Effects of Nicotine on APP Secretion and Aβ- or CT_{105}-Induced Toxicity

Ji-Heui Seo, Seong-Hun Kim, Hye-Sun Kim, Cheol Hyoung Park, Sung-Jin Jeong, Jun-Ho Lee, Se Hoon Choi, Keun-A Chang, Jong-Cheol Rah, Jawook Koo, Eun-Mee Kim, and Yoo-Hun Suh

Several lines of evidence indicated that overexpression or aberrant processing of amyloid precursor protein (APP) is causally related to Alzheimer's disease (AD). Amyloid precursor protein is principally cleaved within the amyloid β protein domain to release a large soluble ectodomain (APPs), known to have a wide range of trophic functions. The central hypothesis guiding this review is that nicotine may play an important role in APP secretion and protection against toxicity induced by APP metabolic fragments (β-amyloid [Aβ], carboxyl terminal [CT]). Findings from our experiments have shown that nicotine enhances the release of APPs, which has neurotrophic and neuroprotective activities in concentration-dependent (>50 μmol/L) and time-dependent (>2 hours) manners. In addition, pretreatment of nicotine (>10 μmol/L for 24 hours) partially prevented Aβ or CT_{105}-induced cytotoxicity in primary cultured neuron cells, and the effects of nicotine-induced protection were inhibited by the pretreatment with a nicotine α-bungarotoxin. Nicotine (>10 μmol/L for 24 hours) partially inhibited CT_{105}-induced cytotoxicity when PC12 cells was transfected with CT_{105}. From these results, we proposed that nicotine or nicotinic receptor agonist treatment might improve the cognitive functions not only by supplementation of cholinergic neurotransmission, but also by protecting Aβ- or CT_{105}-induced neurotoxicity probably through the increased release of APPs and the activation of nicotinic receptors. Biol Psychiatry 2001;49:240–247 © *2001 Society of Biological Psychiatry*

Key Words: Alzheimer's disease (AD), nicotine, amyloid precursor protein (APP), secreted form of APP (APPs), C-terminal fragment of APP (CT_{105}), Aβ

Introduction

Alzheimer's disease (AD) is characterized by distinct neuropathologic lesions, including intracellular neuritic plaque made of amyloid β protein (Aβ), neurofibrillary tangles, and cerebrovascular amyloid deposits (Selkoe 1994). It seems that Aβ is derived from amyloid precursor protein (APP), which are 695 to 770 amino acids long and large membrane-spanning glycoproteins. Amyloid precursor protein is processed with at least two kinds of pathways (Checler 1995). In a constitutive secretory pathway, an unidentified enzyme, "α-secretase," makes a cleavage of large ectodomain (secreted form of APP [APPs]) directly in the outer edge of the membrane and a smaller membrane-associated carboxyl-terminal (CT) fragment. The form of APP secreted by cleavage of α-secretase is known to have neurotrophic activity through lowering of the intraneuronal calcium concentration and neuroprotective activity against glucose deprivation and glutamate toxicity (Mattson et al 1993). A second pathway of APP metabolism has been identified in the endosomal–lysosomal system, resulting in larger potentially amyloidogenic CT fragments of APP by β-secretase (Estus et al 1992; Golde et al 1992; Haass et al 1992a, 1992b; Shoji et al 1992; Tamaoka et al 1992; Kozlowski et al 1992) and subsequently possibly cleaved by γ-secretase to release soluble Aβ (Koo and Squazzo 1994).

Many studies have shown that Aβ is toxic to neurons in vitro (Yankner et al 1990) and in vivo (Fukuchi et al 1994). However, a relatively high concentration (20 μmol/L) of Aβ is needed to induce toxicity, and some studies still failed to demonstrate the toxicity of Aβ in vivo (Clemens and Stephenson 1992). Amyloid β protein deposition has been found without accompanying neurodegeneration, and neurodegeneration could occur in areas with no Aβ deposition. Furthermore, it has been reported that under certain culture conditions Aβ promotes neurite outgrowth (Yankner et al 1990) instead of exerting toxic action. Thus Aβ may not be the sole fragment in the neurotoxicity associated with AD. Consequently the possible effects of other cleaved products of APP need to be explored.

From the Department of Pharmacology, College of Medicine, National Creative Research Initiative Centre for Alzheimer's Proteins and Neuroscience Research Institute, Medical Research Centre, Seoul National University, Seoul, Korea.
Address reprint requests to Yoo-Hun Suh, Seoul National University, College of Medicine, Department of Pharmacology, 28 Yongon-dong, Chongno-gu, Seoul 110-799, Korea.
Received May 30, 2000; revised November 2, 2000; accepted December 8, 2000.

0006-3223/01/$20.00
PII S0006-3223(00)01124-0

Recently it has been reported that CT fragments of APP are found in media and cytosol of lymphoblastoid cells obtained from patients with early- or late-onset familial AD (Matsumoto 1994; Matsumoto and Matsumoto 1994) and Down's syndrome (Kametani et al 1994). Transgenic mice that overexpressed the CT_{100} peptide showed extensive neuronal degeneration in the hippocampal area, with cognitive impairments (Kammesheidt et al 1992) and impairment of long-term potentiation (LTP) (Nalbantoglu et al 1997). Lu and colleagues very recently reported that cytotoxic properties of CT may be due to the generation and release of CT31 and its subsequent amplification effect on the cell death program (Lu et al 2000). In addition, we previously have reported that a recombinant 105-amino acid CT (CT_{105}) itself caused direct neurotoxicity in PC12 cells and primary cortical neurons (Kim and Suh 1996; Suh et al 1996), induced strong nonselective inward currents in *Xenopus* oocytes (Fraser et al 1996; Suh et al 1996), planar lipid bilayers (Kim et al 1999a), and Purkinje cells (Hartell and Suh 2000) and blocked the later phase of LTP in the rat hippocampus in vivo (Cullen et al 1997). CT_{105} impaired calcium homeostasis by inhibiting microsomal calcium uptake by Mg^{2+}-Ca^{2+} adenosine triphosphatase in the rat brain microsome and Na^{+}-Ca^{2+} exchanger activity in SK-N-SH cells, but Aβ did not (Kim et al 1998, 1999b). In addition, we found that intracerebroventricular injection of CT_{105} impaired learning and memory and was toxic to animals (Song et al 1998). These lines of evidence postulate that CT_{105} is an alternative toxic element important in the generation of the symptoms of AD.

