alpha 7 nicotinic acetylcholine receptors on GABAergic interneurons evoke dendritic and somatic inhibition of hippocampal neurons.

Amber V. Buhler  
Pacific University

Tom V. Dunwiddie

Follow this and additional works at: http://commons.pacificu.edu/phrmfac
Part of the Molecular and Cellular Neuroscience Commons, Pharmacy and Pharmaceutical Sciences Commons, and the Systems Neuroscience Commons

Recommended Citation
http://commons.pacificu.edu/phrmfac/21

This Article is brought to you for free and open access by the School of Pharmacy at CommonKnowledge. It has been accepted for inclusion in Faculty Scholarship (PHRM) by an authorized administrator of CommonKnowledge. For more information, please contact CommonKnowledge@pacificu.edu.
alpha 7 nicotinic acetylcholine receptors on GABAergic interneurons evoke dendritic and somatic inhibition of hippocampal neurons.

Abstract
GABAergic interneurons in the hippocampus express high levels of α7 nicotinic acetylcholine receptors, but because of the diverse roles played by hippocampal interneurons, the impact of activation of these receptors on hippocampal output neurons (i.e., CA1 pyramidal cells) is unclear. Activation of hippocampal interneurons could directly inhibit pyramidal neuron activity but could also produce inhibition of other GABAergic cells leading to disinhibition of pyramidal cells. To characterize the inhibitory circuits activated by these receptors, exogenous acetylcholine was applied directly to CA1 interneurons in hippocampal slices, and the resulting postsynaptic responses were recorded from pyramidal neurons or interneurons. Inhibitory currents mediated by GABAA receptors were observed in 27/131 interneuron/pyramidal cell pairs, but no instances of disinhibition of spontaneous inhibitory events or GABAB receptor-mediated responses were observed. Two populations of bicuculline-sensitive GABAA receptor-mediated currents could be distinguished based on their kinetics and amplitude. Anatomical reconstructions of the interneurons in a subset of connected pairs support the hypothesis that these two populations correspond to inhibitory synapses located either on the somata or dendrites of pyramidal cells. In 11 interneuron/interneuron cell pairs, one presynaptic neuron was observed that produced strong inhibitory currents in several nearby interneurons, suggesting that disinhibition of pyramidal neurons may also occur. All three types of inhibitory responses (somatic-pyramidal, dendritic-pyramidal, and interneuronal) were blocked by the α7 receptor-selective antagonist methyllycaconitine. These data suggest activation of these functionally distinct circuits by α7 receptors results in significant inhibition of both hippocampal pyramidal neurons as well as interneurons.

Keywords
neurons, acetylcholine, hippocampus

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

Rights
Terms of use for work posted in CommonKnowledge.

This article is available at CommonKnowledge: http://commons.pacificu.edu/phrmfac/21
α7 Nicotinic Acetylcholine Receptors on GABAergic Interneurons Evoke Dendritic and Somatic Inhibition of Hippocampal Neurons

A. V. BUHLER1 AND T. V. DUNWIDDIE1–3,*

1Department of Pharmacology and 2Neuroscience Program, University of Colorado Health Sciences Center, Denver 80262; and 3Department of Veterans Affairs Medical Research Service, Denver, Colorado 80220

Received 18 April 2001; accepted in final form 24 August 2001

Buhler, A. V. and T. V. Dunwiddie. α7 nicotinic acetylcholine receptors on GABAergic interneurons evoke dendritic and somatic inhibition of hippocampal neurons. J Neurophysiol 87: 548–557, 2002; 10.1152/jn.00316.2001. GABAergic interneurons in the hippocampus express high levels of α7 nicotinic acetylcholine receptors, but because of the diverse roles played by hippocampal interneurons, the impact of activation of these receptors on hippocampal output neurons (i.e., CA1 pyramidal cells) is unclear. Activation of hippocampal interneurons could directly inhibit pyramidal neuron activity but could also produce inhibition of other GABAergic cells leading to disinhibition of pyramidal cells. To characterize the inhibitory circuits activated by these receptors, exogenous acetylcholine was applied directly to CA1 interneurons in hippocampal slices, and the resulting postsynaptic responses were recorded from pyramidal neurons or interneurons. Inhibitory currents mediated by GABA_A receptors were observed in 27/131 interneuron/pyramidal cell pairs, but no instances of disinhibition of spontaneous inhibitory events or GABA_B receptor-mediated responses were observed. Two populations of bicuculline-sensitive GABA_A receptor-mediated currents could be distinguished based on their kinetics and amplitude. Anatomical reconstructions of the interneurons in a subset of connected pairs support the hypothesis that these two populations correspond to inhibitory synapses located either on the somata or dendrites of pyramidal cells. In 11 interneuron/neuron cell pairs, one presynaptic neuron was observed that produced strong inhibitory currents in several nearby interneurons, suggesting that disinhibition of pyramidal neurons may also occur. All three types of inhibitory responses (somatic, dendritic, and interneuronal) were blocked by the α7 receptor-selective antagonist methyllycaconitine. These data suggest activation of these functionally distinct circuits by α7 receptors results in significant inhibition of both hippocampal pyramidal neurons as well as interneurons.

