Nicotine Enhances the Depressive Actions of Aβ₁₋₄₀ on Long-Term Potentiation in the Rat Hippocampal CA1 Region In Vivo

D. B. Freir and C. E. Herron

Department of Human Anatomy and Physiology, Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin 2, Ireland

Submitted 1 November 2002; accepted in final form 6 January 2003

Freir, D. B. and C. E. Herron. Nicotine enhances the depressive actions of Aβ₁₋₄₀ on long-term potentiation in the rat hippocampal CA1 region in vivo. J Neurophysiol 89: 2917–2922, 2003; 10.1152/jn.00996.2002. Hippocampal long-term potentiation (LTP) is a form of synaptic plasticity used as a cellular model of memory. Beta amyloid peptide (Aβ) is involved in Alzheimer’s disease (AD), a neurodegenerative disorder leading to cognitive deficits. Nicotine is also claimed to act as a cognitive enhancer. Aβ is known to bind with high affinity to the α7-nicotinic acetylcholine receptor (nAChR). Here we have investigated the effect of intracerebroventricular (icv) injection of the endogenous peptide Aβ₁₋₄₀ on LTP in area CA1 of urethan-anesthetized rats. We also examined the effect of Aβ₁₂–₂₈ (icv), which binds with high affinity to the α7-nAChR and the specific α7-nAChR antagonist methyllycaconitine (MLA) on LTP. We found that Aβ₁₂–₂₈ had no effect on LTP, whereas MLA depressed significantly LTP, suggesting that activation of the α7-nAChR is a requirement for LTP. Within the in vivo environment, where other factors may compete with Aβ₁₂–₂₈ for binding to α7-nAChR, it does not appear to modulate LTP. To determine if the depressive action of Aβ₁₋₄₀ on LTP could be modulated by nicotine, these agents were also co-applied. Injection of 1 or 10 nmol Aβ₁₋₄₀ caused a significant depression of LTP, whereas nicotine alone (3 mg/kg) had no effect on LTP. Co-injection of nicotine with Aβ₁₋₄₀ 1 h prior to LTP induction caused a further significant depression of LTP compared with Aβ₁₋₄₀ alone. These results demonstrate that nicotine enhances the deficit in LTP produced by Aβ₁₋₄₀. This then suggests that nicotine may exacerbate the depressive actions of Aβ on synaptic plasticity in AD.

INTRODUCTION

Beta amyloid peptide (Aβ) is known to be involved in Alzheimer’s disease (AD), a neurodegenerative disorder. The cognitive impairments associated with AD have also been linked to a decline in cholinergic function (McGeer et al. 1984) with a reported decrease in the number of nicotinic acetylcholine receptor (nAChRs) in the hippocampus and cortex of AD brains (for review, see Schroder and Wevers 1998). Nicotine has been reported to act as a memory enhancer in both humans (for review, see Schroder and Wevers 1998). Nicotine is also known to act as a memory enhancer in both humans and animals (Abdulla et al. 1993). A negative correlation between cigarette smoking and the smoking of AD has been reported (Brenner et al. 1993); however, smokers have also been reported to have a higher incidence of AD (Shalat et al. 1987). The effectiveness of nAChRs as a therapeutic target for the treatment of AD is therefore controversial but potentially beneficial (Kem 2000). Aβ-induced amnesia in mice is attenuated by nicotine administration (Maurice et al. 1996) whereas nicotine injected intravenously has been shown to enhance memory in AD patients (Newhouse et al. 1988). However, improved attention and awareness, without a corresponding enhancement of memory, has also been reported (Jones et al. 1992).

High-affinity binding between the α7-nAChR and Aβ has been reported in vitro with the binding domain between amino acids 12–28 (Wang et al. 2000a,b). Aβ₁₋₄₂ and Aβ₁₂–₂₈ have also been shown to reduce carbachol-induced currents in stratum radiatum interneurons in hippocampal slices (Pettit et al. 2001) via a decrease in the probability of nAChR-gated channel opening. Aβ₁₋₄₂ has also been shown to reduce α7-nAChR-mediated current in cultured cortical neurons (Liu et al. 2001).

