

Inhibition and Disinhibition of Pyramidal Neurons by Activation of Nicotinic Receptors on Hippocampal Interneurons

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Ji, Daoyun and John A. Dani. Inhibition and disinhibition of pyramidal neurons by activation of nicotinic receptors on hippocampal interneurons. *J. Neurophysiol.* 83: 2682–2690, 2000. Nicotinic acetylcholine receptors (nAChRs) are expressed in the hippocampus, and their functional roles are beginning to be delineated. The effect of nAChR activation on the activity of both interneurons and pyramidal neurons in the CA1 region was studied in rat hippocampal slices. In CA1 stratum radiatum with muscarinic receptors inhibited, local pressure application of acetylcholine (ACh) elicited a nicotinic current in 82% of the neurons. The majority of the ACh-induced currents were sensitive to methyllycaconitine, which is a specific inhibitor of $\alpha 7$ -containing nAChRs. Methyllycaconitine-insensitive nicotinic currents also were present as detected by a nonspecific nAChR inhibitor. The ACh-sensitive neurons in the s. radiatum were identified as GABAergic interneurons by their electrophysiological properties. Pressure application of ACh induced firing of action potentials in ~70% of the interneurons. The ACh-induced excitation of interneurons could induce either inhibition or disinhibition of pyramidal neurons. The inhibition was recorded from the pyramidal neuron as a burst of GABAergic synaptic activity. That synaptic activity was sensitive to bicuculline, indicating that GABA_A receptors mediated the ACh-induced synaptic currents. The disinhibition was recorded from the pyramidal neuron as a reduction of spontaneous GABAergic synaptic activity when ACh was delivered onto an interneuron. Both the inhibition and disinhibition were sensitive to either methyllycaconitine or mecamylamine, indicating that activation of nicotinic receptors on interneurons was necessary for the effects. These results show that nAChRs are capable of regulating hippocampal circuits by exciting interneurons and, subsequently, inhibiting or disinhibiting pyramidal neurons.

INTRODUCTION

Nicotinic acetylcholine receptors (nAChRs) play roles in modulating cognitive functions, including learning and memory (Levin and Simon 1998). Nicotine administration facilitates learning and memory on many behavioral tasks in both young and old mammals, and some of the improvements suggest the hippocampus is one target for nicotinic modulation (Arendash et al. 1995; Levin and Torrey 1996; Socci et al. 1995).

The hippocampus is a center for learning and memory and is especially important for spatial learning (Eichenbaum 1996; Wilson and McNaughton 1993; Wood et al. 1999). The hippocampus receives extensive cholinergic innervation from the medial septum-diagonal band complex (Alonso and Amaral 1995; Woolf 1991; Yoshida and Oka 1995), and there is strong expression of

nAChRs in the hippocampus (Martin and Aceto 1981). In the mammalian hippocampus, $\alpha 7$ and $\beta 2$ subunits are widely distributed, but other subunits also are present (Deneris et al. 1988; Rubboli et al. 1994; Séguéla et al. 1993; Wada et al. 1989). In cell culture rat hippocampal neurons express three types of nicotinic currents (Alkondon and Albuquerque 1993). The vast majority of these neurons, however, display a rapidly activating and desensitizing Type IA current, which is sensitive to α -bungarotoxin and methyllycaconitine (MLA). The pharmacology indicates that the Type IA current is mediated by $\alpha 7$ -containing nAChRs (Alkondon et al. 1994; Zarei et al. 1999). That interpretation was verified because Type IA currents are absent from hippocampal neurons derived from $\alpha 7$ -null mutant mice (Orr-Urtreger et al. 1997). A more rare current with slower kinetics in hippocampal cultures is called the Type II current, and it is inhibited by dihydro- β -erythroidine or high concentrations of mecamylamine. Mutant mice lacking $\beta 2$ do not display Type II nAChR currents (Zoli et al. 1998).

Recent advances have begun to reveal the functional roles of nAChRs in the hippocampus. The high calcium permeability of the $\alpha 7$ -containing nAChR (Castro and Albuquerque 1995; Rathouz et al. 1996; Séguéla et al. 1993) enables it to enhance the release of both glutamate and GABA via presynaptic or preterminal mechanisms in the hippocampus (Alkondon et al. 1997; Gray et al. 1996; Radcliffe and Dani 1998; Radcliffe et al. 1999). On the basis of these results, a predominantly presynaptic role has been assigned to the hippocampal nicotinic receptors. Recently, however, fast nicotinic synaptic transmission and somatic and postsynaptic nicotinic responses have been discovered on hippocampal interneurons (Alkondon et al. 1998, 1999; Frazier et al. 1998a,b; Hefft et al. 1999; Jones and Yakel 1997; McQuiston and Madison 1999). These results suggest that postsynaptic nAChRs might influence hippocampal circuits via GABAergic pathways. The aim of the present study was to determine whether nicotinic activation of CA1 interneurons has the ability to produce inhibition or disinhibition of pyramidal neurons. We found that exogenous activation of interneurons by a nicotinic agonist can inhibit CA1 pyramidal neurons via GABA_A synaptic activity or disinhibit pyramidal neurons by reducing spontaneous GABA_A synaptic activity.

METHODS

Slice preparation and electrophysiology

Sprague-Dawley rats (16–30 day old) were anesthetized with a mixture of ketamine (42.8 mg/ml), xylazine (8.6 mg/ml), and

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acepromazine (1.4 mg/ml) at a dosage of 0.05 ml/10g and were decapitated. Coronal or horizontal slices (300–400 μ m thick) were cut in ice-cold cutting solution of the following composition (in mM): 220 sucrose, 2.5 KCl, 30 NaHCO₃, 1.25 KH₂PO₄, 10 dextrose, 7 MgCl₂, and 1 CaCl₂, bubbled with 95% O₂–5% CO₂. Slices were transferred into a holding chamber, containing the external solution (in mM): 125 NaCl, 2.5 KCl, 25 NaHCO₃, 1.25 KH₂PO₄, 25 dextrose, 1 MgCl₂ and 2 CaCl₂, oxygenated with 95% O₂–5% CO₂. After a 30-min recovery at 35°C, slices were maintained at room temperature and were used for recording in the following 5 h.