INTRODUCTION

The α7 subtype of nicotinic acetylcholine receptor (nAChR) is expressed at high levels in the hippocampus, and disruption of the normal function of this receptor has been implicated in auditory gating deficits in schizophrenia (Freedman et al. 1994), in Alzheimer’s disease (Guan et al. 2000; Wang et al. 2000), and in juvenile myoclonic epilepsy (Elmslie et al. 1994), in Alzheimer’s disease (Guan et al. 2000; Wang et al. 2000), and in juvenile myoclonic epilepsy (Elmslie et al. 1994). In support of this hypothesis, it has recently been reported that activation of α7 nAChRs on interneurons results in GABA_A receptor-mediated inhibitory postsynaptic currents (IPSCs) in CA1 pyramidal cells (Buhler and Dunwiddie 2000; Fujii et al. 2000; Ji and Dami 2000). Although it is clear that activation of α7 nAChRs can generate inhibitory responses in the hippocampus, there are several functionally distinct forms of inhibition, each with unique physiological consequences, and thus the net effect of α7 nAChR activation on hippocampal activity is still unclear. As far as hippocampal pyramidal cells are concerned, there are two types of anatomically and functionally distinct GABA_A inhibitory synapses. Inhibitory synapses on the somata, proximal dendrites, and initial axonal segments of pyramidal cells produce large amplitude currents (Pearce 1993; Vida et al. 1998) and are highly effective in inhibiting action potential discharge (Cobb et al. 1995; Miles et al. 1996), whereas synapses on the distal dendrites of pyramidal cells elicit smaller amplitude currents at the soma (Pearce 1993; Vida et al. 1998) but are particularly effective in inhibiting dendritic excitatory inputs (Yanovsky et al. 1997) and may play an important role in selective synaptic integration (Staley and Mody 1992). It is clear that anatomically distinct interneuronal subtypes produce these two types of inhibitory synapses; basket and chandelier cells are primarily responsible for the somatic synapses, whereas there are many subtypes of “dendritically targeted” interneurons that extend axonal ramifications primarily within the stratum lacunosum moleculare (SLM), st. radiatum, or st. oriens layers (Freund and Buzsaki 1996). The clear anatomical segregation of the synaptic termi-
nations of these interneurons has been confirmed using both light as well as electron microscopy (Buhl et al. 1994a; Cobb et al. 1995; Vida et al. 1998). Electrophysiologically, these two synaptic populations can be distinguished by a consistent difference in kinetics, with dendritically originating IPSCs displaying rise and decay times two to three times slower than those seen in somatically-originating currents (Buhl et al. 1994a; Miles et al. 1996; Pearce 1993; Vida et al. 1998). Although it is still unclear how much of these kinetic differences are due to differences in the kinetic properties of the GABA_{A} receptors at these synapses (Pearce 1993) versus cable filtering (Karnup and Stelzer 1999; Soltész et al. 1995), it is widely accepted that somatic and dendritically originating responses can be distinguished by these kinetic parameters (but see Ouardouz and Lacaille 1997). In addition to these two major types of pyramidal cell inhibitory synapses, there are also inhibitory synapses onto other interneurons. The effects of activation of these latter synapses would be disinhibitory as far as pyramidal neurons are concerned, i.e., they would result in the inhibition of interneurons that normally inhibit pyramidal cells (Buckmaster and Soltész 1996; Freund and Buzsáki 1996), and there is evidence that this type of inhibition may also be produced through α7 activation of interneurons (Alkon et al. 1999). Finally, there are other, somewhat less-characterized inhibitory circuits, such as those involving interneurons that produce pyramidal cell inhibition via activation of G-protein-coupled GABA_{B} receptors (Samulack and Lacaille 1993; Williams and Lacaille 1992); at present, the nicotinic regulation of these cells is unknown.

