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α7 Nicotinic acetylcholine receptors and modulation of gabaergic synaptic transmission in the hippocampus

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Abstract

The present report provides new findings regarding modulation of γ-aminobutyric acid (GABA) transmission by α7 nicotinic receptor activity in CA1 interneurons of rat hippocampal slices. Recordings were obtained from tight-seal cell-attached patches of the CA1 interneurons, and agonists were delivered to the neurons via a modified U-tube. Application for 6 s of the α7 nicotinic receptor-selective agonist choline (≥ 1 mM) to all CA1 interneurons tested triggered action potentials that were detected as fast current transients. The activity triggered by choline terminated well before the end of the agonist pulse, was blocked by the α7 nicotinic receptor antagonist methyllycaconitine (50 nM) and was concentration dependent; the higher the concentration of choline the higher the frequency of events and the shorter the delay for detection of the first event. In 40% of the neurons tested, choline-triggered action potentials decreased in amplitude progressively until no more events could be detected despite the presence of the agonist. Primarily, this finding could be explained by Na^+-channel inactivation associated with membrane depolarization induced by α7 nicotinic receptor activation. In 60% of the neurons, the amplitude of choline-induced action potentials was sustained at the intial level, but again the activity did not last as long as the agonist pulse, in this case apparently because of agonist-induced receptor desensitization. These results altogether demonstrate that agonists interacting with α7 nicotinic receptors, including the natural transmitter acetylcholine and its metabolite choline, influence GABAergic transmission, not only by activating these receptors, but also by controlling the rate of Na^+-channel inactivation and/or by inducing receptor desensitization.

Keywords: Hippocampus; Choline; Interneuron; Nicotinic receptor; Excitation; Methyllycaconitine; GABA (γ-aminobutyric acid)

1. Introduction

An increasing body of evidence supports the involvement of neuronal nicotinic acetylcholine receptors in normal health, disease states, and cigarette smoking, and a variety of experimental approaches have been used to unveil the diversity and function of these receptors. Nicotinic acetylcholine receptors that contain α7 subunits are prevalent in the mammalian brain, and have received special attention because of their linkage to schizophrenia and cognitive functions (Leonard et al., 1996; Freedman et al., 1997; Levin and Simon, 1998). The availability of pharmacological tools such as α-bungarotoxin, methyllycaconitine, and choline greatly facilitated the identification and characterization of α7 nicotinic receptors in various regions of the peripheral and central nervous systems (Alkondon et al., 1992; Alkondon and Albuquerque, 1993; Papke et al., 1996; Yum et al., 1996; Alkondon et al., 1997, 1999). Further, the use of systems that allow for rapid agonist application with minimal leak enabled successful and reproducible recordings of α7 nicotinic receptor-mediated responses from neurons in culture as well as in slices (Alkondon and Albuquerque, 1993; Alkondon et al., 1999). Electrophysiological studies have shown that

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α7 nicotinic receptors have diverse functions including mediation of fast synaptic transmission (Ullian et al., 1997; Alkondon et al., 1998; Frazier et al., 1998; Chang and Berg, 1999) and modulation of synaptic transmission mediated by transmitters such as acetylcholine, glutamate and γ-aminobutyric acid (GABA) (McGehee et al., 1995; Gray et al., 1996; Alkondon et al., 1996, 1997, 1999).

The α7 nicotinic receptors exhibit unique features such as rapid desensitization upon exposure to agonists, high calcium permeability, large single-channel conductance and brief mean channel open time (Castro and Albuquerque, 1993, 1995; Mike and Albuquerque, 1999; Arpad Mike, Newton G. Castro and Edson X. Albuquerque, manuscript in preparation). Choline, a precursor and a metabolite of acetylcholine, activates α7 nicotinic receptors to the same extent as does acetylcholine, and induces desensitization at micromolar concentrations (Papke et al., 1996; Alkondon et al., 1997, 1999; Mike and Albuquerque, 1999). Also, it has been observed that single-channel conductance and mean open time of α7 nicotinic receptor channels in hippocampal neurons are similar regardless of whether the agonist is choline or acetylcholine (Mike et al., 1999). It remains to be demonstrated, however, whether both acetylcholine and choline interact with the native α7 nicotinic receptor under in vivo conditions. Further, it is unclear whether α7 nicotinic receptors mediate their functions in vivo via only ionotropic mechanisms (Na^+-flux leading to excitation) or also via Ca^{2+}-flux-related metabotropic pathways. To gain further insights regarding the function of α7 nicotinic receptors and the role of choline in controlling the functions of α7 receptors, we have evaluated the consequences of activation of these receptors in a simple experimental paradigm that mimics normal physiological conditions. In this study, using tight-seal, cell-attached recordings, choline is shown to modulate the excitability of rat hippocampal CA1 interneurons.

