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Nicotinic modulation of glutamatergic synaptic transmission in region CA3 of the hippocampus

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Abstract

Cholinergic modulation of synaptic transmission in the hippocampus appears to be involved in learning, memory and attentional processes. In brain slice preparations of hippocampal region CA3, we have explored the effect of nicotine on the afferent connections of stratum lacunosum moleculare (SLM) vs. the intrinsic connections of stratum radiatum (SR). Nicotine application had a lamina-selective effect, causing changes in synaptic transmission only in SLM. The nicotinic effect in SLM was characterized by a transient decrease in synaptic potential size followed by a longer period of enhancement of synaptic transmission. The effect was blocked by γ-aminobutyric acid (GABA)ergic antagonists, indicating the role of GABAergic interneurons in the observed nicotinic effect. The biphasic nature of the nicotinic effect could be due to a difference in receptor subtypes, as supported by the effects of the nicotinic antagonists mecamylamine and methyllycaconitine. Nicotinic modulation of glutamatergic synaptic transmission could complement muscarinic suppression of intrinsic connections, amplifying incoming information and providing a physiological mechanism for the memory-enhancing effect of nicotine.

Introduction

A variety of experimental results suggest that nicotinic receptors play a role in attentional and memory processing. In humans, nicotine chronically administered to both smokers and non-smokers enhances their performance on a variety of demanding attentional tasks (Rusted & Warburton, 1992; Levin *et al*., 1998; Mancuso *et al*., 1999). Nicotine also appears to improve memory function in normal human subjects. Experimental research has demonstrated that oral administration of nicotine enhances the performance of participants on variations of a classical free recall task (Warburton *et al*., 1992; Phillips & Fox, 1998), and specifically improves the recall of paired associates, possibly due to an increase in the attention of participants during the presentation of associated words (Warburton *et al*., 2001).

In animals, nicotine and nicotinic agonist administration improves learning by both non-aged and aged rats on a variety of memory tasks, such as the Morris water-maze (Arendash *et al*., 1995; Socci *et al*., 1995), and improves the performance of young and old non-human primates on delayed non-match-to-sample tasks (Elrod *et al*., 1988; Buccafusco *et al*., 1995, 1998; Prendergast *et al*., 1997). Additionally, application of nicotine can, in rats, reverse some of the memory deficits that occur after brain lesions (Muir *et al*., 1995), and nicotinic antagonists, such as mecamylamine (MEC), have been shown to cause impairment in working memory tasks such as the 16 radial arm maze (Levin *et al*., 1997; Levin & Simon, 1998), suggesting a role for nicotinic receptors in various memory processes.

Clinical behavioral data also indicate a role of nicotinic acetylcholine receptors in memory and cognition. Patients with Alzheimer's disease show a significant loss of high-affinity nicotinic receptors, an effect correlated with a loss of memory processing (Whitehouse & Au, 1986), and the application of nicotine patches can initially improve the rate of learning and attention in patients with Alzheimer's (Grigoryan *et al*., 1994; Newhouse *et al*., 1997).

Cellularly, nicotinic enhancement of afferent input into the hippocampus could complement muscarinic presynaptic inhibition of intrinsic connections (Hasselmo *et al*., 1995). Physiological data from slices have demonstrated that nicotinic receptors selectively enhance afferent thalamocortical input vs. intrinsic neocortical connections (Gil *et al*., 1997). Enhancement of afferent input may occur as the result of direct, selective nicotinic enhancement of glutamatergic synaptic transmission (Vidal & Changeux, 1993; Radcliffe & Dani, 1998). Direct glutamatergic enhancement, modulated by nicotinic acetylcholine receptors (nAChRs), has been observed for thalamocortical input into the prefrontal cortex (Gioanni *et al*., 1999), and nicotine has been shown to enhance fast, excitatory transmission in the hippocampus (Gray *et al*., 1996; Chiodini *et al*., 1999).

In addition, enhancement of afferent input in the hippocampus could result from a disinhibition of synaptic activity due to suppression of interneuron activity by either α7 or α3β4 nAChRs located on hippocampal interneurons (Jones & Yakel, 1997; Alkondon *et al*., 2000; Alkondon & Albuquerque, 2001). In the experiments described here, we consider the complementary role of nicotinic receptors in the modulation of synaptic transmission in the hippocampus.

Materials and methods

All experiments were performed using brain slices prepared from young (4–8 weeks old), male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA, USA), which were deeply anesthetized with halothane (Sigma-Aldrich, St. Louis, MO, USA) and decapitated. Techniques were reviewed and approved by the Institutional Animal Care and Use Committee at Boston University. The brain was removed under 4 °C artificial cerebrospinal fluid (ACSF)

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oxygenated by bubbling 95% O_2/5% CO_2 through the solution (concentration in mM): NaCl, 124.0; KCl, 2.5; $MgSO_4$, 1.3; Dextrose, 10.0; $NaHCO_3$, 26.0; KH_2PO_4, 1.2; $CaCl_2$, 2.4). The brain was mounted on its dorsal surface, offset by 10–15° from horizontal to effectively preserve Schaffer Collaterals. Four hundred-micron-thick slices were cut in oxygenated 4 °C ACSF using a vibroslicer (World Precision Instruments, Sarasota, FL, USA). The slices near the middle range of the septo-temporal axis were kept and the hippocampal regions were dissected from other tissue.