Long-term potentiation (LTP) is regarded as a cellular model for learning and memory (Bliss and Collingridge 1993). Administration of Aβ peptides (intracerebroventricularly, icv) is known to impair LTP in vivo (Cullen et al. 1997; Freir et al. 2001). Nicotinic agonists have also been shown to facilitate LTP in area CA1 in vitro (Fujii et al. 1999; Hunter et al. 1994), whereas nicotine has been shown to enhance synaptic transmission in area CA3 (Gray et al. 1996). Intraperitoneal injection of nicotine also produces a form of LTP in mouse dentate gyrus in vivo (Matsuyama et al. 2000). The aim of our study was to investigate the effects of nicotine, the endogenous peptide Aβ₁₋₄₀, and agents that bind to the α7-nAChR including Aβ₁₂–₂₈ (Wang et al. 2000a) and the specific α7-antagonist methyllycaconitine (MLA) on synaptic transmission and plasticity in the form of LTP. Part of this work has been presented previously in abstract form (Freir and Herron 2001).

METHODS

In vivo preparation

All experiments were carried out in accordance to guidelines under license from the Department of Health, Ireland (86/609/EEC). Male Wistar rats (175–200 g) were surgically prepared for acute electrophysiological recordings. Briefly, rats were anesthetized with intraperitoneal injections of 1.5 g/kg urethan (ethyl carbamate), and supplementary injections (0.2–0.5 g/kg) were given when necessary to ensure full anesthesia. Heating pads (Braintree scientific) were used to ensure full anesthesia. Heating pads (Braintree scientific) were used to ensure full anesthesia.
maintain the temperature of the animals at 36.5 ± 0.5°C. Deep body temperature was recorded throughout the experiment using a rectal thermometer (Precision Instruments). Small holes were drilled in the skull at the positions of the reference stimulating and recording electrodes. Additionally, in some experiments, a separate hole was drilled to introduce a guide cannula for ivc injection of drug/vehicle. The cannula was secured using acrylic dental cement (Emperor CC) to avoid interference with the stimulating/recording electrodes. Animals were placed in a stereotaxic frame for all recordings. The recording electrode was positioned in the stratum radiatum of area CA1 (3 mm posterior, 2 mm lateral to bregma). A bipolar stimulating electrode was placed in the Schaffer-collateral/commissural pathway distal to the recording electrode (4 mm posterior, 3 mm lateral to bregma). The cannula was positioned above the lateral ventricle in the opposite hemisphere to that of the recording/stimulating electrodes (1 mm posterior, 1.2 mm lateral to bregma).

In vivo electrophysiology

Physiological and stereotactic indicators were used to lower the electrodes through the cortex and into area CA1 of the hippocampus. Test stimuli were delivered to the Schaffer-collateral/commissural pathway every 30 s (0.033Hz). Electrodes were positioned to record a maximal field excitatory postsynaptic potential (EPSP). Baseline EPSPs were recorded at 35–40% of maximal response. LTP was induced using a high-frequency stimulus protocol (HFS: 10 trains of 10 stimuli at 200 Hz, intertrain interval of 2 s) at a stimulus intensity that evoked a field EPSP of approximately 80% of maximum response. This protocol was repeated three times to produce a robust LTP. Field EPSPs were evoked in the CA1 region using low-frequency stimulation (0.033 Hz). Baseline synaptic potentials were recorded for ≥30 min prior to injection of drug/vehicle to ensure a steady state response. Rats were injected with nicotine (3 mg/kg ip). A series of high-frequency stimuli (HFS) were used to measure synaptic efficacy (E). Averaged EPSPs were filtered at 5 kHz, digitized, and recorded using MacLab software acquisition system. The EPSP slope was used to measure synaptic efficacy. EPSPs are expressed as a percentage of the mean initial slope measured during the last 10 min of the preinjection baseline-recording period. LTP data were analyzed using ANOVA measured over a 5-min period (55–60 min) after the induction of LTP. PPF was analyzed prior to and 1 h after drug/vehicle injection using a paired Student’s t-test. The significance level was set at P < 0.05. Error bars on the graphs shown represent the standard error of the mean (SE). Data points in each figure were an average of four consecutive EPSPs taken at 30-s intervals to present our findings in a clear and concise manner. Sample traces are averages of four consecutive EPSPs recorded at the time indicated on each graph.

Materials and chemicals

Rats were obtained from the Biomedical Facility, University College Dublin, Belfield. Stimulating (bi-polar stainless steel; 0.125 mm diam) and recording (mono-polar stainless steel; 0.125 mm diam) electrodes were obtained from Plastics One. Aβ1–40 and Aβ12–28 were purchased from Biosource; nicotine and methyllycaconitine (MLA) were obtained from Sigma (RBI) (Ireland). All other reagents were obtained from Sigma (Ireland). All drugs were dissolved in distilled water and stock solutions were maintained at −20°C.