The neuronal nicotinic acetylcholine receptor (nAChR) is a ligand-gated ion channel that consists of at least eight α-like subunit isoforms (α_2–α_9) and three β-like subunit isoforms (β_2–β_4) that exhibit distinct temporal and tissue-specific patterns of expression. Neuronal nAChR channels characteristically have a greater Ca^{2+} permeability than muscle nAChRs (Levin 1992; McGehee and Role 1995) and have been found to elicit diverse behavioral effects including arousal, attention, anxiolytic activity, analgesia, and cognitive enhancement. Moreover, many studies have indicated a substantial loss of nicotinic receptor population in the brains of AD patients (Kellar and Wonnacott 1990), and the degree of cognitive impairments in AD has been reported to correlate well with the central cholinergic deficits (Bierer et al 1995). In addition, there are epidemiologic data showing a negative correlation between smoking and the onset of AD (Smith and Giacobini 1992), and pilot clinical data indicated that acutely administered nicotine might be beneficial for the treatment of the deficits in attention and information processing associated with AD (Jones et al 1992; Smith and Giacobini 1992). Thus our recent research has focused on nicotine's effect on APP processing and the neuronal cytotoxicity induced by APP metabolites to get additional benefits of nicotinic receptor agonists as theurapeutic agents in AD, and we previously reported that the release of APPs was enhanced by nicotine in PC12 cells (Kim et al 1997). In addition, our recent findings suggest that nicotine or nicotinic receptor agonist treatment might improve the cognitive functions not only by supplementation of cholinergic neurotransmission but also by protecting Aβ- or CT_{105}-induced neurotoxicity, probably through the increased release of APPs.

Enhanced Release of APPs by Nicotine

Time- and Dose-Dependent Release of APPs

We employed PC12 cells, a rat pheochromocytoma cell line, as a model system. They have been shown to constitutively express APP and contain functional nAChRs (Sands and Barish 1992) and express α3, α5, α7, β2, and β4 subunit isoforms that are similar to those in the sympathetic ganglion (Henderson et al 1994). As shown in Figure 1, nicotine increased the release of APPs in a concentration-dependent manner. The levels of APPs after treatment with 50 μmol/L and 100 μmol/L nicotine were significantly different from those of a control group ($p < .05$, Duncan analysis of variance [ANOVA]). The amount of APPs in the conditioned media began to increase at 30 min following application of nicotine (100 μmol/L), reached a maximal level at 3 hours, and tended to decrease thereafter (Figure 2). The maximal stimulation of APPs release by nicotine was 2.9 times the basal level. The levels of APPs after 1- and 2-hour treatment with 100 μmol/L nicotine were significantly different from that of the control group ($p < .05$, Duncan ANOVA). The effect of nicotine on the secretion of APPs is attenuated by cotreatment with mecamylamine, a noncompetitive antagonist of nAChRs, especially the ganglionic-type nAChR. These results indicated nicotine could enhance the release of APPs through the specific interaction with nAChRs.

Mechanism of the Nicotine-Induced APPs Release

To determine whether the increase of APPs by nicotine was due to enhanced transcription of APP, we extracted total RNA from the PC12 cells treated with nicotine and performed reverse transcription polymerase chain reaction. However, there were no significant changes in the expression levels of three major isoforms of APP (APP695, APP751, and APP770) relative to β-actin from 30 min to 4 hours after nicotine treatment (data not shown). Therefore, the enhanced release of APPs by nicotine probably arises from an accelerated proteolytic processing rather than an increased transcription of APP. Cotreatment of mecamylamine, a specific nicotinic recep-