The slices of the hippocampus were stored in room temperature ACSF for a minimum of 1 h before they were transferred to a recording chamber (Fine Science Tools, North Vancouver, Canada). For recording, the slice was submerged in a standard slice chamber with continuously flowing ACSF at 27–29 °C. Unipolar stimulating electrodes (World Precision Instruments) were placed either in stratum radiatum (SR) of CA3 to activate recurrent fibers or in stratum lacunosum moleculare (SLM) of CA3 to activate perforant path fibers and cause evoked potentials in SLM. Recording electrodes were pulled from 1 mm borosilicate capillary tubes (World Precision Instruments) using a Sutter Instrument model P-87 pipette puller and filled with 2 M NaCl (3–6 MΩ resistance), and placed in either SR or SLM of CA3, corresponding to the placement of the stimulating electrode. Figure 1 depicts typical placement in SR and SLM of the electrodes for all experiments presented here.

Paired-pulse stimulation was delivered with a 100-ms interstimulus interval, with pulse pairs applied every 10 s (using a Neuro Data Instruments PG4000 digital stimulator and SIU90 stimulus isolation unit, Cygnus Technology, DE, USA). Data were amplified using an A-M Systems Model 1800 AC Amplifier, which was connected to a Micro1401 ADC that provided input to Spike2 software (Cambridge Electronic Designs, Cambridge, UK) on a computer running Windows 2000 (Microsoft, Redmond, WA, USA).

Once potentials were established, they were allowed to stabilize for at least 30 min before experimental runs. All suppression and enhancement measures are reported in percentage form as a mean relative to baseline ± SEM. Any trials in which the potential failed to return to less than 110% of baseline during wash were discarded.

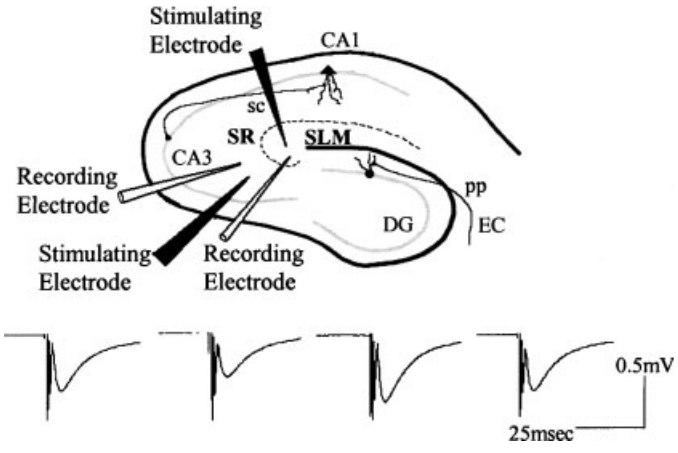

FIG. 1. Top: Typical placement of electrodes for all experiments. Recording electrodes were placed in either stratum radiatum (SR) of CA3 at the same level as the stimulation electrode, in order to record evoked synaptic potentials in the recurrent connections, or in stratum lacunosum-moleculare (SLM) of CA3 at the same level as the stimulating electrode, in order to record evoked synaptic potentials in the afferent connections of the perforant path. Bottom: Average of 10 consecutive fEPSPs seen during bath perfusion of 100 μM nicotine during baseline, suppression of synaptic transmission, enhancement of synaptic transmission and the return to baseline. Portions of the artifact were removed.

Dose–response curve and lamina-specific effects

After the potential stabilized, recording began with a 10-min baseline, followed by a 20-min perfusion of either 1 μM, 5 μM, 10 μM, 50 μM, 100 μM or 500 μM (–)-nicotine hydrogen tartrate (Sigma-Aldrich), and ended with a 30–40-min washout period to ensure field excitatory postsynaptic potentials (fEPSPs) returned to less than 110% of baseline. Each slice was perfused with only one concentration of nicotine, and no more than one trial of each concentration was conducted on a single slice to avoid desensitization. For the lamina-selective experiments, 100 μM nicotine was perfused by bath application and fEPSPs were recorded from either SLM or SR, following the above experimental protocol. For data analysis, the baseline amplitude was calculated by averaging the amplitude of 10 fEPSPs before perfusion. The average of the 10 trials across the peak of the suppression effect was used to generate the average percentage (± SEM) suppression for each experiment. The average of the last 10 trials after the potentials reached a maximum from the perfusate and before switching solutions was used to generate an average percentage enhancement for each experiment. The time points chosen to measure the suppression and enhancement values were consistent for different slices within each set of experiments. In addition, time course plots were made for every experiment. Time course plots for each experiment were matched for the application of nicotine and averaged together using Microsoft Excel (2000). SEM bars were computed for every 10th point. For each concentration of nicotine, paired-pulse facilitation (PPF) was calculated as a percentage of the baseline values. Lamina-selective effects were analysed with a two-tailed Student's *t*-test (Microsoft Excel). In addition, the percentage suppressions and enhancements for each set of experiments were analysed with an ANOVA statistic (SPSS 11.0), with planned *post-hoc* comparisons of each dose and group.