Neurons in CA1 stratum radiatum were recorded at 32–34°C, but CA1 pyramidal neurons were recorded at room temperature to reduce spontaneous GABAergic activity. In all of the experiments, 0.5–1 μ M atropine was added to the external solution to block muscarinic acetylcholine receptors. We use the term “block” rather than “inhibition” in these circumstances to prevent confusing receptor inhibition and GABAergic-mediated inhibitory synaptic currents. When GABAergic currents were recorded from CA1 pyramidal neurons, 6-cyano-7-nitroquinoxaline-2,3-dione disodium (CNQX, 25 μ M) and (\pm 12)-2-amino-5-phosphonopivalic acid (AP-5, 50 μ M) were added to block glutamatergic activity. To record from interneurons, the internal solution in the recording pipettes contained the following (in mM): 115 K-gluconate (KGlu), 20 KCl, 10 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, 10 ethylene glycol-bis (β -aminoethyl ether) *N,N,N',N'*-tetraacetic acid (EGTA), 4 ATP (magnesium salt), 0.3 GTP (sodium salt), and 7 phosphocreatine, adjusted to pH 7.3–7.4 with KOH. Normally the distribution of chloride makes GABAergic synaptic activity difficult to detect when the holding potential of the voltage clamp is near the resting potential because that holding potential is also near the reversal potential for chloride. To make the GABAergic synaptic currents in CA1 pyramidal cells easier to detect, 115 KGlu and 20 KCl were replaced by 135 CsCl to raise the intracellular Cl[−] while also improving the space clamp. Under conditions of high intracellular Cl[−], however, GABA_A activity could depolarize the cell and thus activate voltage-dependent regenerative currents in unclamped distal dendrites. To minimize this problem while keeping chloride relatively high, in 18 experiments, 75 CsCH₃SO₃ and 60 CsCl replaced 115 KGlu and 20 KCl and lidocaine *N*-ethyl bromide was added to inhibit voltage-dependent sodium current. When antagonists (MLA, mecamylamine, bicuculline, tetrodotoxin) were used, they were applied via bath perfusion. The solution flowing rate was adjusted to \sim 3 ml/min.

The patch-clamp recording pipettes had resistances of 2–4 M Ω when filled with the internal solution. Data were acquired with an Axopatch amplifier and stored on an hard drive. Series resistance and input resistance were monitored by injecting a small negative voltage or current step throughout the experiment. Series resistances were usually in a range of 5–30 M Ω and were left uncompensated. Data were discarded if series resistance or input resistance changed by \geq 30%.

Recording sites and local delivery of agonists

Figure 1 shows the locations of the recorded neurons and the arrangement of the recording pipette (R) and the “puffer” pipette (P). By pressure injection, the puffer pipette locally delivered the agonist (ACh) to a desired location. We used two recording paradigms. As shown in Fig. 1A, neurons located in the CA1 s. radiatum 100–300 μ m away from the pyramidal cell layer were recorded, and ACh was locally delivered onto their soma. Most of these neurons were identified as interneurons based on their electrical properties. Some recorded neurons in this paradigm were located near the border between s. radiatum and s. lacunosum-moleculare. In the second recording paradigm (Fig. 1B), ACh was applied locally to an interneuron while recording from a pyramidal neuron. ACh often was applied to several interneurons in the s. radiatum before we recorded a response from the pyramidal neuron that was whole cell clamped. In this paradigm, the interneuron was located 50–400 μ m lateral and $>$ 100 μ m vertical to

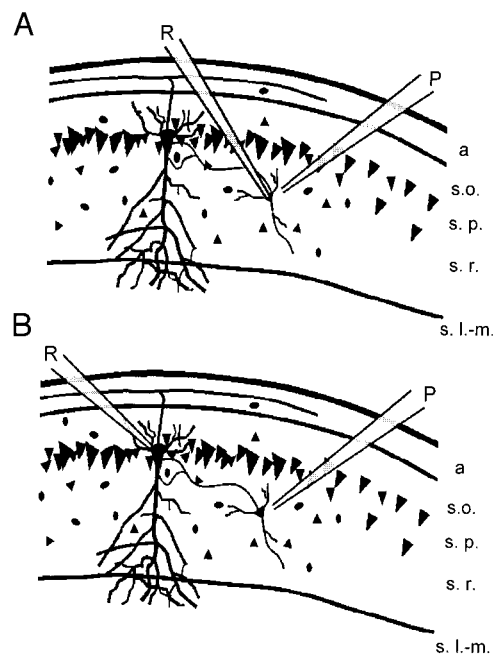


FIG. 1. Arrangement of the recording pipette (R) and the agonist puffer pipette (P) in the hippocampal CA1 region. A: interneuron in stratum radiatum was recorded while acetylcholine (ACh) was pressure-delivered through a puffer pipette onto the soma of the recorded neuron. B: pyramidal neuron in s. radiatum was recorded while ACh was pressure-applied by a puffer pipette onto an interneuron in s. radiatum. a, alveus; s.o., s. oriens; s.p., s. pyramidale; s.r., s. radiatum; s.l.-m., s. lacunosum-moleculare.

the patch-clamped pyramidal neuron. The puffer usually pointed directly onto the single visualized interneuron, and the agonist was diluted and spread with time as it was washed away in the bath. The arrangement optimized activation of the interneuron's soma, but other local areas would experience a slower raise of a lower ACh concentration. The injection pipette was arranged to parallel the pyramidal cell layer to avoid ACh application directly onto or near the patch-clamped pyramidal neuron's soma. This application method is much faster and focal than other application methods because the puffer can be placed into the slice, very near the target, and brief pressure applications are used to produce the desired effect.