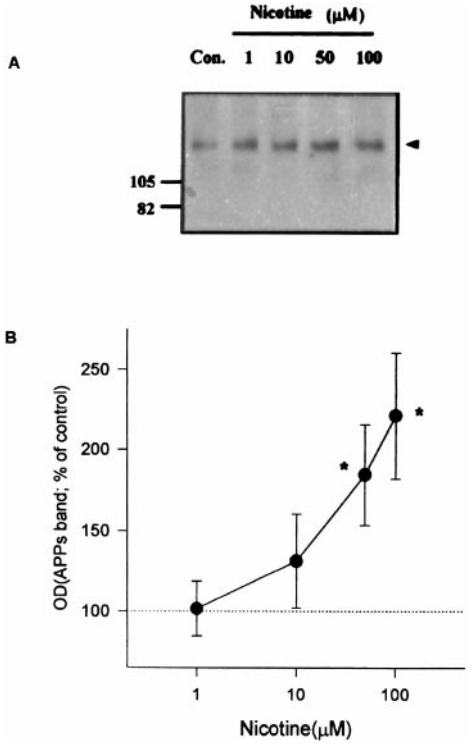

Figure 1. Concentration-dependent release of the secreted form of amyloid precursor protein (APPs) from PC12 cells in response to nicotine. **(A)** Representative immunoblot showing the effects of various concentrations (1, 10, 50, and 100 μmol/L) of nicotine on the release of APPs in PC12 cells. After incubation of the cells with the indicated concentration of nicotine for 4 hours, media was collected and analyzed. The same amounts of extracellular proteins (15 μg) were run on 5–14% gradient sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblotted with 22C11 antibody (antibody against the *N*-terminal portion of APP). Molecular weight of prestained marker is shown at left. The band representing APPs (about 130 kd) is indicated by the arrowhead. (Reproduced with permission from Kim et al 1997.) **(B)** Quantitation of the APPs band in the blots. The amount of APPs was expressed as a percentage of control value without nicotine treatment in the same experiment. Data are mean ± SEM (bar) values of three to seven different experiments. *Significantly different from control value ($p < .05$, Duncan analysis of variance). (Reproduced with permission from Kim et al 1997.) OD, optical density.

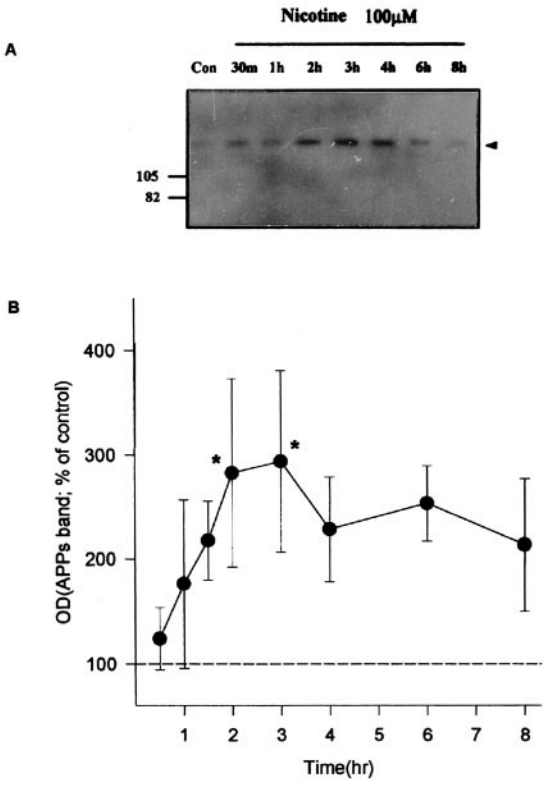

Figure 2. Time-dependent enhancing effects of nicotine on the secretion of the secreted form of amyloid precursor protein (APPs). **(A)** Representative immunoblot demonstrating the effect of nicotine(100 μmol/L) on the production of APPs at various time points in PC12 cells. Molecular weight of the prestained marker is shown at left, and the band representing APPs (about 130 kd) is indicated by the arrowhead. (Reproduced with permission from Kim et al 1997.) **(B)** Results of the densitometric measurements of the APPs band. The amount of APPs was expressed as a percentage of control value in the same experiment. Data are mean ± SEM (bar) values of three to five different experiments. *Significantly different from control value ($p < .05$, Duncan analysis of variance). (Reproduced with permission from Kim et al 1997.) OD, optical density.

tor antagonist, significantly attenuated the release of APPs induced by nicotine ($p < .05$, Wilcoxon rank sum test). Thus it was thought that the effect of nicotine on APP processing was specifically mediated by nAChRs. In addition, ethyleneglycoltetra-acetic acid (EGTA), a calcium chelator, almost completely abolished the enhancing effect of nicotine on APPs release ($p < .01$, Student *t* test) (Figure 3), implying that Ca^{2+} entry through the nAChR is essential in the enhanced release of APPs by nicotine. Mecamylamine or EGTA itself had little effect on APP processing. Then we examined whether the increase in APPs release by nicotine is accompanied by the decrease in the secretion of Aβ in the conditioned media to an easily detectable level. We transiently transfected Swedish mutant APP695 to PC12 cells; however, nicotine (100 μmol/L) treatment did not significantly change the amount of Aβ production in the transfected cells. The mechanism of enhancement of APPs release by nicotine is not clear at present. However, with the fact that the effect of nicotine on APP processing was almost completely abolished by the calcium chelator, EGTA, it is probably related to calcium entry through nAChRs. Neuronal nAChR channels have a greater Ca^{2+} permeability (P_{Ca}:P_{Na} = 20 for the α7 homomeric channel and 1–1.5 for other neuronal heteromeric channels) than muscle nAChRs (P_{Ca}:P_{Na} = 0.2) (McGehee and Role 1995). In PC12 cells, P_{Ca}:P_{Na} is approximately 2.5 (Sands and Barish 1992). Calcium entry

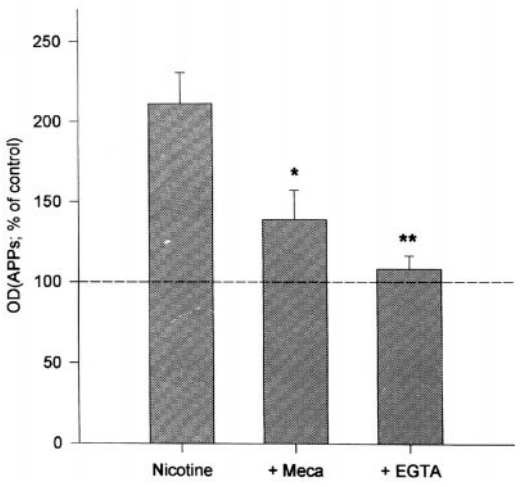

Figure 3. Blocking effects of mecamylamine, a nicotinic receptor antagonist, and ethyleneglycoltetra-acetic acid (EGTA) on the enhancement of release of the secreted form of amyloid precursor protein (APPs) by nicotine in PC12 cells. After incubation of the cells with nicotine alone (100 μmol/L) or in the presence of mecamylamine (10 μmol/L) or EGTA (2.5 mmol/L) for 4 hours, media were collected and analyzed. The amount of APPs was expressed as a percentage of control value in the same experiment. Data are mean ± SEM (bar) values of four independent experiments. Significant difference from the nicotine-alone group: *$p < .05$, Wilcoxon rank sum test; **$p < .021$, Student *t* test. (Reproduced with permission from Kim et al 1997.) OD, optical density.