2. Materials and methods

2.1. Recordings from hippocampal slices

Slices (250-μm thickness) of the rat hippocampus were prepared from 15- to 26-day-old rats according to the methods described earlier (Alkondon et al., 1999). Whole-cell voltage-clamp and current-clamp recordings were also performed as described previously using Cs-methanesulfonate-containing and K-gluconate-containing pipette solutions, respectively (Alkondon et al., 1999). For the cell-attached recordings, a Cs-methanesulfonate-containing internal solution was used. Slices were continuously perfused with artificial cerebrospinal fluid (ACSF) bubbled with 95% O_2 and 5% CO_2 that had the following composition (in mM): NaCl, 125; $NaHCO_3$, 25; KCl, 2.5; NaH_2PO_4, 1.25; $CaCl_2$, 2, $MgCl_2$, 1; and glucose, 25. Agonists were applied to the neurons via a U-tube (Alkondon et al., 1999). Processing and drawing of biocytin-filled interneurons were done according to the methods described earlier (Alkondon et al., 2000).

2.2. Recordings from hippocampal neurons in culture

Cultures of hippocampal neuron were prepared according to the methods described earlier (Alkondon and Albuquerque, 1993). Neurons were continuously perfused with an external solution which had the following composition (in mM): NaCl, 165, KCl, 5, $CaCl_2$, 2; glucose, 10 and HEPES 5, pH adjusted to 7.3 using NaOH. Whole-cell voltage-clamp recordings were obtained from individual neurons that were synaptically connected to neighboring neurons. Field stimulation was achieved using a bipolar platinum electrode placed within a distance of about 100 μm from the recording electrode. Supramaximal voltage pulses (~10 V) were applied at 20 μs duration to evoke synaptic responses. Glutamate currents were eliminated by addition of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10 μM) and DL-2-amino-5-phosphonovaleric acid (APV; 50 μM) to the external solution. Muscarinic receptors were blocked using 1 μM atropine. All recordings were done at room temperature.

2.3. Data analysis

Data were sampled and analyzed using the pClamp 6 program (Axon Instruments, CA).

3. Results

Most experiments were carried out on interneurons located in the stratum radiatum of the CA1 field of hippocampal slices. Neurons were initially identified as interneurons based on their location and morphology using live infrared images. Post-recording reconstruction of biocytin-filled neurons (Fig. 1), and the appearance of large after-hyperpolarizations in the action potential transients (see Fig. 2), also confirmed the identity of the interneurons. The interneuron illustrated in Fig. 1 had dendrites in the stratum radiatum, and the axon projecting to stratum radiatum, stratum pyramidale and stratum oriens.

Application of choline (10 mM) to voltage-clamped interneurons induced inward currents (Fig. 2A). These inward currents persisted in the presence of tetrodotoxin (200 nM), CNQX (10 μM), APV (50 μM), and bicuculline (10 μM), indicating that they originated from the activation of nAChRs on the somatodendritic membrane of the interneurons.

In current-clamped interneurons, choline (10 mM) elicited a short-lasting burst of action potentials (Fig. 2B) that persisted in the presence of the $GABA_A$ receptor antagonist bicuculline. However, in many interneurons, in the absence of bicuculline, a high frequency of sponta-

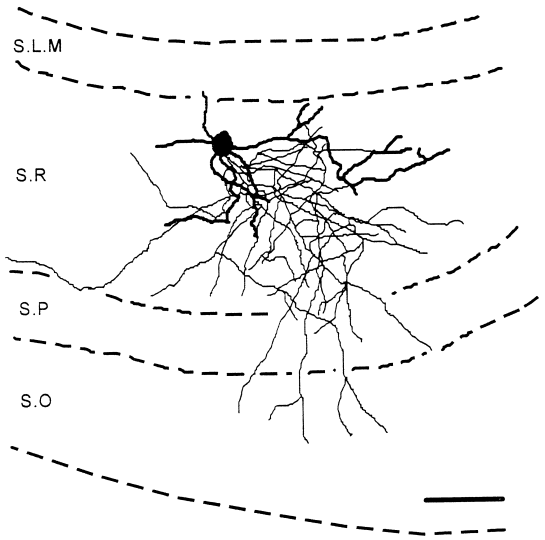


Fig. 1. Morphology of a rat hippocampal CA1 interneuron. Neurolucida drawing of a biocytin-filled interneuron in the CA1 field of a hippocampal slice is shown. The cell body is indicated by the largest filled object, the dendrite is represented by thick processes, and axons by thin processes. Dashed lines are also drawn to indicate different layers of the hippocampus. S.L.M = stratum lacunosum moleculare; S.R = stratum radiatum; S.P = stratum pyramidale; S.O = stratum oriens. Calibration bar = 100 μm.

neous action potentials made it difficult to analyze the choline effects. The firing of spontaneous action potentials was inevitable under the present experimental conditions in the absence of bicuculline, because spontaneous GABAergic synaptic currents could easily depolarize the neuron.

