completely blocked the action potentials elicited by ACh pressure application.

**Identification of ACh-responsive and non-responsive interneurons in stratum oriens**

Because there are several distinct anatomical and functional subtypes of GABAergic interneurons in stratum oriens of the CA1 region (Parra et al., 1998; Freund and Buzsáki, 1996; Buckmaster and Soltesz, 1996), and because not all cells in this layer exhibited nicotinic
receptor-mediated currents, the morphology of these cells was compared to their ACh responsiveness to determine whether there were unique subclass(es) of non-responsive cells that could be differentiated based upon morphological or physiological criteria. Previous studies with a small number of reconstructed cells had suggested that interneurons with axons terminating near the soma or proximal axons of pyramidal cells (soma-tically targeted interneurons) might be unresponsive to nicotinic activation (McQuiston and Madison, 1999). To characterize the projections of ACh-responsive and non-responsive cells, a subset of interneurons were filled with biocytin and anatomically reconstructed following the physiological experiments. Of 15 interneurons located in stratum oriens in which successful and extensive fills were obtained, four cells showed no functional response to pressure application of ACh; of these, two had axonal fields that terminated within and along the pyramidal cell layer (consistent with basket/chandelier type somatically targeted interneurons), one was a bistratified cell, with the axon ramifying in both stratum oriens and radiatum but not pyramidalne, and one had terminations in all layers, consistent with the trilaminar cell type. The 11 reconstructed cells that exhibited fast nicotinic currents with ACh pressure application were also a heterogeneous group. Eight of these cells had axonal arborizations within and just adjacent to stratum pyramidale, consistent with somatically targeted interneurons (e.g. Fig. 2). Other ACh-responsive interneurons had axons that projected primarily to the dendritic regions of CA1. These included an O-LM cell, with axon arborization in the distal part of stratum radiatum and stratum lacunosum.

Table 1. Properties of ACh-responsive and non-responsive interneurons (mean ± S.E.M.)

<table>
<thead>
<tr>
<th></th>
<th>ACh-responsive</th>
<th>ACh non-responsive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cells</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Resting membrane potential (mV)</td>
<td>−50 ± 2</td>
<td>−55 ± 3</td>
</tr>
<tr>
<td>Spike amplitude (mV)</td>
<td>76 ± 5</td>
<td>71 ± 5</td>
</tr>
<tr>
<td>Spike width (ms)</td>
<td>4.2 ± 0.6</td>
<td>3.8 ± 0.7</td>
</tr>
<tr>
<td>Fast after-hyperpolarization (mV)</td>
<td>−11.7 ± 2.1</td>
<td>−14.6 ± 3.9</td>
</tr>
<tr>
<td>Depolarizing after-potential (mV)</td>
<td>0.5 ± 0.3*</td>
<td>1.9 ± 1.7*</td>
</tr>
<tr>
<td>Medium after-hyperpolarization (mV)</td>
<td>−9.4 ± 2.8*</td>
<td>−5.7 ± 2.4*</td>
</tr>
<tr>
<td>Spikes per 50 ms*</td>
<td>4.5 ± 0.2</td>
<td>5.8 ± 0.8</td>
</tr>
<tr>
<td>Spike frequency adaptation (%)**</td>
<td>52 ± 4</td>
<td>23 ± 5</td>
</tr>
<tr>
<td>Slow after-hyperpolarization (mV)</td>
<td>−7.4 ± 1.5</td>
<td>−6.8 ± 2.4</td>
</tr>
<tr>
<td>Sag ratio</td>
<td>0.80 ± 0.04</td>
<td>0.77 ± 0.09</td>
</tr>
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*a*Reflects data from the subset of cells exhibiting depolarizing after-potentials and medium after-hyperpolarizations; the percentage of these cells was similar in both ACh-responsive and non-responsive groups.

*P* < 0.05, Student’s *t*-test.