Antagonist studies

For experiments using mecamylamine (MEC) (Sigma), a non-selective nicotine antagonist, a 10-min baseline was recorded after the 30-min stabilization period. MEC (10 μM) was perfused 15 min prior to, during and 15 min after the perfusion of 100 μM (–)-nicotine hydrogen tartrate. Experiments run with 100 nM methyllycaconitine (MLA) (Sigma) followed the same experimental protocol as those run with MEC. During experiments involving picrotoxin (Sigma), a γ-aminobutyric acid $(GABA)_A$ antagonist, 100 μM picrotoxin was applied throughout the entire course of the experiment. Experiments run with SGS-742 (Lacey & Curtis, 1994; Pozza *et al*., 1999; Green *et al*., 2000), a selective $GABA_B$ antagonist, followed the same experimental protocol as experiments involving picrotoxin. Analyses for all antagonist studies were conducted as above. Individual and combined time course plots were also created and averaged following the procedures described above.

Results

Effect of nicotine in CA3, SLM

Twenty-minute bath application of nicotine was characterized by a transient decrease in evoked synaptic potentials followed by a long-lasting increase in synaptic transmission for all nicotine concentrations above 1 μM. No significant change in evoked synaptic potentials was observed for the nicotine concentration of 1 μM. For all other nicotine concentrations, the amount of suppression and enhancement of synaptic transmission was dose-dependent, and the full dose–response effects are described below. The baseline measurement for every

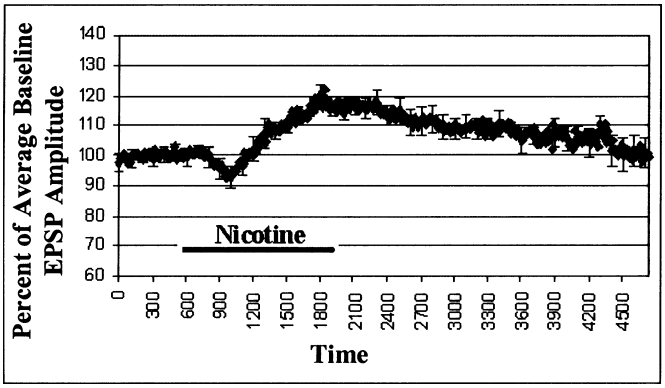

FIG. 2. The time course plot for eight combined experiments involving the 20-min bath application of 100 μM nicotine. For all combined plots, the average percentage of baseline excitatory postsynaptic potential (EPSP) amplitude was plotted against time. Error bars were plotted every 10 points. The 100 μM cumulative plot is typical of all the plots examined for the dose–response experiments conducted in SLM of CA3. The combined plots of nicotine application in SLM were characterized by a transient suppression followed by a long-lasting enhancement of synaptic transmission that returned to baseline 30–40 min after application of the nicotine had ceased.

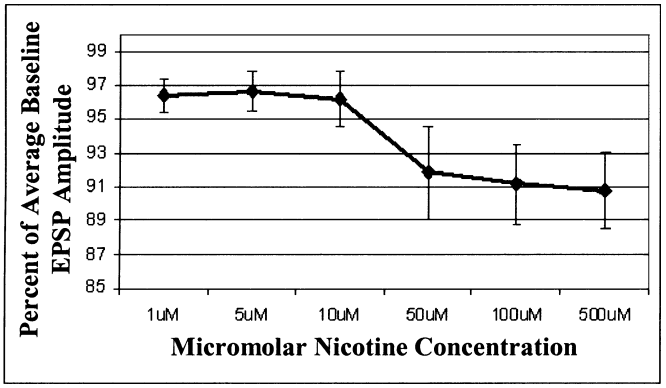

FIG. 3. A dose–response curve for the initial transient suppression was calculated for each micromolar concentration of nicotine applied in SLM of CA3. Values were determined by averaging the amplitude of the 10 field excitatory postsynaptic potentials (fEPSPs) that spanned the trough of the suppression effect. Compared with the later enhancement effect there was more between-experiment variance in the transient suppression values, as indicated by the larger error bars for each point on the dose–response curve. The curve illustrates that for the lower concentrations of nicotine the percentage suppression was usually about 3%, while for the larger concentrations of nicotine the suppression values reached a maximum value of approximately 10%.

experiment was typically stable for the 10 min prior to the application of nicotine, with very little change observed in the synaptic potentials during the 3-min stabilization period prior to the baseline recording. Washing the nicotinic effect to at least 110% of baseline took approximately 30 min for the lower concentrations of nicotine and up to 40 min for the higher concentrations of nicotine.

The time courses for each individual experiment run at 10 μM, 50 μM, 100 μM and 500 μM were combined into cumulative time course plots. As illustrated by the 100 μM time course plot shown in Fig. 2, cumulative time course plots were characterized by the same transient suppression and long-lasting enhancement of synaptic transmission as the individual experimental trials. For each combined time course of a specific nicotine concentration, standard error bars were calculated and plotted every 10th data point. Combined cumulative plots demonstrate that the individual time course plots were strikingly similar, indicated by the small cumulative error bars, evidence that the effect was consistent between slices and highly reproducible.

Dose–response curve of nicotine in CA3 SLM

The effect of nicotine on afferent connections was tested at a wide range of concentrations. The effects of six different concentration levels in CA3 SLM were tested in six–eight slices for each dose, from at least three different animals for each dose. Dose–response curves were constructed for both the transient suppression of EPSPs and the later enhancement of EPSPs. The change in fEPSPs was determined by taking the difference between the average amplitude of 10 fEPSPs just prior to perfusion (baseline) and the average amplitude of 10 fEPSPs after the potentials reached the minimum and maximum values and before switching solutions.