The major focus of the present experiments was to determine what types of inhibitory responses are elicited in pyramidal cells as a result of α7 nAChR activation of a wide variety of interneuronal subtypes. Disinhibition, although potentially physiologically important, would be difficult to observe under these conditions, in part because of the lower percentage of disinhibitory interneurons in the hippocampus (Freund and Gulyás 1997) but also because it would only be observable on a background of inhibitory activity. For this reason, we have explored the possibility of disinhibitory effects by characterizing inhibitory responses in interneurons elicited by α7 nAChR activation of other local interneurons.

**METHODS**

**Slice preparation and recording conditions**

Housing and treatment of all animals were designed to minimize any animal suffering as well as the number of animals used and were in accordance with protocols approved by the University of Colorado Animal Care and Use Committee. Young (18- to 27-day-old) unanesthetized male Sprague-Dawley rats were decapitated, and 300 µM thick coronal hippocampal slices were made with a Vibratome (Pelco, Redding, CA). Slices were incubated for at least 1 h at 35°C in an oxygenated ACSF containing (in mM) 120 KCl, 1.2 KH_{2}PO_{4}, 2.54 K_{2}HPO_{4}, and 25.9 NaHCO_{3} before recordings were made and to identify possible sites of synaptic connectivity with reconstructed pyramidal cells. Agonist application was achieved by pressure application once every 30 s of 600 µM ACh or 600 µM glutamate through a drug application pipette with a Picospritzer (General Valve, Fairfield, NJ) at pressures of 15 psi and durations of 3–10 ms directly onto the interneuron cell body and proximal dendrites. Bath superfusion of other drugs was achieved by addition of stock solutions to the perfusion medium through syringe pumps (Razel Scientific Instruments, Stamford, CT). Responses were acquired on a microcomputer using NeuroPro software (RC Electronics) at a digitization frequency of 3.4 kHz and analyzed in Microsoft Excel with a custom add-in (courtesy of Jason Frazier). Statistical data are presented as means ± SE. All statistical tests (Student’s t-test, linear correlations, sign test, χ^{2}) were considered significant at a level of P ≤ 0.05.

**Kinetic analyses**

Kinetic analyses of evoked GABAergic IPSCs were completed in MiniAnalysis (Synaptosoft). Responses were well fit using an iterative curve-fitting algorithm to the monoexponential equation: y = A_{1} x \exp(-x/t_{a1}). Rise times were always measured from the first IPSC only, when IPSCs occurred as trains.

**Anatomical reconstruction**

Cells were passively filled through the recording pipette with 0.5% biocytin for between 40 min to 2 h. Tissue was then immediately chilled in 4% paraformaldehyde and stored until later processing. Following one 24-h rinse in TBS (Tris-HCl 50 mM and NaCl 150 mM at pH 7.4–7.6), tissue was incubated for 1 h in TBS-X (TBS and 0.5% triton X) and 0.1% streptavidin-cy3 (Jackson Labs) and then rinsed for 15 min in TBS. Slices were mounted with Vectashield (Vector Labs) fade-resistant medium and visualized with epifluorescence on a Nikon PCM 2000 confocal microscope. Images were acquired and stored with the imaging program Simple PCI (Compiix) and drawn for clarity.

**Drugs**

Chemicals were obtained from the following sources: ACh, glutamate, methyllycaconitine (MLA), bicuculline methiodide (BMI), 6,7-dinitroquinoxaline-2,3-(4H,4H)-dione (DNQX), DL-(−)-2-amino-5-phosphonovaleric acid (APV), and biocytin were obtained from either Sigma (St. Louis, MO) or Research Biochemicals (Natick, MA).
RESULTS

Pressure application of ACh to CA1 interneurons evokes synaptic currents in connected pyramidal cells

In the initial series of experiments, whole cell recordings were made from CA1 pyramidal cells using KCl-filled patch electrodes, while ACh was applied directly onto visually identified interneurons via ACh-filled pressure application pipettes (Fig. 1, A and B). Interneurons were selected to have no close neighbors, and the ACh pipette was placed within 10 μm of the targeted interneuron to minimize activation of any other cells. Using this procedure, pressure application of 600 μM or 1 mM ACh to CA1 layer interneurons produced postsynaptic currents in 27 of 131 cell pairs. These events were relatively consistent within each coupled cell pair, but showed considerable diversity between cells (Fig. 2, A–D). Although occasionally single evoked IPSCs were observed, the most common response was a train of IPSCs, possibly indicative of repetitive firing in the interneuron (but see DISCUSSION). These responses were graded in the sense that increasing the duration of application or decreasing the distance between the ACh pipette and the interneuron typically produced an increase in the number/frequency of IPSCs while having little effect on the amplitude of individual events (Fig. 3C). In separate recordings from interneurons, patterns of firing were observed that were qualita-

tively similar to the patterns of IPSCs produced by ACh application in the pyramidal neurons (Fig. 2, E and F). IPSCs were observed in pyramidal neurons following ACh application to 5/45 (11%) st. oriens, 3/7 (43%) st. pyramidale, 15/65 (23%) st. radiatum, and 4/14 (29%) SLM interneurons.