through the neuronal nAChR channel is sufficient to activate various Ca^{2+}-dependent cellular processes (Vernino et al 1992) such as neurotransmitter release. Depolarization induced by nAChR stimulation further increases the Ca^{2+} influx through voltage-sensitive Ca^{2+} channels. Several studies have indicated that calcium can also regulate APP processing. Buxbaum et al (1994) demonstrated that thapsigargin and cyclopiazonic acid, which inhibit intracellular Ca^{2+} uptake into the endoplasmic reticulum, increased APPs release in a protein kinase C–independent manner. Furthermore, calcium ionophore A23187 was also shown to enhance the release of APPs in differentiated PC12 cells (Loefler and Huber 1993). Electric depolarization, which also raises the intracellular Ca^{2+} concentration, enhances the APPs release from the hippocampal slices (Vernino et al 1992). The exact molecular mechanism by which calcium modulates APP processing still remains unclear. One possibility is that calcium-sensitive proteases might be directly involved in APP processing. The other possibility is that calcium might indirectly influence the activities of other proteases responsible for APP processing. Although little is known about the identity of α secretase(s), several proteases have been suggested as potential candidates. One of them is the calcium-activated, dithiothreitol-sensitive metalloprotease present in the rat brain (Allsop et al 1991). However, the exact identity of α secretase(s) and the role of Ca^{2+} in regulating the activity of the enzyme(s) need to be elucidated further. It is of considerable interest that APPs can stabilize the intracellular Ca^{2+} concentration (Mattson et al 1993) by activating high-conductance K^+ channels. These results raise the possibility that APPs induced by increased intracellular Ca^{2+} may act as a negative regulator to control the intracellular level of Ca^{2+}, an important signaling molecule in the neuron. Although an increased release of APPs has been expected to be accompanied by a decrease in Aβ secretion, this is not always true. Several studies demonstrated a dissociation between APPs release and Aβ generation (Dyrks et al 1994; Loefler and Huber 1993; Querfurth and Selkoe 1994). In the present study, nicotine could not lower the Aβ production from the Swedish mutant APP transfectants, whereas it could stimulate the release of APPs. Thus there might be a complex regulatory mechanism for these two processing events of APP. However, since we only examined the effects of nicotine on the pathologically high production of Aβ, the modulation of the physiologic Aβ production by nicotine needs to be established in future studies.

Effect of Nicotine on the CT_{105}- or Aβ-Induced Cytotoxicity

We previously reported CT_{105}-induced cytotoxicity in various neuronal cells including primary neuronal cells (Kim and Suh 1996). In summary, CT_{105} peptide induced a significant lactic dehydrogenase (LDH) release from cultured rat cortical and hippocampal neurons, PC12 cells, and SHSY5Y cells in a concentration- and time-dependent manner but did not affect the viability of U251 cells originating from human glioblastoma. Moreover, when PC12 cells were induced to differentiate into neurons by pretreatment with nerve growth factor (NGF), the cells became much more sensitive to CT_{105}. The toxic effect of CT_{105} was more potent than any fragments of APP. These results indicate that the toxic effect of CT_{105} is neurospecific. In contrast to CT_{105}, Aβ increased LDH release only slightly, even at 50 μmol/L, but inhibited 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) reduction at submicromolar concentrations (Kim and Suh 1996; Suh 1997).

Recently there has been extensive evidence indicating that nicotine modulates the neurotoxic effect of Aβ (Zamani et al 1997). For example, nicotine receptor stimulation protects neurons against Aβ toxicity (Kihara et al 1997, 1998) and β-amyloid peptide binds to the α7 nicotinic receptor (Wang et al 2000). It has been reported that nicotine inhibits amyloid formation by the Aβ peptide

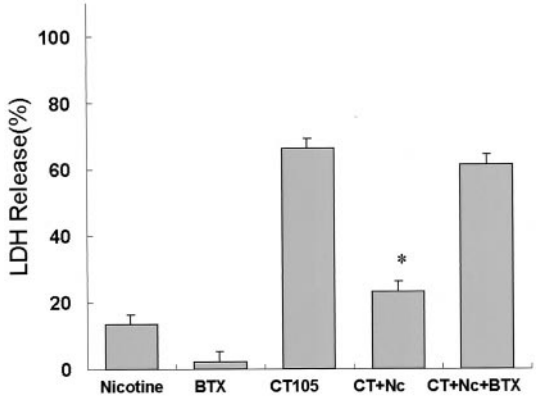

Figure 4. Protective effects of nicotine on the 105–amino acid carboxyl terminal (CT_{105})-induced cytotoxicity in primary neuronal cells, which were pretreated with the α-bungarotoxin (BTX) nicotinic receptor antagonist for 24 hours in the presence of 10 μmol/L nicotine (Nc). Lactic dehydrogenase (LDH) release into the medium is expressed as the percentage of maximal LDH release (positive control), which was obtained upon complete cell lysis by the addition of 0.9% triton X-100, and LDH release obtained upon addition of vehicle was taken as 0% (control). Data are mean ± SEM (bar) values of five independent experiments. *Significant difference from the control group ($p < .01$, SPSS analysis of variance). **Significant difference from CT_{105} peptide-alone group ($p < .05$).