The dose–response curve for the transient suppression of synaptic transmission showed an increase in suppression at higher doses compared with the lower doses of nicotine. As Fig. 3 demonstrates, there was relatively little suppression at the low dose of 1 μM ($-3.63 \pm 1.01\%$, $n = 6$) compared with a larger amount of suppression at the higher doses of 100 μM ($-8.84 \pm 2.35\%$, $n = 8$) and 500 μM ($-9.23 \pm 2.27\%$, $n = 6$). There was not a statistically significant effect of nicotine concentration on synaptic transmission (5, 35; $F = 2.36$, $P = 0.06$). The non-significant suppression effect could be due to the high amount of variance between experiments for the suppression measurement. The estimated inhibitory concentration for 50% response (IC_{50}) was 25 μM.

The dose–response curve for the enhancement effect of nicotine (Fig. 4) demonstrates a clear enhancement effect for the higher doses of nicotine. The enhancement effect peaked at 100 μM ($17.75 \pm 1.69\%$, $n = 8$) and changed relatively little for the very high dose of 500 μM nicotine ($14.22 \pm 3.74\%$, $n = 6$). In addition, statistical analysis indicated a significant enhancement effect of

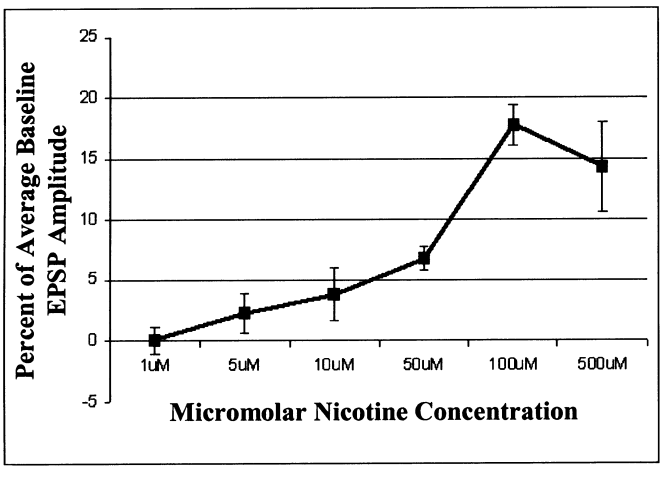

FIG. 4. A dose–response curve for the later enhancement was calculated for each concentration of nicotine applied in SLM of CA3. Values were determined by averaging the amplitude of the last 10 field excitatory postsynaptic potentials (fEPSPs) after the potentials reached a plateau from the perfusion and before switching solutions. Almost no change in synaptic transmission was seen at a dose of 1 μM, with a steady increase in the percentage enhancement of synaptic transmission as the concentration of nicotine increased. The effect peaked at 100 μM, as demonstrated by the lower value for 500 μM.

nicotine concentration on synaptic transmission (5, 35; $F = 11.96$, $P < 0.001$). The estimated excitatory concentration for 50% response (EC_{50}) was 54 μM.

Post-hoc analysis showed a significant difference between the effect of 1 μM and the enhancement effect of 50 μM ($P < 0.05$), 100 μM ($P < 0.001$) and 500 μM ($P < 0.001$). The dose of 100 μM caused significantly more enhancement than all other doses, with the exception of 500 μM ($P = 0.22$). The 5 μM and 10 μM concentrations were significantly different from the effect of 100 μM ($P < 0.001$) and 500 μM ($P < 0.001$).

Lamina-selective effect of nicotine in CA3

Enhancement of synaptic potentials due to application of nicotine selectively affected afferent fiber synaptic potentials while having no effect on intrinsic fiber synaptic potentials in CA3 of the hippocampus.

The bath application of 100 μM nicotine in CA3 SR was characterized by the absence of any predictable change in synaptic transmission, as shown in Fig. 5. Time points for measuring the change in synaptic transmission in CA3 SR were matched for the time points used for measuring the change in synaptic transmission in SLM. EPSPs in intrinsic fibers of CA3 SR showed almost no suppression (−1.17 ± 1.28%, $n = 5$) or enhancement (−2.31 ± 3.92%, $n = 5$). There was some increase in variance due to slow oscillatory changes in amplitude of the synaptic potentials recorded in CA3 SR. Stimulus-induced EPSPs recorded in SR showed significantly less of an enhancement than induced EPSPs in SLM (1, 12; $F = 24.87$, $P < 0.001$). In addition, stimulus-induced EPSPs recorded in SR showed less suppression than induced EPSPs in SLM, but the effect was not statistically significant (1, 12; $F = 4.49$, $P = 0.056$). Only the 100 μM concentration of nicotine was tested in CA3 SR as a clear lamina difference in effect was immediately observed. Figure 5 illustrates the effect of 100 μM nicotine in SR.