A picospritzer (Parker Instrumentation) was used to control the pressure and duration of the puffs that deliver the agonist. When a short (5–20 ms) puff was used, the injection pipette was positioned \sim 10 μ m away from the interneuron's cell body and a pressure of 10 psi was used. When a longer (50 ms to 1 s) puff was used, the injection pipette was positioned 50–80 μ m away and a pressure of 5 psi was used. Control experiments were performed to examine potential artifacts due to the puffing system. When external solution containing no agonist was puffed onto the soma, there was no response under our recording conditions ($n = 30$). However, if the puffer pipette was too close to the recording pipette and a long puff was applied, it could influence the whole cell seal. In that case, a slow and irregular current was seen in 3 of 14 neurons. Neurons were rejected if a slow, irregular current was observed during the pressure application, and we did not position the puffer pipette close to the cell during long agonist applications.

Data analysis

All the values are presented as means \pm SE. Positive and negative current steps were injected and voltage responses were recorded to characterize electrophysiological properties of the recorded neuron. Resting membrane potentials were estimated within 5 min after establishing the whole cell recording configuration. Current steps

(300-ms duration) that were smaller than the threshold required to induce action potential were injected, and input resistances were calculated by dividing the steady-state voltage responses by the injected currents. Action potentials elicited at threshold were used to determine fast afterhyperpolarization-potentials (AHPs). Fast AHPs were calculated as the difference between the most negative potential immediately after an action potential and the threshold potential. Firing frequencies and slow AHPs were determined from the action potential train with the maximum number of action potentials that could be elicited by a 1-s current step (200–1,200 pA). Slow AHPs were calculated as the most negative potentials after the action potential train relative to resting potentials. Negative current steps (300 ms, 100–500 pA) were injected to determine sag ratios, which were calculated from the most negative membrane potential divided by the steady-state potential in response to the injected negative current.

The charge transfer in pyramidal neurons induced by GABA_A receptor synaptic activity was calculated to quantitate the inhibition induced by ACh application (as in Fig. 4B). The charge was integrated through the whole current trace (2 s). The average net charge transferred by ACh-activated GABA_A receptors was calculated as the difference between the average ACh-induced charge transfer and the average charge transfer without ACh application. The ACh application was taken to have produced a significant difference in the calculated charge transfer based on the Student's *t*-test ($P < 0.05$).

RESULTS

The results are based on our recordings from 31 pyramidal neurons and 88 neurons in the s. radiatum of the CA1 region.

ACh-induced nicotinic currents from neurons in the CA1 s. radiatum

Of the 88 recorded neurons in s. radiatum, 72 (82%) displayed a nicotinic response to a somatic pressure application of ACh (0.2–1 mM; Fig. 2). As described in the following text, the ACh-sensitive neurons were identified as GABAergic interneurons. In response to a brief (5–20 ms) application of a high concentration (1 mM) of ACh, a fast activating current, with a profile similar to Fig. 2A, *top*, usually was recorded. When a longer application (200 ms to 2 s) was used, 37 of 45 neurons displayed a fast current with rapid desensitization, 4 neurons responded with a slow current with relatively slower kinetics (Fig. 2A, *bottom*), and 4 neurons showed a current with both the fast component and the slow component. The kinetics of the fast current is similar to the Type IA current, and the slow current is similar to the Type II current in hippocampal cell culture (Alkondon and Albuquerque 1993). Although at least two types of current were recorded, the fast current was the predominant response to the ACh application in the CA1 s. radiatum.

To determine whether the nAChRs on CA1 interneurons contain $\alpha 7$ subunits, MLA sensitivity was tested with 23 neurons. The ACh-induced currents from 20 interneurons were blocked by bath application of 20 nM MLA, and the currents recovered after a 35-min washout (Fig. 2A, *top*). The block was fast and complete. In 2 of 23 interneurons, there were slower nicotinic currents that were completely blocked by 20 nM MLA. In 3 of the 23 neurons, however, there were slower nicotinic currents that were not completely blocked by 20 nM MLA, but that current was inhibited by 25 μ M mecamylamine (MEC) (Fig. 2A, *bottom*). Mecamylamine at that concentration is a nonspecific inhibitor of nAChRs. The result suggests that

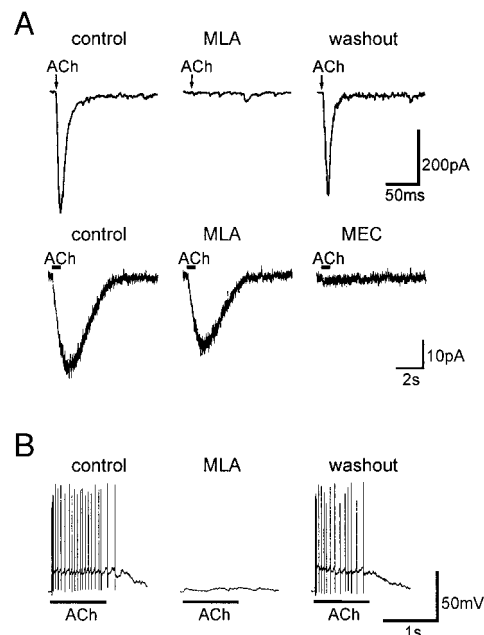


FIG. 2. Local puff application of ACh onto the soma of s. radiatum interneurons activated nicotinic ACh receptor (nAChR) currents. Neurons were voltage-clamped near their resting potentials at -60 mV. A: ACh-induced methyllycaconitine (MLA)-sensitive or mecamylamine-sensitive currents. A fast current response to a 5-ms puff of 1 mM ACh was blocked by 20 nM MLA and recovered after washout (*top*). \downarrow , time at which ACh was applied. A relatively slow current response to a 500-ms puff of 1 mM ACh was partially blocked by 20 nM MLA and completely blocked by 25 μ M mecamylamine (*bottom*). B: 1-s puff application of 200 μ M ACh induced an interneuron to fire action potentials. MLA (20 nM) reversibly blocked the voltage response. \blacksquare , duration of the agonist application. Bicuculline (20 μ M) was present to block GABA_A receptors.