**P** < 0.005, Student’s *t*-test.

Fig. 3. Firing properties of ACh-responsive and ACh-unresponsive stratum oriens interneurons. (A, B) ACh-responsive interneurons (resting membrane potentials −71 mV and −67 mV) and (C) ACh-unresponsive interneuron (resting membrane potential −71 mV). At the top are shown individual examples of responses evoked by ACh recorded in current clamp (calibration is 25 mV and 100 ms). The middle records show averaged responses (six to eight sweeps) to ACh pressure application recorded in voltage clamp in the same cells (scale is 40 pA and 100 ms). Arrows denote the onset of ACh application (~5 ms/10 psi pressure application). The bottom records show action potentials evoked by a 250 ms/500 pA depolarizing pulse in the same cells (scale is 25 mV and 100 ms). During current clamp recordings cells were injected with sufficient hyperpolarizing current to inhibit spontaneous firing, usually requiring no more than a −4 mV change in membrane potential. The axonal terminations of all three cells were identified anatomically: (A) axon terminations in stratum pyramidale (cell shown in Fig. 2B), (B) axon terminations in stratum oriens, (C) bistratified terminations in stratum oriens and stratum radiatum.
moleculare, and two cells with axons which ramified horizontally within stratum oriens. The large percentage of basket/chandelier, somatically targeted interneurons that were observed is consistent with previous studies that suggest that these cells comprise a large percentage of the interneurons in the stratum oriens layer. For example, Sik et al. (1995) reported that 50% of their reconstructed stratum oriens cells were of this type. Additionally, the large cell bodies of these cells make them readily visible under DIC conditions, and thus it is possible that they may even be over-represented in our experiments.

A subset of responsive and non-responsive cells were also compared under current clamp conditions to determine whether they could be distinguished based upon their physiological properties. Several properties were measured (see Experimental procedures for more detail), including the resting membrane potential, action potential amplitude and width, the amplitude of the fast and medium after-hyperpolarizations and the depolarizing after-potential following a single spontaneous action potential. Slow after-hyperpolarizations were measured following a 50 ms train of action potentials, the firing frequency and spike frequency adaptation (accommodation) during a 250 ms depolarizing pulse, and the sag ratio determined during a 250 ms hyperpolarizing pulse. These properties were compared in 12 ACh-responsive and six non-responsive cells (Table 1 and Fig. 3). The only differences that were observed were that the ACh-responsive cells had slower initial firing frequencies (defined as the number of spikes in the initial 50 ms of the response to a depolarizing pulse; $P < 0.05$) and greater frequency adaptation during a 250 ms depolarizing pulse ($P < 0.005$): 12 out of 12 ACh-responsive cells showed >25% adaptation, whereas only two out of six non-responsive cells exhibited comparable adaptation. The difference in firing frequency was due to a subset of non-responsive cells that had especially fast firing rates (seven to eight spikes/50 ms) not seen in the ACh-responsive group; these were also the cells with the least spike frequency adaptation in the entire data set, possibly representing a physiologically distinct group. Anatomically identified basket/chandelier type cells were represented in both the ACh-responsive and non-responsive groups; the four cells of this anatomical type that responded to ACh all showed over 50% spike frequency adaptation, whereas the two cells of this type that did not respond to ACh both showed less than 25% adaptation.

Inhibition of acetylcholinesterase does not affect decay of α7 nAChR currents

There are multiple factors that could control the decay of α7 nAChR-mediated currents, including fast desensitization, rapid hydrolysis of ACh by acetylcholinesterase, and diffusion of ACh away from the site of application/release. To determine the role of catabolism in limiting the response to brief (3–6 ms) local application of ACh, we tested the effect of bath perfusion of an acetylcholinesterase inhibitor, neostigmine, on the decay of α7 nAChR currents. Neostigmine has been shown to have anticholinesterase activity in ventral tegmental area (VTA) and hippocampal brain slices, enhancing nicotinic (VTA) and muscarinic (VTA and hippocampus) currents at concentrations ranging from 100 nM to 10 μM (Calabresi et al., 1989; Zhang et al., 1997). Most of the present experiments were done in the presence of 5 μM neostigmine.
atropine to eliminate any effects mediated by muscarinic receptors. Like other quaternary acetylcholinesterase inhibitors, neostigmine also has direct actions on neuronal nAChRs in the micromolar range (Sherby et al., 1985; Clarke et al., 1994; Yost and Maestrone, 1994; Zheng et al., 1998; Nagata et al., 1997). A wide range of concentrations were investigated in order to clarify the effect of this agent both at low concentrations (10–100 nM) at which there would be sub-maximal inhibition of acetylcholinesterase, but no expected direct actions, as well as at higher concentrations (1–10 μM) at which near maximal (>98%) inhibition of cholinesterase activity should occur (Sherby et al., 1985), but where direct inhibition of the nicotinic receptor might occur. Overall, neostigmine had no significant effect on the amplitude of responses to local ACh application except at the highest concentration tested (ANOVA followed by post-hoc analysis: Tukey–Kramer multiple comparisons test P < 0.05); 10 nM neostigmine, 116 ± 16% of control, n = 4; 100 nM neostigmine, 82 ± 23%, n = 5; 1 μM neostigmine, 104 ± 7%, n = 3; 10 μM neostigmine, 64 ± 8%, n = 5. At no concentration was there any apparent change in the rate of decay of the ACh response (Fig. 4A). This was confirmed by fitting the decay phase of each response to a mono- or bi-exponential decay function (see Experimental procedures; Fig. 4B, C); neither t1 (fast) nor t2 (slow or single mono-exponential decay) was significantly affected by any concentration of neostigmine.