GABAergic antagonists

To determine if the nicotinic enhancement was due to a direct effect on glutamatergic terminals or to modulation of GABAergic influences on glutamatergic terminals, 100 μM picrotoxin was perfused continuously during perfusion with 100 μM nicotine in SLM of CA3. At a concentration of 100 μM, picrotoxin blocked both the suppression

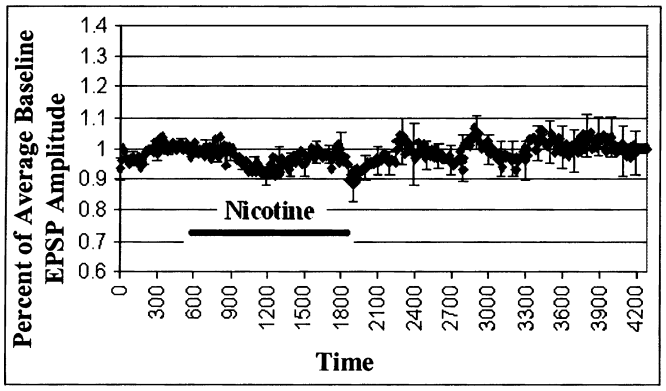

FIG. 5. The lack of effect of 100 μM nicotine in SR can be clearly seen, 100 μM nicotine did not significantly change synaptic transmission in SR of CA3 ($n = 5$). The effect of 100 μM nicotine (Fig. 2) demonstrated the transient suppression followed by the long-lasting enhancement of synaptic transmission.

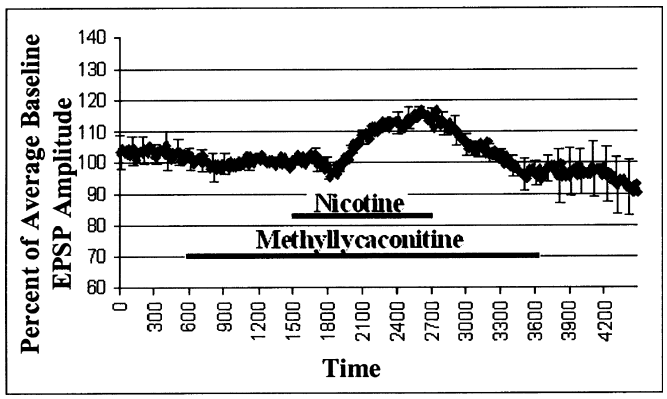

FIG. 6. Combined plot of five experiments involving the bath application of 100 μM nicotine in the presence of 100 μM picrotoxin. Error bars are plotted every 10 points. Only the application of nicotine is indicated in the figure as the picrotoxin was applied continuously throughout the experiment. Application of nicotine in the presence of picrotoxin resulted in no change in synaptic transmission, indicating that picrotoxin completely blocked the effect of the 100 μM nicotine. After the application of the nicotine, the picrotoxin caused some instability in the potentials observed, as indicated by the larger error bars after nicotine was discontinued.

(suppression after blockade: −0.97 ± 2.75%, $n = 5$) of and enhancement (−3.83 ± 2.40%, $n = 5$) of perforant path synaptic transmission due to the application of 100 μM nicotine. The reduction of the enhancement effect was statistically significant (1, 13; $F = 59.22$, $P < 0.001$), while the reduction of the suppression effect approached significance (1, 13; $F = 3.28$, $P = 0.093$). The effect of the continuous application of picrotoxin on the nicotinic effect is illustrated in Fig. 6. The complete blockade by picrotoxin supports the hypothesis that the nicotinic effect seen in SLM is due to modulation of GABAergic transmission. Blockade of $GABA_A$ receptors could cause a reduction in the outward Cl^- current that counterbalances the inward Na current, caused by glutamatergic excitation, resulting in a greater magnitude of excitatory field potentials.

To investigate the possibility of $GABA_B$ receptor involvement in the nicotinic effect we observed, a set of experiments following the same protocol as those for picrotoxin were run using SGS-742, a selective $GABA_B$ antagonist (data not shown). SGS-742 did not block the transient suppression and failed to completely block the enhancement effect of 100 μM nicotine. Infusion of SGS-742 in combination with 100 μM nicotine in SLM resulted in no significant change in the suppression of evoked synaptic potentials (SGS = −8.88 ± 3.18%, $n = 4$; 1, 10; $F = 4.18$, $P = 0.06$), but a significant reduction in the enhancement of synaptic transmission, compared with the enhancement effect of 100 μM nicotine alone (SGS = 6.73 + 1.13%, $n = 4$; 1, 10; $F = 16.48$, $P < 0.01$). Thus, blockade of $GABA_B$ receptors does not seem to result in less presynaptic inhibition of glutamatergic transmission but may block reductions in GABA transmission mediated by presynaptic $GABA_B$ receptors.