nAChR subunits other than $\alpha 7$ can contribute to the nicotinic currents; however, most nAChRs on CA1 s. radiatum interneurons are MLA sensitive and contain the $\alpha 7$ subunit.

To examine the possible contribution from glutamate receptors to the current response, the effect of CNQX and AP-5 was tested on five neurons. Bath application of CNQX (25 μ M) and AP-5 (50 μ M) did not significantly change the amplitudes of the ACh-induced currents ($97 \pm 3\%$ of control, $P = 0.47$, Student's *t*-test, $n = 5$). Therefore the recorded ACh-induced currents were not significantly contaminated by glutamatergic currents arising from glutamate release induced by presynaptic nAChRs (Radcliffe and Dani 1998).

ACh application caused action potentials to be fired in $\sim 70\%$ (50 of 72) of the identified interneurons. In many interneurons, the firing was strong and lasted for seconds in response to a 1-s application of ACh (0.2–1 mM). In the three neurons we tested, the ACh-induced action potential trains were inhibited by 20 nM MLA (Fig. 2B, $n = 3$). This result indicates that stimulation of nAChRs can elicit trains of action potentials in s. radiatum interneurons.

ACh-sensitive and -insensitive neurons in the CA1 s. radiatum displayed different electrophysiological properties

Although the majority of the neurons in CA1 s. radiatum responded with a nicotinic current, there were 16 neurons (18%, $n = 88$) that did not respond with detectable nicotinic current when ACh was pressure-applied onto their somas. We

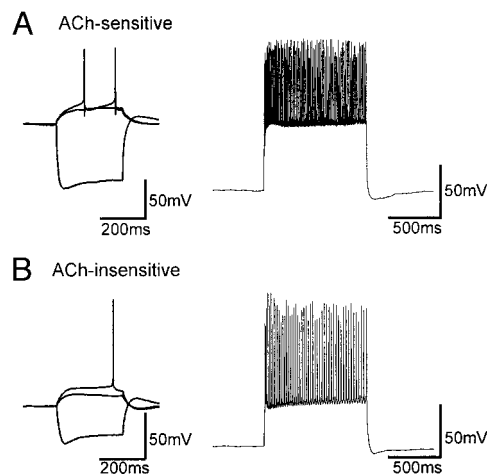


FIG. 3. ACh-sensitive and -insensitive neurons in CA1 s. radiatum displayed distinguishable membrane properties. *A*: voltage responses of an ACh-sensitive neuron to a 300-ms current step with intensities of -500 , 50 , and 60 pA, respectively (*left*) and to a 1-s step of 650 pA (*right*). *B*: voltage responses of an ACh-insensitive neuron to a 300-ms current step with intensities of -500 , 100 , and 150 pA, respectively (*left*) and to a 1-s step of 800 pA (*right*). Responses of the ACh-sensitive neurons are indicative of GABAergic interneurons.

compared the electrophysiological properties of the ACh-sensitive and -insensitive neurons. The voltage responses to current injections of a typical ACh-sensitive and a typical ACh-insensitive neuron in the s. radiatum are shown in Fig. 3. The electrophysiological property of eight ACh-responding neurons and four ACh-insensitive neurons are summarized in Table 1. The ACh-sensitive neurons had significantly larger fast-AHP, higher firing frequency, and higher input resistance. There was no significant difference in the resting potential, the sag ratio, or the slow-AHP between the two cell types. The electrophysiological characteristics displayed by the ACh-sensitive neurons indicate they were GABAergic interneurons (Lacaille et al. 1987; Schwartzkroin and Mathers 1978). The ACh-insensitive neurons, on the other hand, displayed much smaller fast-AHP, lower firing frequency and lower input resistance. Therefore the ACh sensitivity distinguishes two types of neurons in the CA1 s. radiatum.

ACh application onto interneurons can produce direct GABA_A receptor-mediated inhibition of pyramidal neurons

We examined how the ACh-induced excitation of interneurons affects pyramidal neurons using the recording paradigm shown in Fig. 1*B*. When ACh (0.2 – 1 mM) was applied onto an interneuron in the s. radiatum, strong inhibition could be recorded in nearby pyramidal neurons. Special care was taken to avoid ACh application directly onto or near the patch-clamped

pyramidal neuron. A cesium-based solution was used in the recording pipettes, and the intracellular Cl^- concentration was raised to 135 or 60 mM to amplify the GABA_A-mediated current. The same intensity of response by the GABAergic interneurons would be elicited regardless of the solution inside the pyramidal neuron, but we would not easily measure the effect with physiological solutions because the resting potential then would be near to the Cl^- reversal potential.