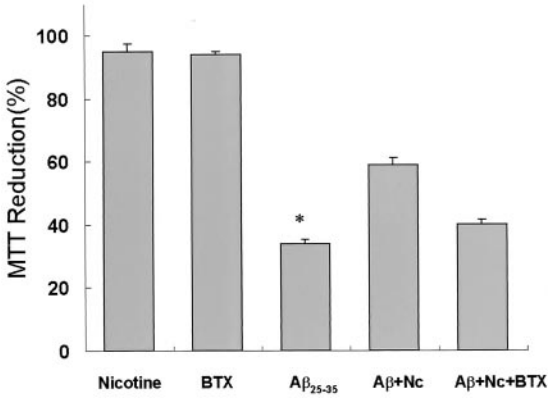

Figure 5. Protective effects of nicotine on the β-amyloid (Aβ)-induced cytotoxicity in primary neuronal cells. Primary neuronal cells were pretreated with the nicotinic receptor antagonist α-bungarotoxin (BTX) for 24 hours in the presence of 10 μmol/L nicotine (Nc) and then were exposed to 10 μmol/L Aβ peptide for 24 hours. Methylthiotetrazole (MTT) reduction activity was determined, assay values obtained upon addition of vehicle were taken as 100% (control), and complete inhibition of MTT reduction (0%, negative control) was defined as the assay value obtained following the addition of 0.9% triton X-100 to completely lyse the cells. Data are mean ± SEM (bar) values of five independent experiments. *Significant difference from the control group ($p < .01$, SPSS analysis of variance).

(Kihara et al 1999; Salomon et al 1996). Additionally, Singh and colleagues reported that nicotine inhibits phospholipases A_2 and D activation by Aβ peptide (Singh et al 1998). These findings strongly suggest that nicotine inhibits its Aβ peptide-induced cytotoxicity and that nicotine might inhibit the cytotoxicity caused by another important APP-derived fragment, CT_{105}. Therefore, we investigated the effects of nicotine on the CT_{105}- or Aβ-induced cytotoxicity. Pretreatment of nicotine (>5 μmol/L for 24 hours) partially inhibited CT_{105}-induced LDH release in primary neuronal cells (≅55%), and the effects of nicotine-induced protection were prevented by cotreatment with nicotine and the α7 neuronal receptor antagonist α-bungarotoxin (αBTX) (≅50%) (Figure 4). Pretreatment of nicotine (>10 μmol/L for 24 hours) partially increased Aβ-induced MTT reduction in primary neuron cells, and the effects of nicotine-induced protection were prevented by the cotreatment with nicotine and αBTX, but there was no significance (Figure 5).

Among the various nicotinic receptor subtypes (Elgoyhen et al 1994; McGehee and Role 1995), α7 neuronal receptors (homo-oligomers made up exclusively of the α7 subunits) and neuronal central nervous system receptors (hetero-oligomers made up of α4 and α2 subunits) are thought to be major subtypes in the central nervous system (Watson et al 1996). In our study, the subtype-specific antagonist (the α7 receptor-selective antagonist αBTX) antagonized the nicotine-induced protection against Aβ-induced cytotoxicity, though its antagonistic effects were not complete. These results suggest that the marked loss of both cholinergic innervation and nAChRs in the cerebral cortex and hippocampus seen in the AD brain may exacerbate the toxic effects of the Aβ peptide or CT_{105} peptide because nicotine and its receptor activation appear to play a neuroprotective role.

Our recent studies using CT_{105} transfection in PC12 cells supported the finding that nicotine inhibited the CT_{105} peptide–induced cytotoxicity. In line with the experiment using extracellularly administered CT_{105}, nicotine (>10 μmol/L) pretreatment partially protected cytotoxicity induced by CT_{105} in CT_{105}-transfected PC12 cells, and the protective effect was blocked by the cotreatment of the nicotinic receptor antagonist αBTX (data not shown). Therefore these results (that the cytotoxicity induced by either extracellular or intracellular CT_{105} is partially blocked by nicotine and reappears with αBTX) indicated that this effect might be mediated by the nicotinic receptor.

In the present study, nicotine showed significant protective effects at a concentration of 10 μmol/L. But it is known that nAChRs, especially those containing α7, desensitize rapidly when exposed to nicotine at more than 10 μmol/L (Zhang et al 1994), and it is possible that the nicotine added to culture media stimulated nAChRs continuously and caused desensitization-elicited receptor dysfunction in at least some of the cells examined. However,

this possibility is not likely, since the concomitant application of nicotine and nicotinic receptor antagonists significantly reduced the neuroprotective action of nicotine and APPs release in our study. Although the protective mechanism of nicotine is still unclear, it has been reported that nicotine activation regulates c-fos transcript levels (Greenberg et al 1986). These findings suggest that persistent receptor stimulation and the increased release of APPs by nicotine are necessary to elicit its protective effect against CT_{105}- or Aβ-induced cytotoxicity.

Conclusions

It is suggested that nicotine protects cortical neurons against CT_{105} peptide- or Aβ-induced neurotoxicity via nicotinic receptor stimulation. Although further study is required to determine the intracellular mechanisms underlying the neuroprotective action of nicotine, nicotine protects neurons against the cytotoxicity of CT and Aβ fragments. Therefore, we propose that acetylcholine acting on nicotinic cholinergic receptors can function as a putative neuroprotective factor against neurodegeneration caused by Aβ or CT_{105} fragments in AD brains.