α7 nAChR-mediated nicotinic currents rapidly desensitize during ACh application or synaptic stimulation at theta frequencies

α7 nAChR-mediated responses are noted for rapid desensitization. To determine how desensitization of these receptors could affect successive responses, the effect of a train of four pressure applications of 1 mM ACh was examined at three frequencies that span the theta rhythm range (4, 7, and 12 Hz). Successive responses to pressure application of ACh were reduced at all three frequencies tested (Fig. 5A–C; Table 2). All three frequencies showed a significant reduction of relative current amplitude with successive responses (one-way ANOVA, P < 0.0001) beginning by the second response. Although desensitization was consistently observed in all cases, there was considerable variability between cells in terms of the amount of cumulative desensitization that occurred during trains of ACh application. In some instances the fourth pressure application produced an appreciable although decreased peak (Fig. 6A), while in other cases no measurable current was elicited (Fig. 6D, E). One possible explanation for these cell to cell differences is that there is a fast and a slow form of desensitization, and that each cell had variable amounts of each component. If we assumed that the time constants for these two types of desensitization were the same in all cells, the rate of decay of successive responses to repeated ACh application could be fit relatively accurately by simply adjusting the amount of each component in individual cells (Fig. 6A–E).

There are substantial differences between activation of synaptic receptors by exogenous vs. endogenous neurotransmitter, which can occur because of presynaptic factors, as well as changes in receptor desensitization that are dependent upon agonist concentration and duration of exposure. Therefore, it was of considerable interest to determine whether synthetically activated α7 nAChR responses showed similar patterns of depression during theta frequency train stimulation. Synthetic responses were evoked in interneurons by electrical activation of cholinergic fibers by stimulation in stratum oriens in the presence of blockers of non-nicotinic fast ligand gated ion-channels: MDL 72222 500 nM for 5HT1 receptors, suramin 100 μM for ATP receptors, BMI

### Table 2. Decrement of α7 nAChR-mediated currents during repetitive stimulation

<table>
<thead>
<tr>
<th>ACh pressure application</th>
<th>First response</th>
<th>Second response</th>
<th>Third response</th>
<th>Fourth response</th>
<th>Fifth response</th>
<th>n =</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 Hz</td>
<td>100%</td>
<td>24 ± 13%</td>
<td>12 ± 7%</td>
<td>7 ± 5%</td>
<td>n/a</td>
<td>4*</td>
</tr>
<tr>
<td>7 Hz</td>
<td>100%</td>
<td>37 ± 14%</td>
<td>13 ± 7%</td>
<td>7 ± 6%</td>
<td>n/a</td>
<td>4*</td>
</tr>
<tr>
<td>12 Hz</td>
<td>100%</td>
<td>39 ± 7%</td>
<td>10 ± 3%</td>
<td>4 ± 2%</td>
<td>n/a</td>
<td>4*</td>
</tr>
<tr>
<td>Synaptic stimulation</td>
<td>100%</td>
<td>64 ± 13%</td>
<td>23 ± 28%</td>
<td>15 ± 17%</td>
<td>10 ± 9%</td>
<td>2</td>
</tr>
</tbody>
</table>

*aOnly those cells in which all three frequencies were tested were included in this data set in order to avoid introducing differences that are attributable to cell to cell differences.