A total of 47 pairs of interneurons and pyramidal neurons were examined. In 14 pairs (30%), application of ACh (0.5 – 1 s) onto the interneuron induced a burst of synaptic current in the pyramidal neuron in the presence of 25 μM CNQX and 50 μM AP-5 (Fig. 4*A*). Under our experimental conditions, the synaptic currents were inward when the neurons were clamped at -50 mV. The duration of the responses varied from seconds to tens of seconds. The longer GABAergic activity may have arisen from activation of more than one interneuron by the ACh application. The ACh-induced synaptic currents were blocked reversibly by 10 μM bicuculline in all of the five tested pairs (Fig. 4*A*), indicating that the inward currents were mediated by GABA_A receptors.

Summarized in Fig. 4*B* are the data from the pairs where the pyramidal neurons responded significantly to the ACh puffed onto the interneurons. If the pairs were connected, ACh application onto the interneuron generated GABAergic synaptic activity in pyramidal neurons as indicated by the large amount of charge transferred by the GABA_A receptors. The ACh-induced responses showed large neuron to neuron variability possibly because of variations in the strength of the connection between the interneuron and the pyramidal neuron. The recorded ACh-induced responses were larger when the pyramidal neurons were perfused by patch electrodes containing 135 mM Cl^- as compared with 60 mM Cl^- (Fig. 4*B*).

Nicotinic pharmacology of the ACh-induced GABAergic synaptic activity

MLA (20 nM) is a specific antagonist of $\alpha 7$ -containing nAChR, and at a concentration of 25 μM , mecamylamine is a nonspecific nAChR antagonist. In three of four experiments, ACh-induced GABAergic synaptic currents recorded from pyramidal neurons were blocked by MLA (Fig. 5*A*), suggesting that most responses were initiated by $\alpha 7$ -containing nAChRs. In one experiment, however, MLA only partially blocked the ACh-induced GABAergic response recorded from the pyramidal neuron (Fig. 5*B*). After the GABAergic response recovered from the MLA blockade, it was abolished completely by the subsequent application of mecamylamine (Fig. 5*B*). The latter experiment indicates that both $\alpha 7$ -containing and non- $\alpha 7$ nAChRs were activated and were capable of contributing significantly to the ACh-induced GABAergic activity.

TABLE 1. *Electrophysiological properties of ACh-sensitive and -insensitive neurons in CA1 stratum radiatum*

Cell Type	<i>n</i>	Resting Potential, mV	Firing Frequency, Hz	Fast-AHP, mV	Sag Ratio	Input Resistance, M Ω	Slow-AHP, mV
ACh-sensitive neurons	8	-60.8 ± 2.1	100.8 ± 8.2	-14.2 ± 1.2	0.82 ± 0.03	390 ± 37	-7.0 ± 1.4
ACh-insensitive neurons	4	-62.5 ± 1.5	58.4 ± 4.4	-1.9 ± 0.3	0.75 ± 0.01	139 ± 12	-4.6 ± 0.6
<i>P</i> value*		0.570	0.001	<0.001	0.107	<0.001	0.138

Values are means \pm SE. * *P* values are the results of Student's *t*-test.

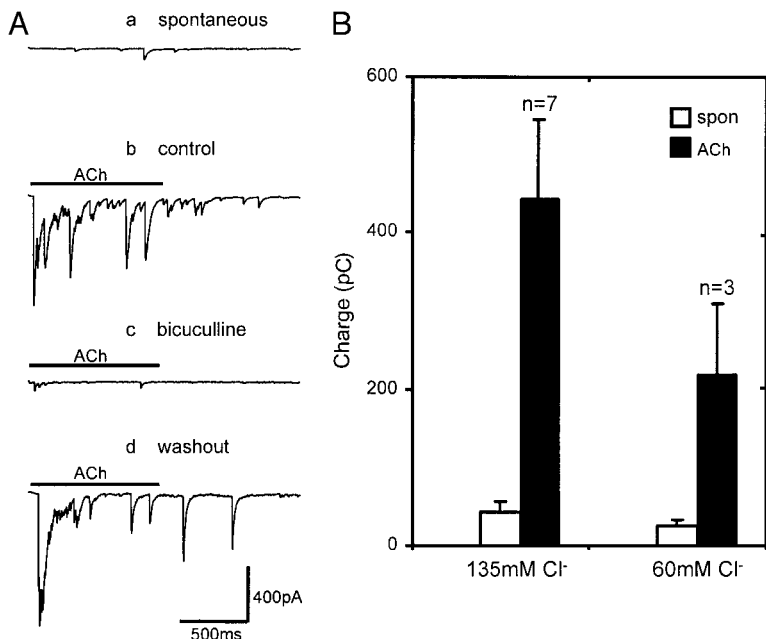


FIG. 4. ACh applied to interneurons in the CA1 s. radiatum produced GABA_A-mediated inhibition of pyramidal neurons. 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 25 μ M) and (\pm 12)-2-amino-5-phosphonopivalic acid (AP-5, 50 μ M) were present in the bath solution. *A*: 1-s trace of spontaneous activity without ACh application. *a*: 2-s trace of spontaneous activity without ACh application. *b*: current response of the pyramidal neuron while 200 μ M ACh was being applied to the s. radiatum interneuron. *c*: blockade of the ACh-induced response by 10 μ M bicuculline. *d*: recovery of the ACh-induced response after a 5-min washout of bicuculline. \blacksquare , duration of the ACh application onto the interneuron. *B*: ACh-application onto interneurons induced GABA_A activity in pyramidal neurons. ACh-induced GABA_A-mediated charge transfer averaged from 7 pyramidal neurons recorded with 135 mM internal Cl⁻ and from 3 pyramidal neurons recorded with 60 mM internal Cl⁻. In these experiments, 200 μ M ACh was applied for 1 s. For comparison, the average charge transfer is also shown for the spontaneous traces (spon) obtained without applying ACh.