ACh evoked currents in pyramidal cells are inhibited by both α7 nAChR and GABA A receptor antagonists

If the currents recorded from pyramidal cells in response to pressure application of ACh to local interneurons were mediated via α7 nAChR activation of the interneurons, followed by the synaptic activation of GABA A receptors on the pyramidal neurons, these responses should be blocked by either α7 nicotinic or by GABA A receptor antagonists. Bath application of the GABA A receptor antagonist BMI (10–20 μM) reversibly inhibited evoked responses in all cells tested (Fig. 3A; mean inhibition of IPSC peak amplitude was 89 ± 5%, n = 9). Similarly, once washout of BMI was completed and the responses recovered, these currents were blocked in an all-or-nothing fashion by the α7 nAChR antagonist, MLA (75 nM; Fig. 3B; the average inhibition was 100 ± 0%, n = 7). This suggests that pressure application of ACh activates α7 nAChRs

FIG. 1. Localization of electrode placements. A: photograph illustrating the position of a drug-filled patch electrode (entering from left) that was used to apply ACh to an interneuron located in stratum radiatum near st. lacunosum moleculare (s.l.m.; not visible at this magnification). A whole cell patch recording electrode was used to make simultaneous recordings from a pyramidal cell located in stratum pyramidale (top). Scale is 100 μM. B: diagram illustrating experimental protocol for ACh application to an interneuron during whole cell recording from a pyramidal cell.

FIG. 2. Responses of CA1 pyramidal cells to ACh application to interneurons. A–D: each section shows 3 successive traces from 4 different pyramidal cells illustrating GABAergic inhibitory postsynaptic currents (IPSCs) evoked by application of 600 μM or 1 mM ACh to hippocampal interneurons. Whole cell recordings were made with KCl-filled electrodes, so IPSCs appear as inward currents in these voltage-clamp recordings. Scale is 30 pA × 50 ms for A–C and 50 pA × 50 ms for D. - - -, time of agonist application. E and F: 3 successive ACh responses evoked from 2 different interneurons and the types of firing that were typically evoked by the application of 1 mM ACh with the protocols used in these experiments. Scale is 50 mV × 50 ms for both. - - -, time of agonist application.
Inhibitory Effects of α7 Activation of Interneurons

ACh application to interneurons elicits responses that are most commonly blocked by antagonists to α7 nAChRs such as MLA, but not by antagonists to other nicotinic receptors (mecamylamine or dihydro-β-erythroidine) (Buhler and Dunwiddie 2001). In a small number of cells, the frequency of spontaneous and evoked IPSCs was analyzed during agonist application sweeps and agonist-free sweeps (also containing a 10 mV/250 ms hyperpolarizing pulse) both before and after bath application of MLA. The frequency of events after local application of ACh but before MLA treatment was significantly higher than after MLA treatment (P = 0.0027) or during the agonist-free sweeps (P = 0.0021); ACh application = 24.4 ± 2.8 Hz; ACh application after MLA = 10.1 ± 4.1 Hz; agonist-free sweep = 9.9 ± 3.8 Hz; agonist-free sweep after MLA = 11.3 ± 4.8 Hz; n = 5.

The possibility that ACh could evoke responses other than fast GABA_A IPSCs was also examined. In all 131 interneuron/pyramidal cell pairs previously described, recordings were also analyzed for the presence of GABA_B receptor-mediated currents or for disinhibitory responses (i.e., a decrease in spontaneous GABAergic activity following ACh application) (see Ji and Dani 2000). In none of these cell pairs were either of these responses observed as a consequence of ACh application, although in control experiments activation of an interneuron by glutamate application or electrical depolarization elicited what appeared to be GABA_B receptor-mediated currents in two postsynaptic pyramidal cells.