To circumvent the depolarizing action of GABA, keep the intracellular milieu intact, and investigate the receptor function in a near-physiological condition, we studied the effects of α7 nicotinic receptor activation using tight-seal cell-attached recordings. Application of choline to the interneurons induced a burst of action potentials that were detected as fast current transients in voltage-clamped cell-attached membrane patches (Fig. 2C). Current transients in the cell-attached patches appeared as inverted action potentials (see traces in Fig. 2D and E), and the after-hyperpolarization component, which is typically observed in interneurons, was seen as prominent outward currents in the cell-attached recordings (Fig. 2E).

The minimal concentration of choline that was effective in eliciting action potentials was also assessed in cell-attached recordings (Fig. 3). Choline at 0.1 and 0.3 mM failed to induce action potentials ($n = 10$ neurons). However, at ≥ 1 mM, it produced action potentials in all the neurons tested ($n = 25$ neurons). The effect of choline was concentration dependent. With increasing concentration of choline, the latency of the first action potential decreased. Further, the number of action potentials was higher with 10 mM choline than with 1 mM choline. Pre-exposure of the neurons to methyllycaconitine (50 nM) inhibited choline-induced action potentials completely, confirming the involvement of α7 nicotinic receptors in this process.

Although choline (10 mM) triggered action potentials in all of the interneurons tested, the kinetics of activation of the neurons by choline were different. In about 40% of the

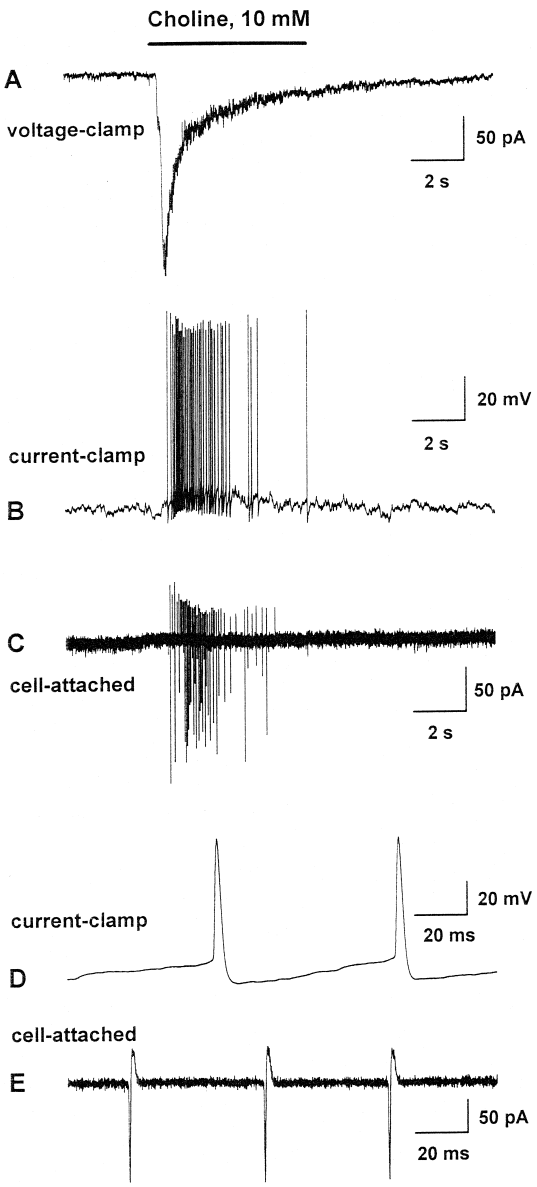


Fig. 2. Experimental conditions to study α7 nicotinic receptor activation in CA1 interneurons in hippocampal slices. (A) Fast-decaying nicotinic current recorded under whole-cell voltage-clamp mode at −68 mV. (B) Burst of action potentials induced by nicotinic receptor activation in a whole-cell current-clamped neuron. (C) Burst of fast current transients (representing action potentials) recorded from neurons under cell-attached configuration. Pipette potential was held at −60 mV. (D) Action potentials recorded from a current-clamped neuron are shown on an expanded scale. (E) Fast current transients recorded in the cell-attached mode are shown on an expanded scale. Note that the after hyperpolarization component of the action potential appears as an outward current in this mode. Choline was applied to the interneurons via a U-tube for 6 s as indicated by the solid line at the top.