Recent studies strongly suggest that APPs has potent neurotrophic and protective activities in several cultured cell models. It can stimulate neurite outgrowth in PC12 cells, promote the proliferation of fibroblasts, and protect cultured neurons from metabolic and excitotoxic insults (Mattson et al 1993). Therefore, APPs may act as a paracrine neurotrophic and neuroprotective factor. Interestingly, nicotine was also shown to attenuate the neuronal degeneration induced by glutamate (Akaike et al 1994) and NGF deprivation (Yamashita and Nakamura 1996) in vitro. These findings were further extended to in vivo studies that demonstrated the protective effects of nicotine against neurotoxin- or mechanically induced degeneration of the nigrostriatal dopaminergic neuron (Janson et al 1989). The evidence that the release of APPs was increased by nicotine suggested that nicotine could play a neuroprotective role against neurodegeneration or neurotoxicity.

The physiologic relevance of the enhanced APPs release by nicotine is not clear. Nicotine has been shown to cause a myriad of psychopharmacologic effects such as cognitive enhancement (Levin et al 1992). The central effects of nicotine were believed to be principally mediated by neuronal nAChRs. Activation of neuronal nAChRs located in the presynaptic sites could facilitate release of neurotransmitters such as glutamate, γ-aminobutyric acid, and dopamine and enhanced the synaptic transmission (McGehee and Role 1995). Our data indicate that the processing of APP can also be modulated by nicotine receptor activation. In AD brains, nicotinic neurotransmission is severely damaged (Bierer et al 1995), which may lead to an altered processing of APP. Reduced APPs release might secondarily contribute to the neuronal loss in AD. Because the degree of cognitive impairments in AD has been reported to correlate well with the deficits of cholinergic neurotransmission in the brain (Smith et al 1992), elevation of acetylcholine level was hypothesized to be helpful in improving the cognitive deficits in AD. Many groups have tried to supplement the cholinergic transmission by administration of acetylcholine precursor, muscarinic or nicotinic receptor agonists, or acetylcholinesterase inhibitors. Although most of them failed to effectively ameliorate the symptoms of AD, pilot clinical studies indicated that nicotine might be beneficial for the treatment of the deficits in attention and information processing associated with the disease (Newhouse et al 1997; Giacobini 2000; Jann 2000). However, nicotine itself has limited utility as a therapeutic agent because of its dose-limiting side effects such as hypertension, tachycardia, and abdominal pain. Thus, many groups are now trying to develop a novel nicotinic receptor agonist that is able to enhance the cognitive functions by specific interaction with neuronal nAChRs without eliciting peripheral side effects. Our results strongly imply that nicotine or nicotinic agonists might be beneficial for the treatment of AD not only by supplementation of the cholinergic neurotransmission but also by protecting Aβ- or CT105-induced neurotoxicity, probably through the increased release of APPs.

This study was supported by grants-in-aid from Korea Ginseng & Tobacco Research Insititute (1999–2000), the BK21 Human Life Sciences project, and the Creative Research Initiative program from MOST.

Aspects of this work were presented at the symposium "Nicotine Mechanisms in Alzheimer's Disease," March 16–18, 2000, Fajardo, Puerto Rico. The conference was sponsored by the Society of Biological Psychiatry through an unrestricted educational grant provided by Janssen Pharmaceutica LP.

References

Akaike A, Tamura Y, Yokota T, Shimohama S, Kimura J (1994): Nicotine-induced protection of cultured cortical neurons against N-methyl-D-aspartate receptor-mediated glutamate cytotoxicity. *Brain Res* 644:181–187.

Allsop D, Yamamoto T, Kamentani F, Miyazaki N, Ishii T (1991): Alzheimer amyloid β/A4 peptide binding sites and a possible "APP-secretase" activity associated with rat brain cortical membranes. *Brain Res* 551:1–9.

Bierer LM, Haroutunian V, Gabriel S, Knott PJ, Carlin LS, Purohit DP, et al (1995): Neurochemical correlates of dementia severity in Alzheimer's disease: Relative importance of the cholinergic deficits. *J Neurochem* 64:749–760.

Buxbaum JD, Rueflil AA, Parker CA, Cypess AM, Greengard P (1994): Calcium regulates processing of the Alzheimer's

amyloid protein precursor in a protein kinase C-independent manner. *Proc Natl Acad Sci U S A* 91:4489–4493.

Checler F (1995): Processing of the beta-amyloid precursor protein and its regulation in Alzheimer's disease. *J Neurochem* 65:1431–1444.

Clemens JA, Stephenson DT (1992): Implants containing beta-amyloid protein are not neurotoxic to young and old rat brain. *Neurobiol Aging* 13:581–586.

Cullen WK, Suh YH, Anwyl R, Rowan MJ (1997): Block of late-phase long-term potentiation in rat hippocampus in vivo by β-amyloid precursor protein fragments. *Neuroreport* 8:3213–3217.

Dyrks TU, Moening K, Beyreuther K, Turner J (1994): Amyloid precursor protein secretion and β/A4 amyloid generation are not mutually exclusive. *FEBS Lett* 349:210–214.

Elgoyhen AB, Johnson DS, Boulter J, Vetter DE, Heinemann S (1994): α9: An acetylcholine receptor with novel pharmacological properties expressed in rat cochlear hair cells. *Cell* 79:705–715.

Estus S, Golde TE, Kunishita TL, Blades D, Lowery D, Eisen J, et al (1992): Potentially amyloidogenic, carboxyl-terminal derivatives of the amyloid protein precursor. *Science* 255: 726–728.