There are several possible ways in which local ACh application might induce IPSCs in hippocampal pyramidal neurons; these include activation of α7 nAChRs on GABAergic nerve terminals, activation of the cell bodies of the interneurons to which ACh was applied, or possibly the activation of other nearby interneurons via spread of ACh to neighboring cells. To distinguish between these possibilities, simultaneous whole cell recordings were made from interneuron/pyramidal cell pairs, and ACh or glutamate was pressure applied to the interneuron via a double-barreled pipette. As in the preceding experiments, only isolated interneurons (i.e., no visible interneurons in the vicinity) were selected, and agonist was applied using small-diameter (2 μm) pipettes located very close to the targeted cell, with low application pressure and duration. In 15 of 15 simultaneous dual recordings obtained in which the cells were not synaptically coupled, no ACh-evoked IPSCs were observed. Thus there is a low probability of ACh activating other interneurons innervating the pyramidal cell. On the other hand, pressure application of glutamate using this protocol did produce IPSCs in noncoupled pyramidal cells in a few instances (3 of 13 pairs). Specificity of the response to ACh application could also be demonstrated by damaging the visualized interneuron while recording from a connected pyramidal cell. If the agonist application pipette was advanced until the interneuron membrane was ruptured, and then returned to its original position and ACh applied, the IPSCs were abolished (Fig. 4). However, this was not always the case when IPSCs were evoked by glutamate application; in two of seven pairs, damaging the interneuron did not abolish the response from the pyramidal neuron.

ACh evokes both fast and slow IPSCs corresponding to putative somatic and dendritic sites of GABAergic innervation

Kinetic analysis of ACh-evoked IPSCs suggested the presence of two distinct types of responses that could be distinguished based on multiple criteria including rise time, decay, and amplitude. For example, fast and slow IPSCs could be identified by rise time as separate populations; fitting the distribution of rise times to a bimodal distribution (rather than unimodal) gave a significantly better fit (likelihood ratio test $\chi^2 = 43.9, 1$ df, $P < 0.0001$; Fig. 5). In addition, these two populations showed significant differences (Student’s t-test; 2-tailed, nonpaired homoscedastic) in mean amplitude ($P =$...
FIG. 4. An intact interneuron soma is required to elicit IPSCs in pyramidal cells. Top: differential interference contrast (DIC) photographs of a double-barreled drug pipette and the target interneuron before (left) and after interneuron rupture (right). Scale bar indicates 6 μm. Bottom: single records of IPSCs elicited in a pyramidal cell by ACh application to the interneuron (left) and a similar set of records showing the absence of evoked currents following interneuron destruction (right). Scale bar represents 20 ms × 20 pA. ---, time of agonist application.

FIG. 5. Fast and slow IPSCs evoked in pyramidal neurons by ACh application to interneurons. A: scatter plot illustrating a statistically significant negative correlation between the average amplitude and the average 10–90% rise time of IPSCs for all cells (n = 23 cells). B: scatter plot illustrating a significant correlation between the average time to 50% decay and the 10–90% rise time of IPSCs for all cells (n = 23 cells). C: an example of a fast IPSC. Top: average of currents evoked in a pyramidal cell by pressure application of ACh onto an interneuron. Scale bar represents 40 pA × 10 ms for both C and D. Pressure application of agonist occurred 15 ms prior to beginning of response. Bottom: frequency histogram showing the rise times of all of the evoked IPSCs recorded from this cell (n = 16). ---, onset and peak of current. D: an example of a slow IPSC. Top: averaged evoked currents show a smaller amplitude as well as a slower rise time than the response shown in C. Pressure application of agonist occurred 13.5 ms prior to beginning of response. ---, onset and peak of current. Bottom: frequency histogram showing the rise times of all evoked IPSCs recorded from this cell (n = 19).
553 INHIBITORY EFFECTS OF α7 ACTIVATION OF INTERNEURONS

Fast IPSCs were observed in 17 of 127 cell pairs in which the quality of the recording conditions was adequate to perform kinetic analyses, and were characterized by fast rise times (10–90% rise time of 1.5 ± 0.1 ms), large amplitude (−48 ± 4 pA), and rapid decay (100–50% decay time of 8.5 ± 0.6 ms; e.g., Fig. 5C). Slow IPSCs were recorded in 6 of 127 cell pairs and were characterized by slow rise times (2.9 ± 0.1 ms), smaller amplitude responses (−14 ± 2 pA), and slower decay (16.4 ± 1.7 ms; e.g., Fig. 5D). Despite differences in absolute numbers due to different recording conditions, these kinetic properties correspond well to previous characterizations of IPSCs originating from the somatic or the dendritic regions of pyramidal cells (Buhl et al. 1994a; Ouardouz and Lacaille 1997; Pearce 1993; Vida et al. 1998).