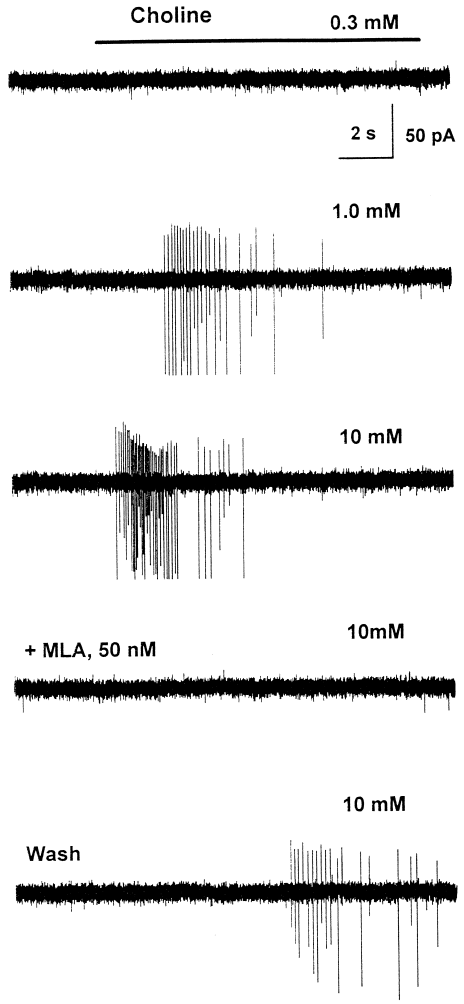


Fig. 3. Choline excites CA1 interneurons in hippocampal slices via activation of α7 nicotinic receptors. Different concentrations of choline were applied to an interneuron (12-s pulse each; solid line at the top) during a cell-attached recording. No response was seen at 0.3 mM, but at 1 and 10 mM, fast current transients were induced. The number of transients was higher and the delay to detect the first transient was shorter at 10 mM than at 1 mM choline. Superfusion of the slice with methyllycaconitine (MLA; 50 nM) for 5 min prevented the action of choline completely, and a partial reversal of the effect of choline was obtained after a 5-min wash.

interneurons tested, choline induced a robust train of action potentials, and the amplitude of choline-triggered transients decreased progressively until no more events could be detected despite the continued presence of the agonist (Fig. 4). In the other 60%, choline induced isolated action potentials; the transients had invariable amplitude and faded away well before the end of the agonist pulse (Fig. 5). However, in both groups of neurons, quisqualate, an agonist for α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, induced trains of action potential transients that exhibited attenuation in amplitude with time of exposure to the agonist. The differences in the effects of choline between the two groups of interneurons argue in favor of differences in α7 nicotinic receptor density and distribution along the neuronal surface. In the interneurons that responded to choline with transients that progressively decreased in amplitude, α7 nicotinic receptors are likely to be present in high density along the neuronal surface. Then, attenuation of the amplitude of the action potential transients could be attributed to the inactivation of Na^+ channels resulting from a strong depolarizing action of choline. It is noteworthy that CA1 interneurons responded similarly to quisqualate, and that this agonist, by activation of AMPA-type glutamate receptors, induces a strong depolarization in the current-clamp

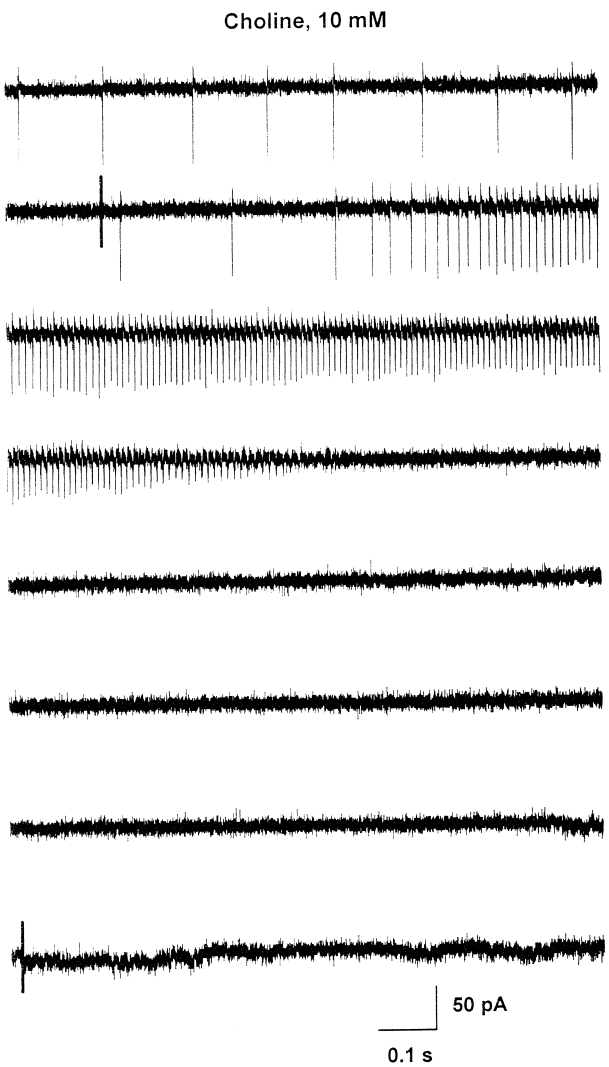


Fig. 4. Choline-induced activation of α7 nicotinic receptors results in inactivation of Na^+ channels. Sample recording obtained from an interneuron under cell-attached configuration. In this neuron, choline induced a robust burst of fast current transients. The amplitude of the transients decreased progressively, and no events could be detected well before the end of the agonist pulse. This attenuation is most likely caused by the depolarization-induced inactivation of Na^+ channels. The duration of application of choline was 6 s, and the start and end of the pulse are indicated by two solid vertical bars in the traces.