Pyramidal neuron responses arose directly from ACh-induced interneuron action potentials

We studied the TTX sensitivity of the ACh-induced GABAergic synaptic currents measured from pyramidal neurons. Bath application of 0.5 μ M TTX blocked the GABAergic synaptic activity induced in pyramidal neurons by ACh application onto interneurons (Fig. 6, $n = 3$). The synaptic response recovered after washout of TTX for 30 min. The data indicate that action potentials arising from the interneurons were required for the ACh-induced effect. Under our experimental conditions, the results suggest that the GABAergic synaptic

activity recorded from the pyramidal neurons arose from the direct excitation of GABAergic interneurons by nAChRs.

ACh-induced interneuron activity can produce disinhibition of pyramidal neurons

During most of this study, we recorded from pyramidal neurons with low spontaneous GABAergic activity. Under those conditions, the most common effect of the ACh application onto interneurons was GABA_A-mediated inhibition of the pyramidal neurons. However, we also recorded a disinhibition of one pyramidal neuron (Fig. 7) that displayed strong spon-

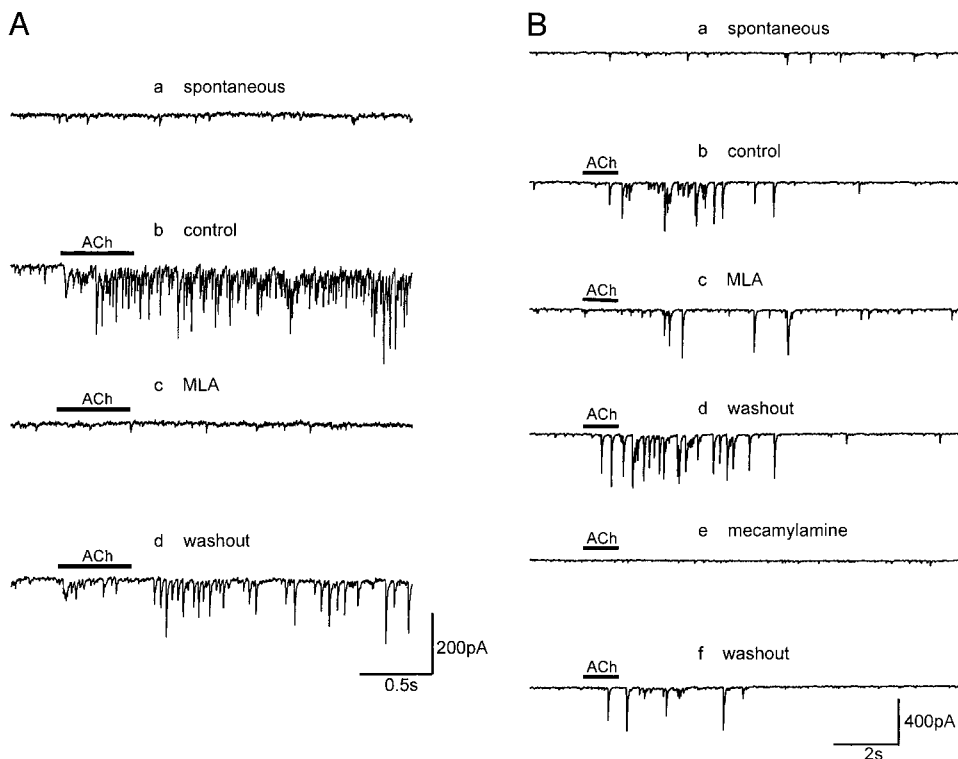


FIG. 5. Nicotinic antagonists blocked the ACh-induced GABAergic synaptic activity recorded from pyramidal neurons. CNQX (25 μ M) and AP-5 (50 μ M) were present in the bath solution. Patch-pipette solution contained 60 mM Cl⁻. *A*: ACh-induced effect recorded from a pyramidal neuron was blocked by 20 nM MLA. *a*: trace of spontaneous GABA activity. *b*: response recorded from the pyramidal neuron while ACh (1 mM) was pressure-applied to an interneuron. *c*: blockade of the response by 20 nM MLA. *d*: partial recovery of the response after washout of MLA. *B*: ACh-induced effect recorded from another pyramidal neuron was blocked partially by 20 nM MLA but was blocked completely by 25 μ M mecamylamine. *a*: trace of spontaneous GABA activity. *b*: response recorded from the pyramidal neuron while ACh (1 mM) was pressure-applied to an interneuron. *c*: partial blockade of the response by 20 nM MLA. *d*: recovery of the response after washout of MLA. *e*: complete blockade of the response by 25 μ M mecamylamine. *f*: partial recovery of the response after washout of mecamylamine. \blacksquare , duration of the agonist application.

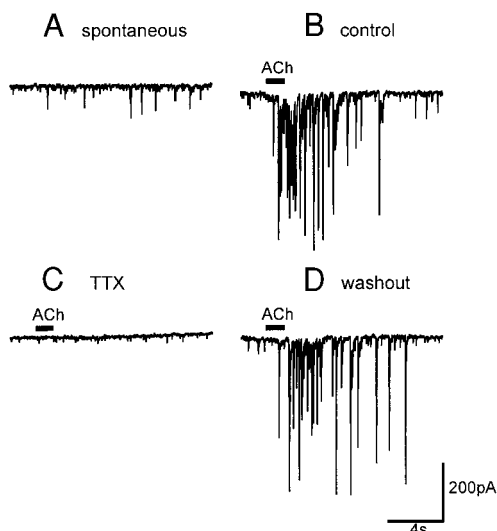


FIG. 6. ACh-induced GABAergic activity recorded from pyramidal neurons was TTX sensitive. CNQX (25 μ M) and AP-5 (50 μ M) were present in the bath solution, and 60 mM Cl^- was included in the patch pipette. *A*: trace of spontaneous GABA activity. *B*: response recorded from the pyramidal neuron while ACh (1 mM) was pressure-applied to an interneuron. *C*: blockade of the ACh-induced response by 0.5 μ M TTX. Also note that TTX blocked the spontaneous, action-potential-dependent synaptic currents but not the small currents arising from the quantal release of GABA. *D*: recovery of the response after washout of TTX. ■, duration of the agonist application.