The effects of nicotinic antagonists

The effect of nicotine appears to be at nicotinic cholinergic receptors based on the fact MEC, a non-selective nicotinic antagonist, entirely blocked the transient suppression and almost completely blocked the enhancement effect of 100 μM nicotine in CA3 SLM (6.14 ± 2.84%, $n = 5$). Time courses for each experiment involving MEC were plotted and then averaged into one cumulative time course plot. Standard error bars were computed for every 10th data point in the

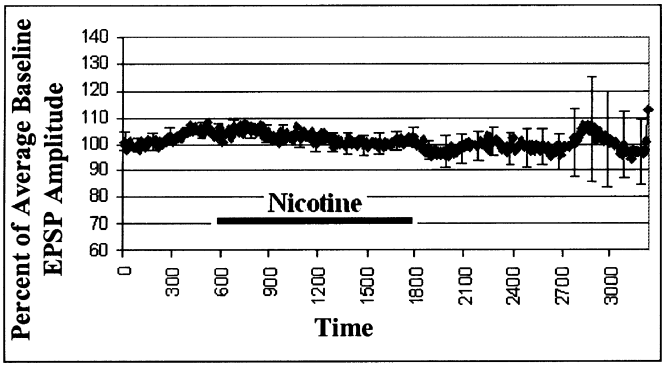

FIG. 7. Combined plot of five experiments involving the bath application of 100 μM nicotine in the presence of 10 μM MEC. Error bars are plotted every 10 points. Application of MEC resulted in the complete absence of any suppression of synaptic transmission, and a significant decrease in the later enhancement effect of nicotine on evoked synaptic potentials.

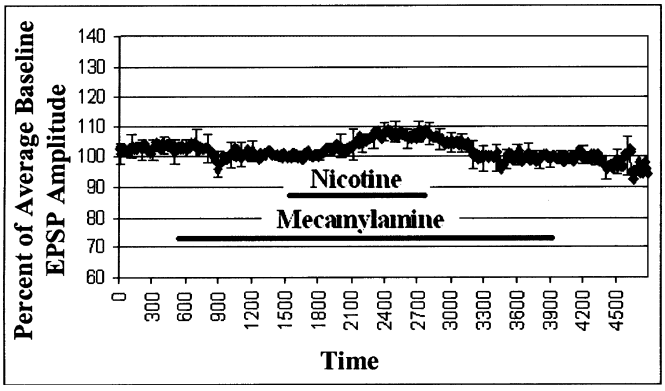

FIG. 8. Combined plot of five experiments involving the bath application of 100 μM nicotine in the presence of 10 nM MLA. Error bars are plotted every 10 points. Application of MLA resulted in a significant decrease in the suppression of synaptic transmission and no significant change in the later enhancement effect of nicotine on evoked synaptic potentials.

cumulative plot. Figure 7 illustrates the characteristic effect of a bath application of 100 μM nicotine in the presence of 10 μM MEC. The suppression of evoked synaptic potentials was almost completely blocked (0.69 ± 1.51%, $n = 5$). This blockade of the suppression of synaptic transmission due to 100 μM nicotine in the presence of MEC was statistically significant (1, 12; $F = 9.89$, $P < 0.01$). In addition, the application of MEC significantly reduced the later enhancement effect of nicotine (Control NIC = 17.75 ± 1.69%; NIC + MEC = 6.13 ± 2.84%; 1, 12, $F = 13.80$, $P < 0.01$).

To investigate the possible involvement of α7 nAChRs, the most common receptor in the rat hippocampus (Buhler & Dunwiddie, 2002; McQuiston & Madison, 1999), 100 nM MLA was applied before, during and after the application of 100 μM nicotine in SLM. Time courses for each experiment involving MLA were plotted and then averaged into one cumulative time course plot (Fig. 8). SEM bars were computed for every 10th data point in the cumulative plot. In the presence of MLA the suppression of evoked synaptic potentials by 100 μM nicotine was reduced (−1.89 ± 0.50%, $n = 5$). This block of suppression was statistically significant relative to baseline suppression (1, 12; $F = 9.89$, $P < 0.01$). There was no significant change in the later enhancement effect of nicotine (15.49 ± 2.49%, $n = 5$).

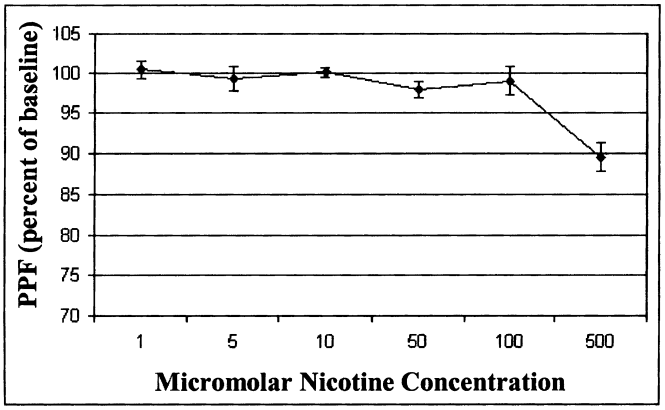

FIG. 9. Plot of paired pulse facilitation (PPF) relative to baseline after perfusion of different concentrations of nicotine. There was no significant PPF effect at any concentrations of nicotine except for 500 μM.

PPF analysis

The primary site of action of nicotine on afferent fiber connections appears to be postsynaptic. Changes in PPF, due to the application of a drug, typically indicate a presynaptic mechanism of drug action. Analysis of PPF data did not indicate a significant dose-dependent effect or a significant change during the infusion of nicotine at almost every concentration applied (Fig. 9). However, PPF analysis of the 500 μM concentration did reveal a significant decrease in PPF during the application of nicotine ($t = -3.02$, $P < 0.05$). As a change in PPF has been suggested to indicate a presynaptic mechanism, the PPF data suggest that the effect of nicotine is due to activation of postsynaptic mechanisms. The change in PPF was unique to the very high dose of 500 μM.