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**Fig. 5. Cumulative decline in α7 nAChR-mediated responses to repetitive ACh application.** Averaged inward currents (four to five sweeps) evoked from a single cell by pressure application of 1 mM ACh at (A) 4 Hz, (B) 7 Hz, and (C) 12 Hz. Arrows indicate time of ACh application, scale bar is 50 pA by 200 ms for all. In each case, the response to the last application of ACh was essentially undetectable.
20 μM for GABA\textsubscript{A} receptors, DNQX 20 μM and APV 40 μM for glutamate receptors. Two cells were found in which synaptic currents that were blocked by MLA could be evoked under these conditions. Both cells showed marked variability in the rate of decay. All cells were tested with 1 mM ACh applied in four pulse trains at 4 Hz. Despite this heterogeneity, all responses could be well fit by varying the amplitudes of the two components of the bi-exponential decay function \( y = (A_1) \exp(-x/140) + (A_2) \exp(-x/1400) \). The individual time constants \( \tau_1 = 140 \text{ ms}, \tau_2 = 1400 \text{ ms} \) were estimated by assuming that the two most extreme examples had only the slow (A) or only the fast (E) component of desensitization. Arrows indicate time of ACh application, scale bars equal 250 ms for all and 200 pA, 108 pA, 163 pA, 260 pA, and 75 pA for A–E. The percentage of the fast (A\textsubscript{1}) and slow (A\textsubscript{2}) components is given for each curve.

**DISCUSSION**

**Pharmacological characteristics of stratum oriens layer interneurons**

Although most previous studies of nicotinic responses in hippocampal interneurons have focused on those located in stratum radiatum, the interneurons found in stratum oriens include subtypes that play unique roles in regulating hippocampal activity (Buckmaster and Soltész, 1996; Freund and Buzsáki, 1996). Nevertheless, the present study has demonstrated that there are considerable similarities in the nicotinic responsiveness of
interneurons in the stratum oriens and stratum radiatum. Both the pharmacology and the kinetics of fast α7-mediated responses were similar in these regions, although the fraction of non-responsive cells was significantly greater in stratum oriens (Frazier et al., 1998b; McQuiston and Madison, 1999).

The fraction of stratum oriens cells responding with slow, non-α7-mediated responses was substantially lower in our studies than in previous work, which may reflect differences in the number and locus of ACh application. Additionally, agonist dissociation and receptor desensitization may have inadvertently led to our recording fewer cells with slow responses. Second, there were substantial differences in the protocol for agonist application in the two studies. The precise cellular distribution of α7 and non-α7 receptors is unclear (Alkondon and Albuquerque, 1993; Frazier et al., 1998b; Freedman et al., 1999). Different methods of applying ACh to interneurons in the slice preparation could readily activate physically and pharmacologically distinct receptor populations. U-tube application (Alkondon and Albuquerque, 1993) would be expected to activate all receptors on a cell regardless of location (Alkondon et al., 1999). Short application periods and lower concentrations of ACh, such as were used in this study (1 mM ACh, 3–10 ms applications within 10 μm of the cell body), would be expected to activate primarily somatic receptors, whereas ejection of larger amounts of ACh from a pressure pipette (3 mM ACh, 100 ms applications from a distance of approximately 30 μm) could lead to activation of additional receptor populations (McQuiston and Madison, 1999). The currents reported by McQuiston and Madison (1999) were ~three-fold larger than the currents that we recorded, which is consistent with the activation of a greater number of receptors under their experimental conditions. This difference in agonist application could explain the low numbers of non-α7 nAChR-mediated currents, as well as the larger percentage of ACh-unresponsive neurons seen in the present study.

Interneurons that project to somatic regions of hippocampal pyramidal neurons exhibit functional nicotinic receptor responses

From a functional standpoint, interneuron projections to pyramidal neurons are either dendritic, which are thought to generate rather weak GABA A receptor-mediated inhibitory post-synaptic potentials (IPSPs) in the cell body, but may play a significant role in controlling dendritic synaptic integration, or somatic, which generate large IPSPs that are likely to inhibit the initiation of action potentials. It has been proposed that ACh-sensitive interneurons project almost exclusively to dendritic subregions of CA1 (McQuiston and Madison, 1999). However, in the subset of stratum oriens interneurons that we examined, many ACh-responsive cells (8/11) project to the pyramidal cell bodies. Our results also suggest that there may be stratum oriens interneurons that project to dendritic regions but which are insensitive to ACh. However, this conclusion must be somewhat tentative, because some of our ‘insensitive’ cells might have responded to application of larger amounts of ACh. There is also evidence that there may be differences in the physiological characteristics of interneurons that correspond to their sensitivity to nicotinic agonists; Ji and Dani (2000) found that in stratum radiatum interneurons, ACh-sensitive neurons exhibited faster firing frequency, higher input resistance, and a larger fast after-hyperpolarization than did ACh-insensitive neurons. Although these cells were not identified morphologically, this suggests that there are physiologically distinct subsets of cells in the stratum radiatum that differ in their sensitivity to nicotinic activation. The present experiments also found physiological differences between ACh-responsive and non-responsive neurons; stratum oriens interneurons that were responsive to ACh exhibited significantly lower spike frequencies and greater adaptation than non-responsive cells, suggesting that the same criteria cannot be used in different CA1 sub-regions to differentiate between responsive and non-responsive cell types. In any case, it is clear from our studies that there are somatically projecting interneurons that are activated by α7 nAChRs, although the proportion of these cells that show such responses is uncertain. Even with the relatively small sample size in the present study, it is clear that most major classes of hippocampal interneurons express functional α7 receptors. Because nAChRs exert a powerful excitatory effect on such a large percentage and on so many varied subtypes of interneurons in the hippocampus (see Freund and Buzsaki, 1996, for review), it would seem likely that they produce a correspondingly complex effect on local inhibition.