taneous GABAergic activity in the presence of CNQX and AP-5. A 1-s pressure application of ACh onto an interneuron potentially reduced the spontaneous GABAergic activity. The spontaneous activity recovered from the ACh application in a few seconds. Bath application of 20 nM MLA partially blocked the ACh-induced reduction of spontaneous GABAergic activity. After MLA was removed from the bath and the effect recovered, subsequent application of 25 μ M mecamylamine totally blocked the ACh-induced disinhibition. Finally, the spontaneous activity was completely abolished by 10 μ M bicuculline, indicating that it was mediated by GABA_A receptors. These results suggest that activation of nAChRs on s. radiatum interneurons also can disinhibit pyramidal neurons by reducing tonic GABA_A receptor mediated inhibition.

DISCUSSION

Nicotinic responses were recorded from interneurons located in the CA1 s. radiatum. The majority of the currents evoked by ACh were MLA sensitive, indicating that most of the nAChRs contain the $\alpha 7$ subunit. In the s. radiatum of the CA1 region, the ACh-sensitive neurons were GABAergic, and most of them fired action potentials in response to ACh application. Localized ACh application onto interneurons produced TTX-sensitive, GABA_A receptor-mediated inhibition of pyramidal neurons. The ACh-induced inhibition was mostly mediated by $\alpha 7$ -containing nAChRs and, to a lesser degree, by non- $\alpha 7$ nAChRs. Our data also indicate that activation of $\alpha 7$ -containing and non- $\alpha 7$ receptors could produce disinhibition of pyramidal neurons by suppressing tonic GABA_A activity. Our experimental conditions enabled us to observe the GABAergic activity as an inward current. With lower internal chloride, the same number of GABA_A receptors would open to hold the cell near the chloride reversal potential, which is near the resting

potential. In that case, however, the GABA_A activity would not be observed as the inward currents shown in Figs. 4–7. The results of this work show that nAChR activity on interneurons has the capacity to produce pronounced inhibition or disinhibition in pyramidal neurons. Thus nicotinic receptors in the CA1 region have the ability to influence the activity of hippocampal circuits.

ACh-sensitive and -insensitive neurons in CA1 s. radiatum

In all of our experiments, muscarinic receptors were inhibited by atropine. ACh application induced nicotinic currents in the majority (82%) of s. radiatum neurons (also see Alkondon et al. 1998, 1999; Frazier et al. 1998a,b; Hefft et al. 1999; Jones and Yakel 1997; McQuiston and Madison 1999). ACh-sensitive and -insensitive neurons in CA1 s. radiatum displayed distinctive electrophysiological properties. ACh-sensitive neurons displayed electrical properties indicative of GABAergic interneurons: high firing frequency, large fast-AHP, and high-input resistance (Lacaille et al. 1987; Schwartzkroin and Mathers 1978). The ACh-insensitive neurons, on the other hand, had a low firing frequency, a small fast-AHP, and a lower input resistance. Most ACh-insensitive neurons electrophysiologically resemble a cell type referred to as s. radiatum

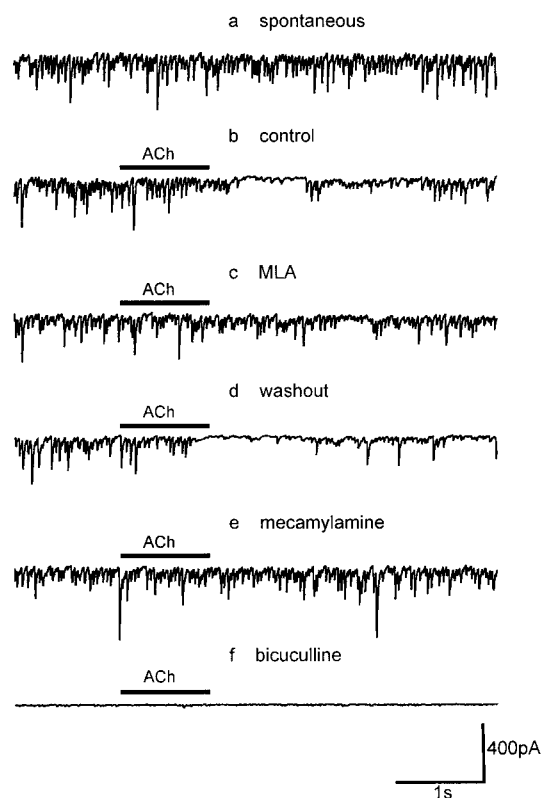


FIG. 7. ACh applied onto an interneuron in the CA1 s. radiatum produced disinhibition of a pyramidal neuron. CNQX (25 μ M) and AP-5 (50 μ M) were present in the bath solution, and 60 mM Cl^- was included in the patching pipette. *a*: trace of spontaneous GABA activity recorded from the pyramidal neuron. *b*: reduction of the spontaneous GABAergic synaptic activity produced by pressure application of ACh (1 mM) onto an interneuron in CA1 s. radiatum. *c*: partial blockade of the ACh-induced reduction by 20 nM MLA. *d*: recovery of the ACh-induced reduction after washout of MLA. *e*: blockade of the ACh-induced reduction by 25 μ M mecamylamine. *f*: blockade of the spontaneous activity by 10 μ M bicuculline. ■, duration of the agonist application.

giant cells (Maccaferri and McBain 1996). Similar to pyramidal neurons and different from other cell types in this region, this cell type is capable of undergoing direct long-term potentiation (LTP). Although most neurons in this region are identified as GABAergic neurons by glutamic acid decarboxylase immunoreactivity (Ribak et al. 1978; Woodson et al. 1989), we cannot exclude the possibility that the ACh-insensitive neurons are a small percentage of excitatory pyramidal neurons displaced into the nearby s. radiatum.