There was a significant correlation between rise time and response amplitude for the entire data set ($r^2 = 0.57$, $n = 23$, $P = 0.00006$; Fig. 5A), but there was no correlation between these parameters within the fast rise time group alone ($r^2 = 0.01$, $n = 17$, $P = 0.71$). There was also a significant correlation between rise time and decay time for the entire population ($r^2 = 0.55$, $n = 23$, $P = 0.00008$; Fig. 5B), as might be expected if both of these parameters reflect the electrotonic distance between the synapse and the recording site. There was no correlation between series access resistance and either rise time ($r^2 = 0.1$, $n = 23$, $P = 0.14$) or IPSC amplitude ($r^2 = 0.02$, $n = 23$, $P = 0.53$), suggesting that population differences based on these parameters were not due to differences in the quality of the recordings. To further ensure that slow responses were not due simply to poor recording conditions, the existence of spontaneous fast IPSCs was confirmed in each cell showing slow responses.

To examine directly the origin of these fast and slow responses, a subset of these interneurons were filled with biocytin to visualize the regions in which their axons terminated. Following ACh application to an interneuron (and recording from a pyramidal neuron), a second patch recording electrode was used to passively fill with biocytin the interneuron to which ACh had been applied. Four interneurons producing fast IPSCs were reconstructed and all appeared to be basket or chandelier cells (somasitically innervating subtypes) with axonal fields predominately restricted to the stratum pyramidale (Fig. 6, A and B). Neurons with similar morphology have been described previously (Buhl et al. 1994b, 1995; Halasy et al. 1996). Cell bodies of the somatically innervating interneurons were located in the st. pyramidale and st. radiatum. Three interneurons that elicited slow IPSCs were successfully filled with biocytin; one appeared to be of the bistratified type (described in Buhl et al. 1994a; Halasy et al. 1996), with a dense axonal field in both st. oriens and st. radiatum, excluding the st. pyramidale (Fig. 6, C and D), the second appeared to be of the trilaminar type (described in Ali et al. 1999; Sik et al. 1995), with axonal arborization in the stratum oriens, pyramidale and radiatum, and the third, which was only was partially filled with biocytin had an axonal field that was predominantly confined to the st. radiatum. Cell bodies of these three interneurons were located in the SLM or st. radiatum.

**FIG. 6.** Anatomical reconstructions of interneuron/pyramidal neuron pairs and associated IPSCs recorded from pyramidal cells. A: the fast, large amplitude IPSCs evoked in a pyramidal neuron by ACh application to an interneuron. B: the anatomical reconstruction of this pair of cells, which confirmed that the pyramidal neuron received somatic input from the interneuron. The pyramidal cell is shown in black, the interneuron soma and dendrites in red, and the axonal field of the interneuron in green. C and D: a slow IPSC recorded from a pyramidal neuron, and the reconstructed cell pair. The axon of this interneuron projected primarily to st. oriens and st. radiatum (bistratified type interneuron). Individual IPSCs such as those shown in C were occasionally of such low amplitude that they were difficult to confirm without signal averaging. Scale for IPSCs is 40 ms × 40 pA (magnified response in inset in C is 40 ms × 13 pA), and the dashed lines indicate the time of agonist application. Scale for reconstructions is approximately 45 μm for both.
Short-term plasticity of evoked IPSCs

Although in some cases pressure application of ACh to an interneuron produced only a single IPSC in the postsynaptic cell, in most cases multiple IPSCs were evoked (Fig. 2), which is consistent with repetitive spiking in CA1 interneurons elicited by ACh application (Fig. 2F). Among cells exhibiting trains of fast putatively somatic type IPSCs, three types of response patterns were observed. Some trains showed decremental properties, with successively smaller responses (Fig. 7A), some showed no change, while others showed synaptic facilitation (Fig. 7B). The percent facilitation of IPSC amplitude (expressed as \(A_2/A_1\)) was not significantly correlated to the interval between responses (Fig. 7C) either for the entire group (\(r^2 = 0.2, n = 14, P = 0.064\)), within the subsets showing depression or facilitation, or within individual cells (a statistically significant correlation was observed in 1/9 cells). Thus the pattern was not simply reflecting some generalized properties of these synapses (e.g., depression at short inter-spike intervals) but appeared to be specific to the synapse being tested. However, there was a significant negative correlation between the amplitude of the initial IPSC and the amount of facilitation within individual cells (i.e., larger amplitude initial responses showed less facilitation or greater depression of the second response: mean \(r = -0.43 \pm 0.13\), \(n = 9\) cells; \(P = 0.05\), nonparametric sign test). Because of the small amplitude of the slow putatively dendritic IPSCs, it was feasible to measure amplitude changes in only one cell that exhibited trains of slow IPSCs, and facilitation was observed in this cell.