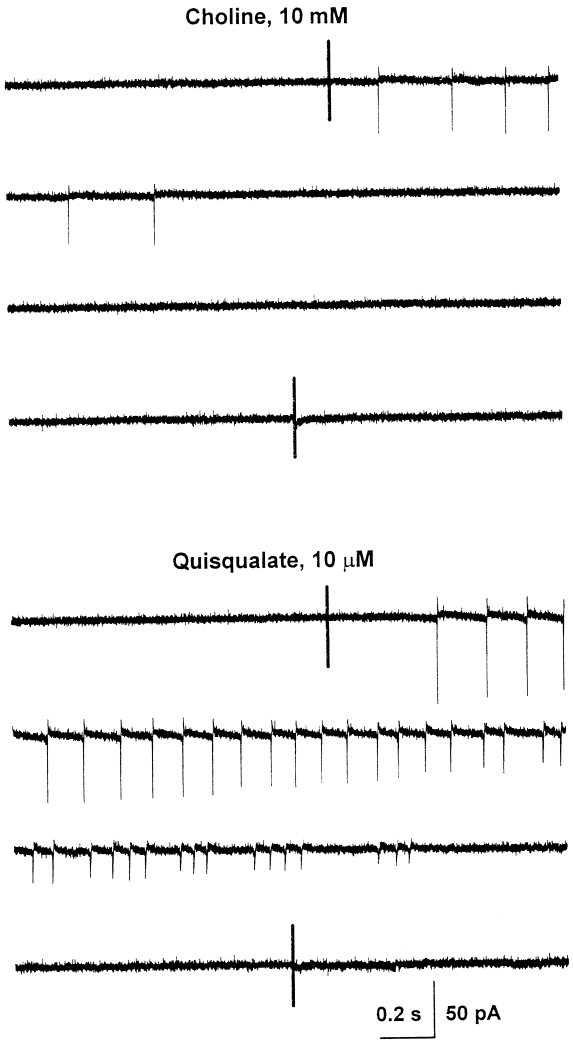


Fig. 5. Choline desensitizes α7 nicotinic receptors prior to the onset of Na^+ channel inactivation in interneurons of hippocampal slices. Sample recordings obtained from an interneuron under cell-attached configuration. In this neuron, quisqualate, but not choline, induced a burst of attenuating action potentials. The start and end of agonist pulses are indicated by two solid vertical bars for each set of traces.

recordings (data not shown). On the other hand, in the interneurons that responded to choline with isolated, non-attenuating fast-current transients, α7 nicotinic receptors are probably at low density on the neuronal surface. In that case, termination of the events could be accounted for by agonist-induced desensitization of α7 nicotinic receptors.

To determine whether choline-induced attenuation of action potential amplitude affects synaptic transmission in the hippocampus, hippocampal neurons in culture rather than in slice were used, because α7 nicotinic receptors are abundantly expressed in cultured neurons (Alkondon and Albuquerque, 1993), and it is easier to record from one neuron while stimulating a nearby neuron in culture. In the cultured neurons, field stimulation of neurons synapsing onto the neuron under study in culture evoked GABAergic synaptic currents in the presence of blockers of glutamate receptors. Evoked GABAergic postsynaptic currents remained fairly stable in amplitude for more than 1 h at stimulation frequencies of 1 Hz under control conditions. However, when a short (1 s) pulse of choline (10 mM) was applied to a large field of neurons including the neuron under study, there was a reduction of the amplitude of evoked GABAergic currents, an induction of nicotinic postsynaptic currents, and an increase in the frequency of spontaneous GABAergic synaptic currents (Fig. 6). The inhibitory effect of choline on the evoked GABAergic currents reversed within a min of continuous washing of the neuron with physiological solution (Fig. 6), and could be reproduced several times in the same neuron. Further, the inhibitory effect of choline on the evoked GABAergic responses was concentration dependent between 1 and 10 mM, and could be observed both at negative and at positive membrane potentials (see traces in Figs. 6 and 7). A pre-application of methyllycaconitine (1 nM) inhibited