Acetylcholinesterase, desensitization and the termination of α7 nAChR receptor currents

Despite a wealth of literature characterizing the single-channel properties of nAChRs, it is still unclear what mechanisms underlie the fast decay kinetics seen in synaptic-aptically activated or exogenously activated responses. At the neuromuscular junction, synaptic concentrations of ACh are thought to decline much more rapidly than does the end-plate potential (Ochoa et al., 1989), and it is believed that synaptic responses are terminated not by receptor desensitization, but by channel closure rate in the absence of ACh (Taylor, 1985; Colquhoun and Sakmann, 1998; Auerbach and Akk, 1998). The factors that could determine the time course of central α7 nAChR-mediated responses include ACh breakdown, diffusion away from the receptor, dissociation of bound agonist, and receptor desensitization. The present results demonstrate that inhibition of acetylcholinesterase has no effect on the time course or decay of currents evoked by fast ACh application, which suggests that the rate of hydrolysis of ACh does not determine the time course of these responses. Additionally, agonist dissociation and diffusion away from the receptors cannot by themselves...
account for the decay of single responses, because successive responses in a train would show no decrement if this were the case. The conclusion that dissociation and diffusion alone do not determine the decay of individual currents is further supported by unpublished data from our laboratory in which we have found that in some cells, the time course of an ACh response appears to be nearly independent of the duration of ACh application (P. Dobelis and T.V. Dunwiddie, unpublished observations). Similar results have been reported by Alkondon et al. (1997), who found that α7 nAChR-mediated currents activated by 1 s long pulses of ACh decayed with time courses similar to the responses shown here, which were activated by 3–10 ms long pulses. At this point, it is not possible to identify the factors that determine the rate of decay of individual α7-mediated responses, although it appears likely that desensitization plays a significant role.

Although fast desensitization appears to play a predominant role in the termination of single α7 nAChR responses, the mechanism of cumulative decay of these same responses is more complex. An analysis of the currents shown in Fig. 6A–E shows that the magnitude of successive responses can be fit reasonably well by a bi-exponential decay function. The two components of this function could reflect the entry of receptors into a fast desensitized state, and a second, slower onset component of receptor desensitization or inactivation, although there are many other possibilities, such as multiple populations of receptors. It is of note that during trains, the amplitude of the second response as a percent of the first becomes smaller as the time between responses is lengthened (Fig. 5); this observation suggests that receptors that are fully recovered and activatable at short intervals become inactivated at longer intervals. This could be explained several ways. One possible mechanism is that receptors continue to cycle through agonist bound states during the decay phase and accumulate into the (fast) desensitized state. A second possible mechanism is that there exists a slower onset non-conducting state that could be due to accumulation into a secondary (deep) desensitized or inactivated state of the receptor (Ochoa et al., 1989; Paradiso and Brehm, 1998; Fenster et al., 1999). The results illustrated in Fig. 6 demonstrate that there are substantial differences in the rate of cumulative depression in different cells, and that these differences can be qualitatively explained by the presence of fast and slow decaying components. We hypothesize that cells exhibiting rapid and extensive cumulative depression (Fig. 6D, E) are ones in which the fast component predominates, and that the decay of these individual responses is primarily attributable to desensitization, whereas in other cells (Fig. 6A, B) this is not the case. Whether this hypothesis is correct, and the extent to which the proposed rapid and slow components of decay can be independently regulated in the same cell are interesting issues that must be resolved by future experiments. That the cumulative depression of synaptic responses can be fit approximately by these same components suggests that pressure-evoked and synaptic responses may decay during repetitive stimulation for similar reasons, although additional presynaptic factors such as transmission failure or presynaptic depression may contribute as well.