Discussion

Nicotinic effects specific to afferent fiber synapses

Our results demonstrate a dose-dependent effect of nicotine in SLM characterized by a brief, transient suppression and followed by a long-lasting enhancement of evoked synaptic potentials. Nicotine selectively enhanced synaptic transmission in the afferent fiber system in SLM of the CA3 region in the hippocampus and had no effect on the intrinsic fiber synapses in SR. The role of nicotine in enhancing certain memory processes in both humans (Rusted & Warburton, 1992; Levin *et al*., 1998; Mancuso *et al*., 1999) and animals (Arendash *et al*., 1995; Buccafusco *et al*., 1995, 1997; Socci *et al*., 1995) could be due to the nicotinic enhancement of the afferent transmission of new sensory information.

Previously, nicotine has been shown to selectively enhance afferent connections in thalamo-cortical slices (Gil *et al*., 1997; Gioanni *et al*., 1999), but this is the first demonstration of selective nicotinic enhancement of perforant path fibers, compared with intrinsic connections, in the hippocampus. A study by Psarropoulou *et al*., 2003) also found a lamina-selective enhancement by nicotine in CA3 of the hippocampus; however, the enhancement was observed in SR rather than SLM. Although contrary to the results of the current study presented, the study by Psarropoulou *et al*. (2003) found long-lasting enhancement in SR only in the presence of bicuculline, a selective $GABA_A$ antagonist. None of the experiments presented here was conducted in the presence of bicuculline, differentiating the current results from the previous finding of Psarropoulou *et al*. (2003).

The lamina-selective enhancement observed involves GABA receptors, as supported by the absence of the nicotinic effect in the presence of the $GABA_A$ antagonist picrotoxin and the reduction of the nicotine effect in the presence of the $GABA_B$ antagonist SGS-742. The distribution of GABAergic interneuron subtypes, categorized on the basis of their response to nicotine, varies significantly between cellular layers of the hippocampus (McQuiston & Madison, 1999). This lamina organization of GABAergic interneuron subtypes could underlie a lamina-selective nicotinic effect. Additionally, previous research demonstrated that the activation of α7 and α4β2 nAChRs, in the hippocampus, resulted in greater inhibition of interneurons in SLM compared with stratum oriens, stratum pyramidal and SR (Alkondon & Albuquerque, 2001). The significantly greater inhibition of interneurons in SLM could cause disinhibition of pyramidal neuron dendrites in SLM and underlie lamina-selective nicotinic enhancement.

Nicotinic enhancement of glutamatergic transmission has been observed in a variety of brain structures (McGehee & Role, 1995; Gioanni *et al*., 1999; Metherate & Hsieh, 2003), including the thalamus (Guo *et al*., 1998), neocortex (Vidal & Changeux, 1993) and the hippocampus (Alkondon *et al*., 1996; Radcliffe & Dani, 1998). In the hippocampus, long-lasting glutamatergic enhancement by nicotine has been observed at the mossy fibers (Radcliffe & Dani, 1998). Application of nicotine has also been shown to facilitate long-term potentiation and enhance *N*-methyl-D-aspartate (NMDA) receptor-mediated synaptic transmission (Aramakis & Metherate, 1998; Ji *et al*., 2001; Fujii *et al*., 1999), supporting the idea that nicotine has a modulatory effect on glutamatergic transmission.

The disinhibition of GABAergic interneurons

As previously suggested, one possible mechanism for the long-lasting enhancement seen is disinhibition of glutamatergic transmission. The lamina-selective effect of nicotine application appears to act mostly via GABA interneurons, as suggested by the complete blockade of both the transient suppression and later enhancement effect by the selective $GABA_A$ antagonist, picrotoxin. One possible mechanism for the blockade of the effect, due to the application of GABA antagonists, is that the antagonist raises the baseline to the level of the enhancement of synaptic transmission. Blocking $GABA_A$ receptors could result in less postsynaptic $GABA_A$ Cl^- outward current. In field potential recordings, the Cl^- current may counterbalance the excitatory glutamatergic inward Na current so that reduction of the Cl^- current may enhance the size of glutamatergic field potentials. The application of the $GABA_B$ antagonist SGS-742 also significantly reduced the nicotinic enhancement effect, suggesting that $GABA_B$ receptors could contribute to the enhancements, possibly by suppressing GABAergic transmission via $GABA_B$ receptors on GABA terminals. The results observed in these experiments suggest that the application of nicotine results in a reduction of GABAergic inhibition, via either $GABA_A$ or $GABA_B$ mechanisms, resulting in a disinhibition of excitatory field potentials.

Nicotine has been shown to inhibit GABAergic interneurons in SLM of the CA1 area in the hippocampus, which resulted in the disinhibition of pyramidal neurons (Ji & Dani, 2000; Alkondon & Albuquerque, 2001; Buhler & Dunwiddie, 2002). More specifically, nicotine application resulted in greater inhibition of interneurons in SLM, compared with other cell layers of the hippocampus, suggesting that nicotine may more selectively disinhibit feed-forward zones of the hippocampus (Alkondon & Albuquerque, 2001). Our results support the idea that the nicotinic inhibition of GABAergic interneurons in SLM, resulting in a disinhibition of activity, is more selective for the feed-forward connections of the perforant path, and that the disinhibition of activity could modulate excitatory glutamatergic synaptic transmission in the perforant path.