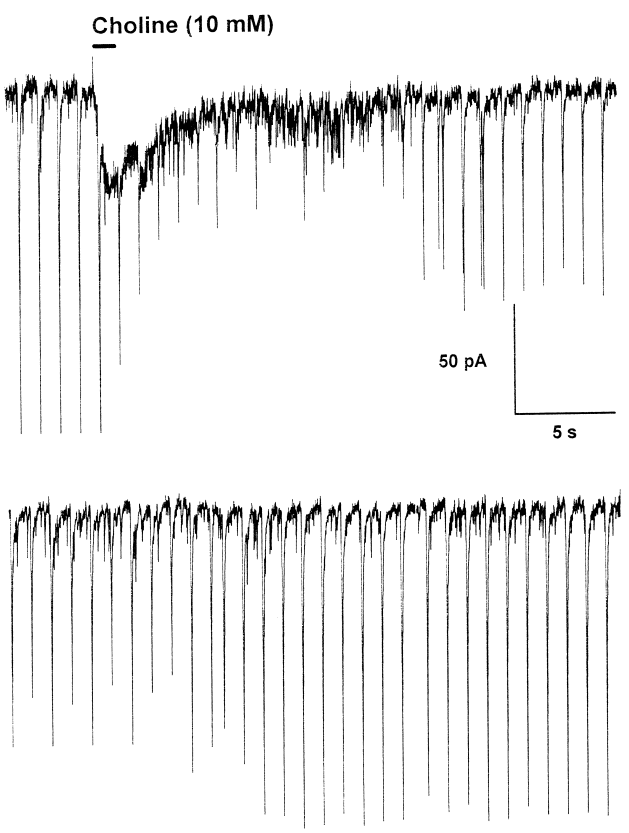


Fig. 6. Choline inhibits evoked GABAergic synaptic transmission in cultured hippocampal neurons. Sample recordings obtained from a cultured hippocampal neuron under whole-cell voltage-clamp configuration. GABAergic synaptic currents were induced at 1 Hz by field stimulation of nearby neurons in the presence of the glutamate blockers CNQX (20 μM) and APV (50 μM) at −60 mV. U-tube application of 1 s pulse of choline induced an inward nicotinic current along with an increase in the number of miniature postsynaptic currents, but suppressed the amplitude of evoked GABAergic synaptic currents. This inhibition was reversed within a min of washing of the neuron with choline-free solution.

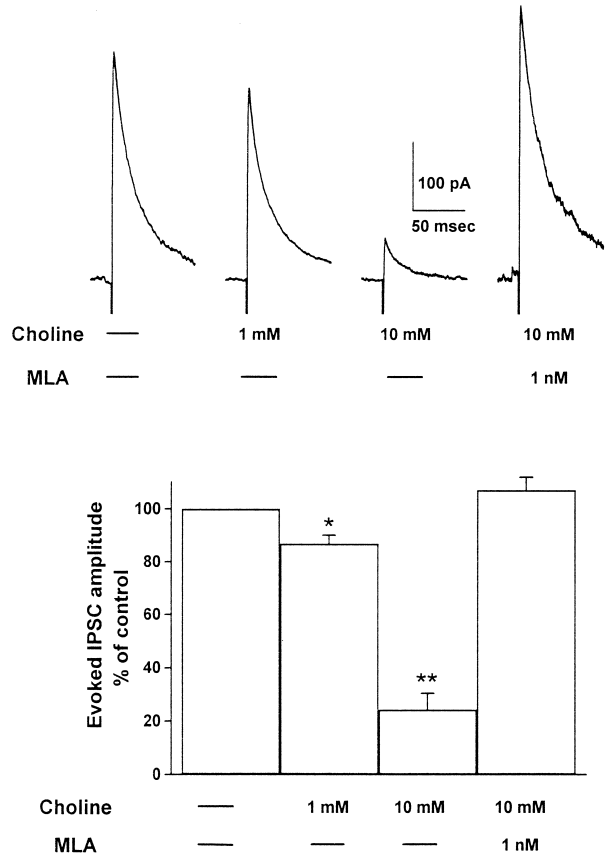


Fig. 7. Choline inhibits GABAergic synaptic transmission via activation of α7 nicotinic receptors. Evoked GABAergic synaptic currents were recorded at +40 mV from a hippocampal neuron in culture. Inhibitory effect of 1 and 10 mM choline, and the ability of methyllycaconitine (MLA) to prevent the inhibitory effect of choline are shown. Results obtained from five neurons are shown on the bottom as mean ± S.E.M (*, $p < 0.05$; **, $p < 0.01$, according to the student's t-test).

completely the ability of choline to suppress evoked GABAergic synaptic transmission (Fig. 7), confirming the involvement of α7 nicotinic receptors.

4. Discussion

The present study highlights the mode of excitation of interneurons in a nearly physiological condition by choline-induced activation of α7 nicotinic receptors. Choline triggered action potentials in interneurons, and this effect was concentration dependent. Desensitization of α7 nicotinic receptors curtailed the spike activity induced by choline in most neurons, but in a sizable population of interneurons desensitization was preceded by Na^+ channel inactivation, which abbreviated the active propagation of the action potentials. Evidence is provided, suggesting that choline-induced inactivation of the Na^+ channels is responsible for inhibition of synaptic transmission between groups of neurons that express α7 nicotinic receptors. The various facets of action of choline and the multiple functions of α7 nicotinic receptors are discussed below.