ACh-evoked IPSCs in CA1 interneurons

Because some hippocampal interneurons project to other interneurons, preliminary experiments were conducted on pairs of interneurons in which ACh was applied to one interneuron, while whole-cell recordings were made from another nearby interneuron. In 11 interneuron/interneuron cell pairs (8 individual presynaptic cells), one presynaptic interneuron was identified that produced strong IPSCs in two of four local interneurons (Fig. 8). These responses were identified as IPSCs and not direct responses to ACh application because there were clearly multiple events evoked by single applications of ACh (Fig. 8A) and because when the slice was superfused with 75 nM MLA, each peak dropped out in an all-or-nothing fashion during the onset of the drug effect (Fig. 8B) as opposed to the gradual reduction in response amplitude that occurs when direct responses to ACh are antagonized by this agent.

**DISCUSSION**

**Activation of \(\alpha_7\) nAChRs on interneurons inhibits pyramidal cells**

In previous studies, our laboratory has demonstrated that \(\alpha_7\) nAChR-mediated currents can be evoked from CA1 st. oriens interneurons that project to both dendritic (i.e., st. oriens, st. radiatum, and SLM) and somatic (st. pyramidale) subregions of the hippocampal CA1 region (Buhl and Dunwiddie 2001). Although these data suggest that activation of nicotinic inputs to the hippocampus could produce both somatic and dendritic inhibition in CA1 pyramidal neurons, it is also possible that the dendritic projections are to other interneurons not pyramidal neurons. However, the present experiments have shown directly that there are two kinetically distinct types of GABA<sub>A</sub> receptor-mediated currents evoked in pyramidal cells by \(\alpha_7\) nAChR activation of local inhibitory interneurons. The kinetic differences that we observed between these slow and fast IPSCs are consistent with the differences reported by other laboratories in comparisons of these physically distinct synaptic populations (Buhl et al. 1994a; Pearce 1993). The anatomical reconstructions of interneurons in the present set of experiments provided direct anatomical support for the conclusion that the two types of physiologically distinguishable responses reflect activation of anatomically distinct sets of synapses. The ability of \(\alpha_7\) nAChRs to activate interneurons that provide both somatic and dendritic GABAergic input to the pyramidal neurons suggests that cholinergic input to the hippocampus plays a complex role in regulating hippocampal inhibition, which could include both inhibition of cell firing as well as more specific attenuation of excitatory inputs that terminate near dendritic inhibitory synapses.

There are several mechanisms by which local application of ACh could produce GABA release in these experiments, including activation of somatic/dendritic receptors, terminal receptors, or activation of nearby nontargeted interneurons through agonist diffusion. At least two pieces of evidence would suggest that the GABA release described here is action potential dependent, and likely arising from somatic/dendritic receptors. First, previous work describing \(\alpha_7\) receptor-mediated GABA release in the hippocampus (Alkondon et al. 1999) found this phenomenon to be tetrodotoxin sensitive, and second, the data presented here (Fig. 4) illustrate the requirement of an intact cell body for IPSC generation, suggesting that in at least some of our cells, activation of terminal \(\alpha_7\) nAChRs was not sufficient to produce the observed phenomenon. However, the possibility that terminal \(\alpha_7\) nAChRs contributed to the generation of IPSCs in some of our recordings cannot be completely ruled out.
Inhibitory effects of α7 activation of interneurons

The question of cell specificity of agonist application was also addressed in this work, and it appears that with local application of ACh, IPSCs are generated almost exclusively through activation of the targeted cell rather than through nonselective activation of other local interneurons. The less selective nature of IPSC generation by local application of glutamate compared with ACh could be explained by several mechanisms, including direct activation of other interneurons due to a longer active lifetime and diffusion distance with glutamate, or greater sensitivity of pyramidal neurons to glutamate than to ACh (Frazier et al. 1998b; McQuiston and Madison 1999), resulting in the activation of pyramidal neurons and induction of IPSCs through secondary activation of other interneuron populations.

Despite the large number of cell pairs investigated in this study, no instances of ACh-evoked GABA_B receptor-mediated currents were observed. However, there are several reasons why GABA_B responses may not have been observed under these conditions, so it would be premature to conclude that nicotinic activation cannot produce GABA_B currents. First, only a small portion of the interneurons activated in this study were likely to be of the two identified subsets of interneuron producing GABA_B responses, i.e., the SLM (Williams and Lacaille 1992) and oriens/alveus border interneurons (Samulack and Lacaille 1993; Yanovsky et al. 1997). Second, it is possible that the postsynaptic CA1 GABA_B Receptor system is not yet fully functional in the immature rats used in this study (Janigro and Schwartzkroin 1988). A more extensive investigation of the ability of nicotinic cholinergic activity to activate the GABA_B system would be needed to resolve this issue.