Thus, the mechanisms of decay of α7 nAChR-mediated responses to exogenous ACh appear to differ somewhat from neuromuscular junction synaptic responses in that receptor desensitization may play a greater role in the termination of the former type of response. The differences between these systems are further emphasized by

![Fig. 7. Cumulative decay of synaptically activated α7 nAChR-mediated responses.](image-url)
the effects of acetylcholinesterase inhibitors. Such inhibitors typically produce striking increases in the amplitude and duration of end-plate currents (Hobbiger, 1976; Taylor, 1985), but had no effect on our responses to locally applied ACh. These results are consistent with the results cited above, suggesting that prolonging the presence of ACh, either by longer duration of application or by inhibiting its breakdown, does not substantially alter the time course of α7 nAChR-mediated currents. If in vivo synaptic responses respond similarly to the exogenously activated responses tested in this study, cholinesterase inhibitors used clinically in the treatment of Alzheimer’s disease (Whitehouse, 1998; Guan et al., 2000) and proposed for the treatment of schizophrenia (Freedman et al., 1994) would be unlikely to significantly extend or enhance α7 nAChR-mediated responses. Our results further suggest that at the nanomolar blood concentrations used clinically (Sherby et al., 1985; Yost and Maestrone, 1994), neostigmine is unlikely to directly inhibit α7 nAChRs.

α7 nAChRs are unlikely to maintain high frequency phasic inhibition during septo-hippocampal theta activity

Previous studies have shown that one role of α7 nAChRs in the hippocampus is to activate hippocampal interneurons (Frazier et al., 1998a,b), which subsequently produce a strong inhibition of downstream cells, predominantly pyramidal neurons (Buhler and Dunwiddie, 2000; Ji and Dani, 2000). This suggests that nicotinic activation of interneurons through α7 nAChRs could be an important determinant of tonic inhibitory tone, and might also mediate phasic inhibition of hippocampal neurons as well (Miller and Freedman, 1993). However, the pronounced inhibition of the latter responses in trains of α7 nAChR-mediated responses, regardless of whether they were evoked by exogenous or endogenous ACh, suggests that this receptor would be relatively ineffective in driving phasic inhibition of pyramidal neurons at theta frequencies, although the data concerning evoked synaptic responses are at this point preliminary and would need to be expanded in order to draw strong conclusions about the behavior of this synapse. If α7 nAChRs prove to respond similarly in vivo, α7-containing receptors might activate a strong inhibitory response at the beginning of theta bursts or when septal cholinergic neurons fire individual spikes, but they would seem unlikely to contribute significantly to sustaining rhythmic interneuronal excitation. Further maintenance of the oscillatory state could involve other nAChR subtypes in addition to the well known muscarinic cholinergic and GABAergic components of theta (Freund and Gulyas, 1997). In accordance with this idea, the one study investigating the influence of nicotinic activation on hippocampal theta rhythm found that it appeared to be modulated by non-α7 nAChRs (Cobb et al., 1999). It should be noted that these responses differ markedly from those seen at the chick ciliary ganglion where the α7 nAChR can support response rates as high as 25 Hz (Chang and Berg, 1999).

CONCLUSION

These experiments demonstrate that most major anatomical subtypes of inhibitory cells in the CA1 stratum oriens respond to pressure application of ACh with α7 nAChR-mediated responses. This suggests that alterations in nicotinic function in the hippocampus could have a substantial impact on synaptic inhibition through activation of several major classes of GABAergic interneurons. Furthermore, the rapid desensitization of α7 nAChR currents that has been described in various systems plays a significant role in terminating responses to locally applied ACh; if this holds true for synaptic α7 responses as well, it may be difficult to enhance these responses with acetylcholinesterase inhibitors for therapeutic purposes. We also observed that desensitization could not only affect the decay of individual responses, but that it acts in a cumulative fashion to depress these responses during repetitive activation at theta frequencies. These findings suggest that the α7 nAChRs are likely to suffer from cumulative depression during septal theta activity, and are therefore unlikely to play a significant role in mediating phasic GABAergic inhibition during theta activity.

NOTE ADDED IN PROOF

Tom Dunwiddie, scientist, mentor, and friend, was killed July 12th in a climbing accident in Yosemite National Park. We will miss him greatly.

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