Fraser S, Suh YH, Chong YH, Djamgoz MA, (1996): Membrane currents induced in Xenopus oocytes by the carboxyl terminal fragment of the β-amyloid precursor protein. *J Neurochem* 66:2034–2040.

Fukuchi K, Kunkel DD, Schwartzkroin PA, Kamino K, Ogburn CE, Furlong CE, Martin GM (1994): Overexpression of a C-terminal portion of the beta-amyloid precursor protein in mouse brains by transplantation of transformed neuronal cells. *Exp Neurol* 127:253–264.

Giacobini E (2000): Cholinesterase inhibitor therapy stabilizes symptoms of Alzheimer disease. *Alzheimer Dis Assoc Disord* 14(suppl 1):S3–S10.

Golde TE, Estus S, Younkin LH, Selkoe DJ, Younkin SG (1992): Processing of the amyloid protein precursor to potentially amyloidogenic derivatives. *Science* 255:728–730.

Greenberg ME, Ziff EB, Greene LA (1986): Stimulation of neuronal acetylcholine receptors induces rapid gene transcription. *Science* 234(4772):80–83.

Haass C, Koo EH, Mellon A, Hung AY, Selkoe DJ (1992a): Targeting of cell-surface beta amyloid precursor protein to lysosomes: Alternative processing into amyloid-bearing fragments. *Nature* 357:500–503.

Haass C, Schlossmacher MG, Hung AY, Vigo-Pelfrey C, Mellon A, Ostaszewski BL, et al (1992b): Amyloid β-peptide is produced by cultured cells during normal metabolism. *Nature* 359:322–325.

Hartell NA, Suh YH (2000): Effects of fragments of β-amyloid precursor protein on parallel fiber-purkinje cell synaptic transmission in rat cerebellum. *J Neurochem* 74:1112–1121.

Henderson LP, Gdovin MJ, Liu C, Gardner PD, Maue RA (1994): Nerve growth factor increases nicotinic Ach receptor gene expression and current density in wild-type and protein kinase A-deficient PC12 cells. *J Neurosci* 14:1153–1163.

Jann MW (2000): Rivastigmine, a new-generation cholinesterase inhibitor for the treatment of Alzheimer's disease. *Pharmacotherapy* 20:1–12.

Janson AM, Fuxe K, Agnati LF, Jansson A, Bjelke B, Sundstrom E, et al (1989): Protective effects of chronic nicotine treatment on lesioned nigrostriatal dopamine neurons in the male rat. *Prog Brain Res* 79:257–265.

Jones GM, Sahakian BJ, Levy R, Warburton DM, Gray JA (1992): Effects of acute subcutaneous nicotine on attention, information processing and short-term memory in Alzheimer's disease. *Psychopharmacology (Berl)* 108:485–489.

Kametani F, Tanaka K, Tokuda T, Ikeda S (1994): Secretory cleavage site of Alzheimer amyloid precursor protein in heterogeneous in Down's syndrome brain. *FEBS Lett* 351: 165–167.

Kammesheidt A, Boyce FM, Spanoyannis AF, Cummings BJ, Ortegon M, Cotman C, et al (1992): Deposition of beta/A4 immunoreactivity and neuronal pathology in transgenic mice expressing the carboxyl-terminal fragment of the Alzheimer amyloid precursor in the brain. *Proc Natl Acad Sci U S A* 89:10857–10861.

Kellar KJ, Wonnacott S (1990): Nicotinic cholinergic receptors in Alzheimer's disease. In: Wonnacott S, Russel MAH, Stolerman IP, editors. *Nicotinic Psychopharmacology: Molecular, Cellular and Behavioral Aspect.* New York: Oxford Press, 341–373.

Kihara T, Shimohama S, Akaike A (1999): Effects of nicotinic receptor agonists on beta-amyloid beta-sheet formation. *Jpn J Pharmacol* 79:393–396.

Kihara T, Shimohama S, Sawada H, Kimura J, Kume T, Kochiyama H, et al (1997): Nicotinic receptor stimulation protects neurons against beta-amyloid toxicity. *Ann Neurol* 42:159–163.

Kihara T, Shimohama S, Urushitani M, Sawada H, Kimura J, Kume T, et al (1998): Stimulation of alpha4beta2 nicotinic acetylcholine receptors inhibits β-amyloid toxicity. *Brain Res* 792:331–334.

Kim HJ, Suh YH, Lee MH, Ryu PD (1999a): Cation selective channels formed by C-terminal fragment of β-amyloid precursor protein. *Neuroreport* 10:1427–1431.

Kim HS, Lee JH, Suh YH (1999b): C-terminal fragment of Alzheimer's amyloid precursor protein inhibits sodium/calcium exchanger activity in SK-N-SH cell. *Neuroreport* 10: 113–116.

Kim HS, Park CH, Suh YH (1998): C-terminal fragment of amyloid precursor protein inhibits calcium uptake into rat brain microsomes by Mg^{2+}-Ca^{2+} ATPase. *Neuroreport* 9:3875–3879.

Kim SH, Kim YK, Jeong SJ, Haass C, Kim YH, Suh YH (1997): Enhanced release of secreted form of Alzheimer's amyloid precursor protein (βAPP) in PC12 cells by nicotine. *Mol Pharmacol* 52:430–436.

Kim SH, Suh YH (1996): Neurotoxicity of a caroboxy terminal fragment of the Alzheimer's amyloid precursor protein. *J Neurochem* 67:1172–1182.

Koo EH, Squazzo SL (1994): Evidence that production and release of amyloid β-protein involves the endocytic pathway. *J Biol Chem* 269:17386–17389.