Nicotinic receptor subtypes and their role in nicotinic modulation

The observed nicotinic effect in SLM was biphasic in nature, and may be due to the activation of different nicotinic receptor subtypes within SLM. Studies have indicated the presence of α7, α3β2 and α4β2 nAChRs in SLM of the hippocampus (Alkondon *et al*., 1996; Jones & Yakel, 1997; McQuiston & Madison, 1999). Research has suggested α7 nAChRs underlie a fast nicotinic current, while α3β2 and α4β2 underlie slower nicotinic currents (Albuquerque *et al*., 1997; McQuiston & Madison, 1999; Alkondon & Albuquerque, 2004). Although three different types of nicotinic receptors have been consistently identified in the hippocampus (Alkondon & Albuquerque, 1993; Jones & Yakel, 1997; Frazier *et al*., 1998), the presynaptic and postsynaptic α7 receptor appears to be the most widespread receptor subtype in the hippocampus (McQuiston & Madison, 1999; Buhler & Dunwiddie, 2002). For this reason MLA, a selective α7 nicotine receptor antagonist (Sharples & Wonnacott, 2001), was used to determine if the α7 receptor subtype was involved in the transient suppression and long-term enhancement of synaptic transmission in SLM. Application of MLA significantly and selectively reduced the transient suppression of synaptic transmission. Concentrations of MLA above 10 nM can also block α6 receptors (Klink *et al*., 2001), however, the α6 receptor is only seen in high densities in the catecholaminergic nuclei, the thalamus and reticular formation (Le Novere *et al*., 1996). Thus, the reduction of the suppression effect by MLA suggests that the α7 receptor subtype is the major receptor subtype responsible for the suppression of synaptic potentials in SLM, but not for the enhancement. Activation of α7 receptors could result in a short-lived suppression of synaptic transmission due to a transient excitation of GABAergic interneurons. Activation of α7 nAChRs in the hippocampus has been shown to excite interneurons (Alkondon & Albuquerque, 2001). The absence of the suppression effect in the presence of picrotoxin suggests that the increase in GABA release activates $GABA_A$ receptors, perhaps located on pyramidal neurons. The decrease in synaptic transmission due to the activation of α7 receptors may be transient as the α7 receptor has been shown to undergo rapid desensitization (Alkondon & Albuquerque, 1993).

Both the suppression and enhancement of synaptic transmission were blocked by the non-selective nicotinic antagonist MEC. Some recent research points out that MEC may more selectively inhibit the α3β4 receptors at lower doses (Alkondon & Albuquerque, 2004); but MEC is considered a non-selective nicotine receptor antagonist at a concentration of 10 μM or higher (Sharples & Wonnacott, 2001). A concentration significantly higher than the 10 μM concentration of MEC we used would most likely completely block the effect of the 100 μM nicotine; however, higher doses of MEC also transiently inhibit NMDA receptors, which could complicate interpreting the antagonistic effect (Sharples & Wonnacott, 2001). The reduction in both the suppression and enhancement effect by MEC, along with the failure of MLA to reduce the enhancement effect, indicate that non-α7 nAChRs are responsible for the enhancement of synaptic transmission by nicotine in SLM. Activation of different nicotinic receptor subtypes could provide an explanation for the biphasic nature of the nicotinic effect observed.

The change in synaptic transmission during the application of nicotine appears to arise from postsynaptic influences on glutamatergic

terminals, as indicated by the absence of a dose-dependent PPF effect. Receptor localization studies in hippocampal slices support nicotinic responses dependent on postsynaptic nAChRs (Hunt & Schmidt, 1978; Freedman *et al*., 1993).

Role of acetylcholine in memory processing

Experimental research and computational modeling have extensively supported the role for acetylcholine in learning and memory processes. Cholinergic modulation of synaptic transmission in the hippocampus is important for the encoding of new information (Hasselmo, 1999). Specifically, activation of muscarinic acetylcholine receptors causes enhancement of long-term potentiation and selective suppression of intrinsic connections but not afferent connections in the hippocampus (Hasselmo & Schnell, 1994). The selective suppression of intrinsic connections by muscarine has been demonstrated in piriform cortex, auditory cortex and the hippocampus (Hasselmo & Bower, 1992; Hasselmo & Schnell, 1994; Hsieh *et al*., 2000). Computational models suggest that the lamina-selective role of muscarinic receptors in the hippocampus sets the dynamics of cortical circuits. Suppression of transmission prevents retrieval of old information from interfering with the storage of new information (Hasselmo & Schnell, 1994; Hasselmo, 1999). The lamina-selective effect in perforant path synaptic transmission by nicotine could provide a mechanism for the selective modulation of incoming information, complementing the role of muscarinic suppression of intrinsic connections in CA3 (Hasselmo *et al*., 1995).

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Abbreviations

ACSF, artificial cerebrospinal fluid; fEPSP, field excitatory postsynaptic potential; GABA, γ-aminobutyric acid; MEC, mecamylamine; MLA, methyllycaconitine; nAChRs, nicotinic acetylcholine receptors; NMDA, *N*-methyl-D-aspartate; PPF, paired-pulse facilitation; SLM, stratum-lacunosum moleculare; SR, stratum radiatum.

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