4.1. Advantages of cell-attached recordings

Cell-attached recording from a patch of voltage-clamped soma membrane of an interneuron allowed the detection of action potentials as fast current transients without interference from other synaptic currents. The waveform of the current transients resembled an inverted action potential recorded in the current-clamp mode, but more importantly showed an after-hyperpolarization component as distinct outward current. These features enable one to discern interneurons from pyramidal neurons, because the latter group of neurons do not exhibit a large after-hyperpolarization component (Morin et al., 1996). Here, we confirmed the identity of interneurons electrophysiologically considering the waveform of the currents in cell-attached recordings, and morphologically, based on live infrared images of the neurons and their locations in the slices and on post-recording reconstruction of biocytin-filled neurons.

Neurons from which cell-attached recordings were obtained exhibited a low number of spontaneous spikes or no spikes, which permitted the analysis of spike-generating property of depolarizing agents with least interference from other factors. Most importantly, the intracellular ionic composition and the proteins and other structural elements remained intact in the cell-attached recordings, which makes this condition similar to normal physiological conditions than other conventional recording techniques. We demonstrated earlier that in whole-cell voltage-clamped neurons, the amplitudes of α7 nicotinic receptor-mediated type IA currents run down substantially with recording time because of the loss of intracellular high energy phosphates and other cellular components (Alkondon et al., 1994). Further, in the cell-attached recordings, to isolate the nicotinic responses, there was no need to use pharmacological agents, some of which are known to interact with the nicotinic receptors.

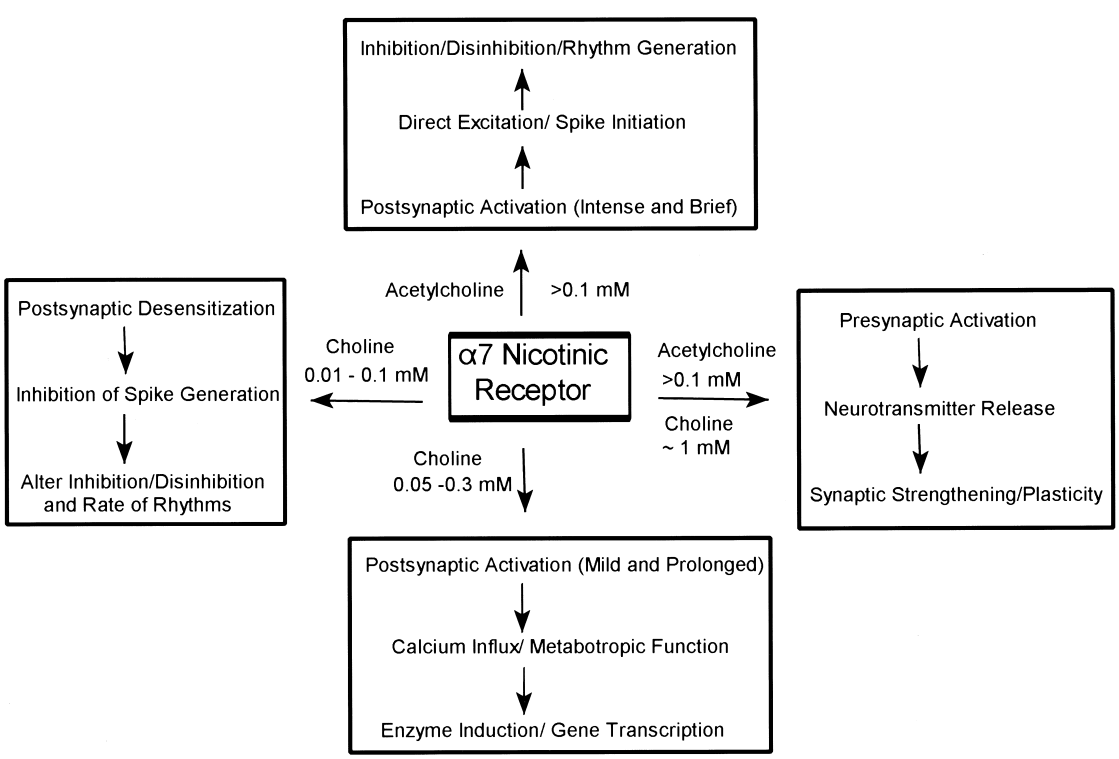
4.2. Significance of the concentration-dependence of choline-induced excitation of interneurons