Kozlowski MR, Spanoyannis AL, Manly SP, Fidel SA, Neve RL (1992): The neurotoxic carboxy-terminal fragment of the Alzheimer amyloid precursor binds specifically to a neuronal cell surface molecule: PH dependence of the neurotoxicity and the binding. *J Neurosci* 12:1679–1687.

Levin ED (1992): Nicotinic systems and cognitive function. *Psychopharmacology* 108:417–431.

Loefler J, Huber G (1993): Modulation of β-amyloid precursor protein secretion in differentiated and nondifferentiated cells. *Biochem Biophys Res Commun* 195:97–103.

Lu DC, Rabizadeh S, Chandra S, Shayya RF, Ellerby LM, Ye X, et al (2000): A second cytotoxic proteolytic peptide derived from amyloid beta-protein precursor. *Nat Med* 6:397–404.

Matsumoto A, Matsumoto R (1994): Familial Alzheimer's disease cells abnormally accumulate beta-amyloid harbouring peptides preferentially in cytosol but not in extracellar fluid. *Eur J Biochem* 225:1055–1062.

Matsumoto A (1994): Altered processing characteristics of amyloid-containing peptides in cytosol and media of familial Alzheime's disease cells. *Biochem Biophys Acta* 1225:304–310.

Mattson MP, Cheng B, Culwell AR, Esch FS, Lieberburg I, Rydel RE (1993): Evidence for excitoprotective and intraneuronal calcium-regulating roles for secreted forms of the beta-amyloid precursor protein. *Neuron* 10:243–254.

McGehee DS, Role DW (1995): Physiological diversity of nicotinic acetylcholine receptors expressed by vertebrate neurons. *Annu Rev Physiol* 57:521–546.

Nalbantoglu J, Tirado-Santiago G, Lahsaini A, Poirier J, Goncalves O, Verge G, et al (1997): Impaired learning and LTP in mice expressing the carboxy terminus of the Alzheimer amyloid precursor protein. *Nature* 387(6632):500–505.

Newhouse PA, Potter A, Levin ED (1997): Nicotinic system involvement in Alzheimer's and Parkinson's diseases. Implications for therapeutics. *Drugs Aging* 11:206–228.

Querfurth HW, Selkoe DJ (1994): Calcium ionophore increases amyloid β peptide procuction by cultured cells. *Biochemistry* 33:4550–4561.

Salomon AR, Marcinowski KJ, Friedland RP, Zagorski MG (1996): Nicotine inhibits amyloid formation by the beta-peptide. *Biochemistry* 35:13568–13578.

Sands SB, Barish ME (1992): Neuronal nicotinic acetylcholine receptor currents in phochromocytoma (PC12) cells: Dual mechanisms of rectification. *J Physiol* 447:467–487.

Selkoe DJ (1994): Alzheimer's disease: A central role for amyloid. *J Neuropathol Exp Neurosci* 60:607–619.

Shoji M, Golde TE, Ghiso J, Cheung TT, Estus S, Shaffer LM, et al (1992): Production of the Alzheimer amyloid beta protein by normal proteolytic processing. *Science* 258:126–129.

Singh IN, Sorrentino G, Sitar DS, Kanfer JN (1998): (−)Nicotine inhibits the activations of phospholipases A2 and D by amyloid beta peptide. *Brain Res* 800:275–281.

Smith CJ, Giacobini E (1992): Nicotine, Parkinson's and Alzheimer's disease. *Rev Neurosci* 3:25–42.

Song DK, Won MH, Jung JS, Lee JC, Kang TC, Suh HW, et al (1998): Behavioral and neuropathologic changes induced by central injection of carboxyl-terminal fragment of beta-amyloid precursor protein in mice. *J Neurochem* 71:875–878.

Suh YH (1997): An etiological ro le of amyloidogenic carboxyl-terminal fragments of the β-amyloid precursor protein in Alzheimer's disease. *J Neurochem* 68:1781–1791.

Suh YH, Chong YH, Kim SH, Choi W, Kim KS, Jeong SJ, et al (1996): Molecular physiology, biochemistry, and pharmacology of Alzheimer's amyloid precursor protein (APP). *Ann N Y Acad Sci* 786:169–183.

Tamaoka A, Kalaria RN, Lieberburg I, Selkoe DJ (1992): Identification of a stable fragment of the Alzheimer amyloid precursor containing the beta-protein in brain microvessels. *Proc Natl Acad Sci U S A* 89:1345–1349.

Vernino S, Amador M, Leutje CW, Patrick J, Dani JA (1992): Calcium modulation and high calcium permeability of neuronal nicotinic acetylcholine receptors. *Neuron* 8:127–134.

Wang HY, Lee DH, D'Andrea MR, Peterson PA, Shank RP, Reitz AB (2000): Beta-amyloid(1-42) binds to alpha7 nicotinic acetylcholine receptor with high affinity: Implications for Alzheimer's disease pathology. *J Biol Chem* 275:5626–5632.

Watson EL, Abel PW, DiJulio D, Zeng W, Makoid M, Jacobson KL, et al (1996): Identification of muscarinic receptor subtypes in mouse parotid gland. *Am J Physiol* 271:C905–C913.

Yamashita H, Nakamura S (1996): Nicotine rescues PC12 cells from death induced by nerve growth factor deprivation. *Neurosci Lett* 213:145–147.

Zamani MR, Allen YS, Owen GP, Gray JA (1997): Nicotine modulates the neurotoxic effect of beta-amyloid protein(25-35) in hippocampal cultures. *Neuroreport* 8:513–517.

Zhang ZW, Vijayaraghavan S, Berg DK (1994): Neuronal acetylcholine receptors that bind alpha-bungarotoxin with high affinity function as ligand-gated ion channels. *Neuron* 12:167–177.