Subtypes of nicotinic receptors in the hippocampus

Our results suggest that the major subtype of nAChR on CA1 s. radiatum interneurons are $\alpha 127$ -containing receptors. Hybridization studies indicated that $\alpha 127$ and $\beta 2$ are widely expressed and that other subunits also are present in the hippocampus (Rubboli et al. 1989; Wada et al. 1993). In cell cultures from the rat hippocampus, fast Type I currents arise from $\alpha 7$ -containing nAChRs (Alkondon and Albuquerque 1993; Alkondon et al. 1994; Zarei et al. 1999). Slower Type II and Type III currents were only recorded from a small percentage of cultured neurons. Although still rare, slow currents were more common in our slice study than in cell culture. In the slice preparation, however, agonist applications are more variable and may not always reveal the true kinetics, especially when the kinetics are fast. Sensitivity to selective antagonists, such as MLA, is a better method to determine the nAChR subtype. In our experiments, only 3 of 23 neurons showed a slow residual current after the MLA blockade. These findings are consistent with other studies (Alkondon et al. 1999; Frazier et al. 1998b; McQuiston and Madison 1999). When pyramidal neurons were recorded and ACh was applied to the interneurons, MLA-insensitive effects were observed in two of five connected pairs.

Inhibition and disinhibition of pyramidal cells by nAChRs on interneurons

Interneurons in the s. radiatum impose powerful inhibition on pyramidal neurons (Sik et al. 1995). There are several types of interneurons in this region that play different roles in hippocampal circuits (Freund and Buzsaki 1996; Miles et al. 1996). Basket cells and axon-axonic cells mainly innervate the soma, the proximal dendrites, and the axon initial segments of principal neurons. Many interneurons in s. radiatum innervate distal dendrites, whereas some interneurons only innervate other interneurons. Because the majority of interneurons in this region responded to ACh application, many different types of interneurons could be activated by nAChRs. Thus the modulation of hippocampal circuits by nAChRs could be complicated and varied. Our study demonstrates that local application of ACh onto interneurons can cause both inhibition and disinhibition of pyramidal neurons. The ACh-induced inhibition and disinhibition were mediated by the direct excitation of interneurons by mainly somatic nAChRs, but our recording paradigm could not prevent the agonist from reaching some preterminal or presynaptic nAChRs. The location of our agonist puffer very near the interneuron soma and the TTX sensitivity of the effect argue that somal nAChRs were the primary site of action in this study. Most probably, activating nAChRs on those interneurons that directly innervate pyramidal neurons

causes the inhibition, and activating nAChRs on those interneurons that innervate other interneurons causes the disinhibition. ACh activation of interneurons that then inhibit other interneurons would release some pyramidal neurons from some of their inhibitory inputs (i.e., disinhibition). Such an indirect excitation of pyramidal neurons is supported by a study that shows nAChR activation can increase GABA activity in some interneurons (see Alkondon et al. 1999).

Roles of nAChRs in learning and memory

One possible role for nAChRs is to influence synaptic plasticity in the hippocampus. LTP and long-term depression (LTD) are candidates for the cellular mechanism of learning and memory. Postsynaptic depolarization is required for certain forms of hippocampal LTP (Larkman and Jack 1995; Magee and Johnston 1997; Markram et al. 1997), and depolarization of the pyramidal neurons is strongly shaped by inhibition. In that way, LTP and LTD are influenced by the GABAergic activity (Paulsen and Moser 1998; Steele and Mauk 1999; Wallenstein and Hasselmo 1997), and our results show that nAChR activity can excite interneurons and inhibit or disinhibit pyramidal neurons. Therefore nAChRs have the capacity to influence LTP/LTD by activating interneurons and, thereby, decreasing or increasing the postsynaptic depolarization of the pyramidal neurons. This modulatory influence of nAChRs will depend on the strength, timing, and connectivity of the interneurons excited by nicotinic activity.

Another possible role for nAChRs is to affect the rhythmic activity in the hippocampus. One prominent property of hippocampal circuits is the production of different rhythmic oscillations. Theta rhythm (4–12 Hz) and gamma activity (40–100 Hz) often occur during paradoxical sleep or while awake rats explore their environments (Chrobak and Buzsaki 1998; Vanderwolf 1969). These rhythms provide important windows for synaptic plasticity underlying learning (Huerta and Lisman 1993, 1995). It is also known that hippocampal interneurons are important for pacing the rhythmic oscillations, which are modulated by septal inputs (Csicsvari et al. 1999; Dragoi et al. 1999; Freund and Buzsaki 1996; Stewart and Fox 1990). Because nicotinic activity can directly excite interneurons (Alkondon et al. 1998; Frazier et al. 1998a; Hefft et al. 1999) and exert inhibition or disinhibition on principal neurons, nAChRs could influence the rhythmic activity of the hippocampus. Although CA1 interneurons and pyramidal neurons may be capable of producing intrinsic theta oscillation (Chapman and Lacaille 1999; Leung and Yim 1991), they need to be driven near the firing threshold to generate the oscillation. The excitatory nAChRs on interneurons have the capacity to depolarize the interneurons toward their firing threshold and to drive the pyramidal neurons back toward their resting potential. In this way, nAChRs could influence the rhythmic activity in the hippocampus and participate in synaptic mechanisms underlying learning and memory.

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