Results

Aβ1–40 causes a depression of LTP

The endogenous peptide Aβ1–40 was injected icv at a concentration of 1 or 10 nmol in 5 μl distilled water. Aβ1–40 had no significant effect on baseline synaptic transmission monitored for 1 h postinjection. After icv injection with 5 μl distilled water (vehicle) LTP was measured (167 ± 3%, n = 12) 1 h post tetanus (Fig. 1). LTP was depressed significantly in animals treated with 1 nmol (141 ± 6%, n = 6, P < 0.01) or 10 nmol (123 ± 8%, n = 6, P < 0.001) Aβ1–40 compared with control values (Fig. 1).

Effect of nicotine on synaptic transmission and LTP

Animals were injected intraperitoneally with distilled water/nicotine after a baseline recording period, and EPSPs were monitored for a further 60 min postinjection (Fig. 2). The nicotine-treated groups (3 mg/kg in 0.5 ml distilled water) showed no significant change in baseline response ±1 h post injection (Fig. 2). LTP was induced 1 h after injection of nicotine/vehicle, and EPSPs were monitored for a further period of 1 h post-HFS. Vehicle-injected animals (0.5 ml distilled water ip) showed stable LTP measured 1 h post-tetanus (164 ± 3%, n = 10). Nicotine (3 mg/kg) delivered 60 min prior to

FIG. 1. Aβ1–40 causes a dose-dependent depression of long-term potentiation (LTP). Baseline recordings were monitored for 30 min prior to injection of drug/vehicle. Excitatory postsynaptic potentials (EPSPs) were then monitored for a period of 1 h postinjection. High-frequency stimulation (HFS) caused a significant potentiation of the EPSP in the vehicle-injected group (○; 167 ± 3%, n = 12). Intracerebroventricular (icv) injection of 1 (▲) and 10 nmol (□) Aβ1–40 caused a significant depression of LTP (141 ± 6%, n = 6, P < 0.01) and (123 ± 8%, n = 6, P < 0.001) respectively, measured 1 h posttetanus. ↑↑, drug/vehicle injection. Ave. field EPSPs are shown 5 min prior to tetanus and 60 min after tetanus for control (1 and 2) 1 nmol Aβ1–40 (3 and 4), and 10 nmol Aβ1–40 (5 and 6). ↑↑, time of injection Aβ1–40/vehicle.

J Neurophysiol • VOL 89 • JUNE 2003 • www.jn.org
tetanus produced no significant change in LTP when compared with control values (153 ± 4%, n = 5; Fig. 2).

### Co-injection of nicotine and Aβ1-40

The effects of co-applying Aβ1-40 (1 nmol icv) and nicotine (3 mg/kg ip) 1 h prior to the induction of LTP were examined (Fig. 3). Drug applications did not alter baseline synaptic transmission; however, a significant depression of LTP was noted in the co-injected group (121 ± 6%, n = 5, P < 0.01) when compared with vehicle controls (167 ± 3%, n = 12, icv; Fig. 3). The depression of LTP reported in the co-injected group was significantly greater than that previously recorded in animals injected with Aβ1-40 (1 nmol) alone (141 ± 6%, n = 6, P < 0.05). This demonstrates that co-injection of nicotine and Aβ1-40 1 h prior to LTP induction caused a further significant impairment of LTP compared with either agent alone.

### Effect of Aβ12-28 on LTP

Injection of 10 nmol Aβ12-28 1 h prior to induction of LTP had no effect on baseline synaptic transmission (Fig. 4). There was also no significant reduction in LTP observed in animals treated with Aβ12-28 (157 ± 3%, n = 6) compared with the icv control group (167 ± 3%, n = 12; Fig. 4).

### MLA depresses LTP

Injection of the α7nAChR antagonist, MLA (5 mg/kg ip), 1 h prior to LTP induction did not alter baseline synaptic transmission. Application of HFS produced LTP (144 ± 5%, P < 0.05) that was depressed significantly compared with control values (164 ± 3%, n = 10; ip controls, Fig. 5).

### Nicotine and Aβ12-28 increase PPF

Nicotine (3 mg/kg) produced an increase in PPF when measured 60 min post-injection (P < 0.05; Fig. 5A). Aβ12-28 injection of 10 nmol Aβ12-28 (1 nmol) alone (141 ± 6%, n = 6) compared with vehicle controls (167 ± 3%, icv; n = 12, B: intraperitoneal injection of 5 mg/kg methyllycaconitine (c) 1 h prior to tetanus had a significant effect on LTP (144 ± 5%, n = 6, P < 0.05) compared with vehicle controls (164 ± 3%, ip control). Averaged EPSPs are shown 5 min prior to tetanus and 60 min after tetanus for control (1 and 2), 1 nmol Aβ1-40 (3 and 4), and Aβ1-40 and nicotine (5 and 6).