Inhibitory vs. disinhibitory effects of α7 nAChR activation

Activation of interneuronal α7 nAChRs in the hippocampus can produce strong inhibitory currents in pyramidal cells (Fujii et al. 2000; Ji and Dani 2000), which might be expected to result in a generalized depression of hippocampal activity. However, the existence of a subpopulation of interneurons in the CA1 that specifically inhibit other interneurons (Acsady et al. 1996; Gulyas et al. 1996) suggests that there may be disinhibitory effects as well. The present experiments, although preliminary in nature, have shown that IPSCs can be evoked in interneurons following the activation of α7 nicotinic receptors on nearby interneurons (see also Alkondon et al. 1999). These experiments, which must be expanded to draw strong conclusions about the frequency and characteristics of this phenomenon, provide a direct, albeit limited, observation of this effect. Further, these experiments support the hypothesis that α7 nicotinic receptors are capable of producing a functional disinhibition of pyramidal cells as suggested by Ji and Dani (2000), who reported disinhibition in a single pyramidal cell following α7 nicotinic activation of a presynaptic interneuron. Both the small percentage of specialized disinhibitory interneurons and the paucity of evidence for disinhibitory effects on spontaneous IPSCs in pyramidal neurons suggest that disinhibition may be a relatively minor effect in this region compared...
with direct inhibition. However, disinhibitory effects may be difficult to observe experimentally because interneuron-selective “disinhibitory” interneurons appear to most commonly innervate other disinhibitory interneurons or interneurons that inhibit pyramidal cells at dendritic sites (Acsady et al. 1996; Gulyas et al. 1996). Thus there would be little disinhibition of spontaneous IPSCs, which are believed to reflect primarily somatic events (Soltetz et al. 1995). Functionally, this would suggest that nicotinic activation of the disinhibitory circuit may be more likely to produce a reduction in dendritic inhibition than a loss of the more powerful somatic inhibition. However, the net effect of cholinergic input to the hippocampus would depend on both the number and type of interneurons activated by this input and on the timing of disinhibitory effects relative to direct pyramidal cell inhibition.

The question of whether the net effect of α7 nicotinic activation will produce general inhibition or excitation in the hippocampus is still unresolved. Attempts to directly activate α7 nAChRs on large numbers of interneurons in the hippocampal slice to show a general effect are frustrated by problems including the very rapid desensitization of these receptors, which probably preclude any kind of experiments involving bath application of nicotinic agonists. Even if this problem could be surmounted, the question would remain as to whether simultaneous activation of the many subsets of α7 nAChR populations present in this region would resemble in any way the physiological patterns of activation of these receptor subpopulations. It would appear more likely that these populations are activated in a phasic manner and that activation of cholinergic inputs in vivo would result in changes in both the pattern as well as overall firing frequency of pyramidal neurons.

Conclusions

These experiments demonstrate that a primary effect of α7 nAChR-mediated activation of CA1 hippocampal interneurons is to produce at least two distinct forms of GABA_A receptor-mediated inhibition in CA1 pyramidal neurons. These two types of inhibitory responses, which appear to correspond to selective activation of somatic and dendritic synapses, would have distinct functional roles; the activation of both suggests that α7 nicotinic circuits may have a complex inhibitory effect involving both strong somatic inhibition and selective inhibition of dendritic inputs. In addition to these direct inhibitory effects, α7 nAChR-mediated activation of some interneurons also evoked IPSCs in other interneurons, providing evidence for an additional role of the α7 nAChR in disinhibitory circuits. The ultimate effect of α7 nAChR activation of CA1 interneurons on hippocampal output clearly will depend on the timing as well as the relative magnitude of the activation of these three inhibitory circuits.

We thank J. Weiner for comments on the manuscript, R. Levinson for the use of the confocal imaging equipment, and D. Young for assistance with statistics. Finally, a deep and abiding thanks to Dr. Tom Dunwiddie; scientist, mentor, and friend.

REFERENCES

Acsady L, Gorcs TJ, and Freund TF. Different populations of vasoactive intestinal polypeptide-immunoreactive interneurons are specialized to control pyramidal cells or interneurons in the hippocampus. Neuroscienece 73: 317–334, 1996.


Elmslie FY, Rees M, Williamson MP, Kerr M, Kielisen MJ, Pang KA, Sundqvist A, Friis ML, Chadwick D, Richens A, Covantis A, Santos M,