The estimation of the concentration of choline that is necessary to induce spike activity in the interneurons can shed light on the role of endogenous choline in controlling the activity of α7 nicotinic receptors. The threshold agonist concentration was close to 1 mM, and concentrations of choline ≤ 300 μM were ineffective in inducing action potentials. Our previous studies indicated that choline can elicit a measurable macroscopic current in the hippocampal neurons even at concentrations ranging from 100 to 500 μM (Alkondon et al., 1997, 1999), and single channel experiments revealed that concentrations between 50 and 100 μM are also effective in activating α7 nicotinic

receptors (Arpad Mike, Newton G. Castro and Edson X. Albuquerque, unpublished data). In fact, recent results from our laboratory (Mike and Albuquerque, 1999) indicate that the total charge flowing through α7 nicotinic receptors is higher with moderate agonist concentration than with high agonist concentration. These observations suggest that at lower concentrations (50–300 μM) choline can activate α7 nicotinic receptors, but this activation is not sufficient to induce excitation/action potential generation in the interneurons. Also, it has been demonstrated that concentrations between 10 and 100 μM of choline can cause desensitization of α7 nicotinic receptors and cause changes in the expression of these receptors (Alkondon et al., 1997, 1999). Altogether, these various observations lead to the following general conclusions regarding the actions of choline. Three ranges of concentration of choline can be identified based on their reported effects. The lowest range (10 to 100 μM) causes receptor desensitization that can limit the frequency of cell firing due to synaptic α7 nicotinic receptor activation. The medium range (50 to 300 μM) causes mild and reasonably prolonged activation of α7 nicotinic receptors that can increase Ca^{2+} influx into the neurons, resulting in a cascade of metabotropic functions. The highest concentration range (≥ 1 mM) causes direct excitation of interneurons that can result in inhibitory/disinhibitory actions. This concentration is also effective in evoking neurotransmitter release in both an action potential-dependent and an action potential-independent manner (Alkondon et al., 1997, 1999; see Scheme 1).

4.3. Predicted mode of activation of α7 nicotinic receptors in vivo

Potentially choline can perform all the functions of acetylcholine at the α7 nicotinic receptors; however, acetylcholine is effective at nearly one-tenth the concentration of choline (Papke et al., 1996; Alkondon et al., 1997). Therefore, the endogenous concentrations of acetylcholine and choline, and the location of the α7 nicotinic receptors would dictate the manner in which the two substances would interact with the α7 nicotinic receptors in vivo. The observations that fast synaptic α7 nicotinic receptor-mediated currents are recorded in a low percent of interneurons, and that most interneurons respond to exogenous application of α7 nicotinic receptor agonists (Frazier et al., 1998; Alkondon et al., 1998, 1999), suggest that this nicotinic receptor subtype is located both synaptically and nonsynaptically. In the absence of any evidence that choline is released from the cholinergic nerve terminals, it is safe to assume that acetylcholine would be the primary neurotransmitter released at the α7 nicotinic receptor-containing synapses. As inferred from the concentration profile of choline shown in this study, acetylcholine would be effective at about 100 μM in inducing action potentials by activating α7 nicotinic receptors. Acetylcholinesterase is



Scheme 1.

present in most regions of the brain. Therefore, the synaptic concentration of acetylcholine cannot be sustained for longer than a few ms. However, once acetylcholine is hydrolyzed, an equimolar concentration of choline will be generated and may not be removed within a ms-time range. This favors a condition in which choline, but not acetylcholine, would remain at the synapse for a longer time, and also would diffuse from the synapse to presynaptic and extra synaptic regions where additional α7 nicotinic receptors are located. The concentrations of choline available for diffusion would be larger at higher frequency of cholinergic stimulation. Choline, generated in this manner, can mediate a variety of actions that do not require direct excitation and action potential generation (Scheme 1). Such actions would be neurotransmitter release from presynaptic sites (McGehee et al., 1995; Alkondon et al., 1996; Gray et al., 1996), mild and sustained activation of postsynaptic α7 nicotinic receptors causing Ca^{2+} influx and mediating Ca^{2+}-dependent functions, as well as desensitization of postsynaptic α7 nicotinic receptors. This latter effect of choline accounts for the ability of choline to control the frequency of action potentials resulting from the initial activation of this receptor by acetylcholine (see Scheme 1). Although both choline and acetylcholine are capable of causing receptor desensitization, there would be a difference depending on whether α7 nicotinic receptors were desensitized by choline or by acetylcholine, because choline-induced desensitization recovers faster than desensitization induced by acetylcholine (Mike and Albuquerque, 1999). At present, the physiological significance of the α7 nicotinic receptor-mediated Na^{+} channel inactivation is unclear. However, it is likely that such mechanisms are operative during high frequency firing of cholinergic neurons, or in situations like cigarette smoking where nicotine delivered to the brain rapidly via the first few puffs of inhalation may be sufficient to cause excitation of interneurons via nicotinic receptor activation.

In conclusion, the present results provide further evidence regarding the concentrations at which choline and acetylcholine are natural neurotransmitters for the activation and desensitization of α7 nicotinic receptors and mediate diverse functions including modulation of glutamatergic and GABAergic synapses.

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