![Figure 2](http://jn.physiology.org/)

**FIG. 2.** Nicotine 3 mg/kg does not effect LTP. Administration of 3 mg/kg nicotine 1 h (●) pretetanus produced an LTP (153 ± 4%, n = 5) similar to that seen in vehicle-injected controls (●; 164 ± 3%, n = 10 intraperitoneal control). Averaged EPSPs are shown 5 min prior to tetanus and 60 min after tetanus for control (1 and 2) and nicotine-treated groups (3 and 4). ➺, time of injection nicotine/vehicle.

![Figure 3](http://jn.physiology.org/)

**FIG. 3.** Effect of nicotine administration on Ab1-40-induced depression of LTP. Co-application of 1 nmol icv Aβ1-40 and 3 mg/kg ip nicotine (●) 1 h prior to LTP induction produced an LTP (121 ± 6%, n = 5) that was similar to that seen after application of 1 nmol Aβ1-40 alone (●; 141 ± 6%, n = 6). The LTP produced in the co-injected group was significantly reduced when compared with vehicle-injected (icv) animals (●; 167 ± 3%, n = 12, P < 0.01). Averaged EPSPs are shown 5 min prior to tetanus and 60 min after tetanus for control (1 and 2), 1 nmol Aβ1-40 (3 and 4), and Aβ1-40 and nicotine (5 and 6). ➺, time of injection of Aβ1-40/vehicle or co-injection of Aβ1-40 and nicotine.

![Figure 4](http://jn.physiology.org/)

**FIG. 4.** Effects of Aβ12-28 and methyllycaconitine on LTP in vivo. A: icv injection of 10 nmol Aβ12-28 (●) 1 h prior to tetanus had no significant effect on LTP (157 ± 3%, n = 6) compared with vehicle controls (●; 167 ± 3%, icv, n = 12), B: intraperitoneal injection of 5 mg/kg methyllycaconitine (c) 1 h prior to tetanus had a significant effect on LTP (144 ± 5%, n = 6, P < 0.05) compared with vehicle controls (●; 164 ± 3%, ip control). Averaged EPSPs are shown 5 min prior to tetanus and 60 min after tetanus for vehicle (1 and 2), Aβ12-28 (3 and 4, A) and MLA (3 and 4, B). ➺, time of injection of Aβ12-28/MLA/vehicle.
caused an increase in PPF; however, Aβ_{1-28} had no effect (Fig. 5B). An increase in PPF is usually associated with a decrease in presynaptic transmitter release.

**DISCUSSION**

We reported previously that the shorter fragment Aβ_{25-35} depresses LTP in vivo (Freir et al. 2001). Here we demonstrate that the endogenous peptide, Aβ_{1-40}, which is neurotoxic in cell culture (Yankner et al. 1990), also depresses LTP in vivo. These results are consistent with in vitro studies showing that amyloid peptides cause impairment of hippocampal LTP (Itoh et al. 1999). LTP is also depressed in transgenic mice that overexpress the mutated human APP gene (Chapman et al. 1999). What then are the potential mechanisms involved in this impairment of LTP by Aβ peptides?

Nicotinic agonists can improve performance in memory-linked behavioral tasks (Abdulla et al. 1993; Levin 1992) and the nAChR antagonist mecamylamine causes impairment of memory in humans (Newhouse et al. 1992). Aβ is also known to bind with picomolar affinity to the α7-nicotinic acetylcholine receptor (nAChR), with the binding domain contained within amino acid sequence 12–28 of the full-length peptide (Wang et al. 2000a,b). We investigated therefore a possible interaction between Aβ and the α7-nAChR.

In the hippocampal CA1 region, α7-nAChRs are present on GABAergic inhibitory interneurons and the soma of CA1 pyramidal cells (Jones and Yakel 1997). Stimulation of presynaptic α7-nAChRs can enhance the release of glutamate due to the high calcium permeability of the α7-gated channel (Gray et al. 1996). Nicotine, at concentrations found in plasma from cigarette smokers (50–500 nM), has been shown to desensitize nAChRs on CA1 GABAergic interneurons (Alkondon et al. 2000), leading to pyramidal cell disinhibition (Dani et al. 2000). Aβ_{1-40}, Aβ_{1-28}, and Aβ_{12-28} can antagonize reversibly α7- and other nAChR-mediated currents in vitro. (Liu et al. 2001; Pettit et al. 2001). Nicotine (Shimohama and Kihara 2001), cytisine, (α4β2 agonist) (Kihara et al. 1998), and the α7-agonist, DMXBA, (Li et al. 1999) have also been shown to inhibit Aβ-mediated toxicity in cultured neurons.

To examine a possible role for the α7-nAChR in LTP, because α7-agonists are known to facilitate LTP (Hunter et al. 1994), we investigated the effects of the specific α7-nAChR antagonist MLA. We found that MLA caused a significant depression of LTP when administered 1 h pretetanus. The concentration of MLA used in our study (5 mg/kg) delivered intraperitoneally should result in a concentration of 50–100 nM in rat brain (Turek et al. 1995). This concentration range (50–100 nM) has been shown to block completely the activation of α7-nAChRs in cultured hippocampal neurons (Alkondon et al. 1992).

Because Aβ_{12-28} was reported to cause a block of the α7-channel (Pettit et al. 2001) in a similar manner to Aβ_{1-42}, we investigated the effects of Aβ_{12-28} on LTP. We found that injection of Aβ_{12-28} icv had no significant effect on baseline synaptic transmission or LTP after the injection of nicotine. Co-injection of nicotine and Aβ_{1-40} 1 h pre-HFS caused a further significant impairment of LTP compared with Aβ alone.

Our result suggests that activation of α7-nAChRs is involved in LTP in the CA1 region in vivo. Although both Aβ_{1-40} and Aβ_{12-28} have been shown to bind to the α7 receptor with high affinity in vitro, in our study these peptides had differing effects on LTP in vivo. Assuming a role for α7-nAChRs in LTP, because MLA reduces LTP, it is difficult to interpret the depression of LTP by Aβ_{1-40} in terms of an interaction with the α7-binding site. Although Aβ_{1-40} at picomolar concentrations was shown to inhibit binding of MLA to synaptic membranes (Wang et al. 2000a), there was no interaction unless the membranes had been washed extensively. This suggests the possible existence of a soluble endogenous factor/protein that binds to either Aβ_{1-42} or the α7-nAChR. It has been shown that a variety of albumins can bind to and potentiate α7-nAChR-mediated responses in vitro (Conroy et
We found that interneurons, this is likely to lead to increased excitability in factor that competes for $\alpha 7$-nACHRs. This result would desensitization of these receptors, may explain reduced trans-depression of LTP in vivo is not likely due to an interaction with the $\alpha 7$-nACHR.

We have also examined neurotransmitter release in the form of PPF in area CA1. Injection of nicotine (3 mg/kg) 60 min pretetanus caused a significant increase in PPF. An increase in PPF is usually associated with a decrease in presynaptic neurotransmitter release. An increase in neurotransmitter release due to activation of calcium permeable presynaptic $\alpha 7$ nACHR channels on mossy-fiber terminals has been demonstrated (Gray et al. 1996). Desensitization of these receptors, however, may lead to decreased neurotransmitter release. GABAergic inhibition may also play a role in apparent alteration of PPF. Because long-term exposure to nicotine will cause desensitization of the $\alpha 7$nACHRs (Alkondon et al. 2000) on inhibitory interneurons, this is likely to lead to increased excitability in the CA1 region and subsequent increases in amplitude of the second EPSP that is evoked at a 50-ms interval. We found that $\alpha B_{12-28}$ caused a significant increase in PPF indicative of decreased neurotransmitter release. This correlates with a report demonstrating that $\alpha B_{12-28}$ reduces Ca$^{2+}$ influx via the $\alpha 7$nACHR channel (Pettit et al. 2001). Blockade of presynaptic $\alpha 7$-nACHRs onto CA3 pyramidal neurons, similar to nicotine desensitization of these receptors, may explain reduced transmitter release onto CA1 excitatory neurons. This result would not agree however with the existence of a soluble endogenous factor that competes for $\alpha B$ binding and is under further investigation.

These results suggest that although $\alpha B_{1-40}$ causes an impairment of LTP in vivo, the effects mediated via binding to nACHRs may be reduced due to endogenous proteins present in cerebrospinal fluid. Surprisingly, because nicotine has been reported to act as a cognitive enhancer, we found that co-application of nicotine and $\alpha B_{1-40}$ produced a level of LTP significantly depressed compared with that recorded in $\alpha B_{1-40}$ alone. This observation, which is under further investigation, indicates that nicotine can exacerbate the effects of $\alpha B$ and may account for the slightly higher incidence of AD among smokers (Shalat et al. 1987).

This research was supported by Enterprise Ireland, Department of Physiology University College Dublin (UCD), and UCD Presidents Research Award 2001.

REFERENCES


McGeer PL, McGeer EG, Suzuki J, Dolman CE, and Nagai T. Aging,
Alzheimer’s disease, and the cholinergic system of the basal forebrain. 


