Nicotine Breaks Down Preformed Alzheimer's β-Amyloid Fibrils in Vitro

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Background: *Cerebral deposition of amyloid β-peptide (Aβ) is a major neuropathologic feature in Alzheimer's disease (AD). A consistent protective effect of smoking on AD has been documented by many case-control studies. It has been suggested that nicotine, a major component of cigarette smoke, protects neurons against Aβ toxicity via the upregulation of nicotinic receptors, as well as via the inhibition of β-amyloid fibril (fAβ) formation from Aβ.*

Methods: *We used fluorescence spectroscopy with thioflavin T and electron microscopy to examine the effects of nicotine, pyridine, and N-methylpyrrolidine on the formation, extension, and disruption of fAβ(1-40) and fAβ(1-42) at pH 7.5 at 37°C in vitro.*

Results: *Nicotine dose-dependently inhibited fAβ(1-40) and fAβ(1-42) formation from fresh Aβ(1-40) and Aβ(1-42), respectively, as well as the extension reaction of both fAβs. Moreover, nicotine disrupted preformed fAβ(1-40) and fAβ(1-42). These effects of nicotine were observed at concentrations above 10 mmol/L and were similar to those of N-methylpyrrolidine.*

Conclusions: *The antiamyloidogenic effect of nicotine may be exerted not only by the inhibition of fAβ formation but also by the disruption of preformed fAβ. Additionally, this effect may be attributed to N-methylpyrrolidine moieties of nicotine.* Biol Psychiatry 2002;52: 880–886 © *2002 Society of Biological Psychiatry*

Key Words: Alzheimer's disease, nicotine, β-amyloid fibrils, thioflavin T, electron microscopy, disruption

Introduction

The intracerebral accumulation of the amyloid β-peptide (Aβ) as senile plaques or vascular amyloid plays a key role in the pathogenesis of Alzheimer's disease (AD; Selkoe 2000). Despite recent progress in the symptomatic therapy with cholinergic drugs (Doody et al 2001), an effective therapeutic approach that interferes directly with the neurodegenerative process in AD, especially the accumulation of Aβ in the central nervous system (CNS) is eagerly awaited.

An inverse relationship between smoking and AD has been suggested (Graves and Mortimer 1994; Lerner et al 1997; Ulrich et al 1997). Nicotine in tobacco provokes health problems in humans, but in its pure form it has the potential to be a valuable pharmaceutical agent (Jarvick 1991). Nicotine administration via injection or skin patches has been shown to significantly improve attention and learning in animals (Levin and Simon 1998), as well as in patients with AD (Emilien et al 2000; White and Levin 1999; Wilson et al 1995). These beneficial effects have been attributed to the protection of neurons against Aβ toxicity via the upregulation of nicotinic receptors deficient in the AD brain (Kihara et al 1997; Zamani et al 1997). On the other hand, Zagorski's group reported that nicotine inhibits β-amyloid fibril (fAβ) formation from Aβ, based on circular dichroism, ultraviolet, and nuclear magnetic resonance spectroscopic studies (Salomon et al 1996; Zeng et al 2001).

Based on a nucleation-dependent polymerization model to explain the mechanisms of fAβ formation in vitro (Jarrett and Lansbury 1993; Lomakin et al 1997; Naiki and Gejyo 1999; Naiki et al 1997), we previously reported that nordihydroguaiaretic acid (NDGA) and rifampicin (RIF) inhibit fAβ formation from Aβ and fAβ extension dose-dependently in vitro (Naiki et al 1998). Recently, we reported that NDGA disrupts fAβ(1-40) and fAβ(1-42) within a few hours at pH 7.5 at 37°C, based on fluorescence spectroscopic analysis with thioflavin T (ThT) and electron microscopic studies (Ono et al 2002). The activity of NDGA to break down fAβ(1-40) and fAβ(1-42) in comparison with other molecules reported to inhibit fAβ formation from Aβ, to disrupt preformed fAβ both in vivo and in vitro was on the order of NDGA $\gg$ RIF = tetracycline $>$ poly(vinylsulfonic acid, sodium salt) = 1,3-propanedisulfonic acid, disodium salt $>$ β-sheet breaker peptide (iAβ5). Moreover, in cell culture experiments, fAβ disrupted by NDGA was significantly less toxic than intact fAβ.

In this study, we used fluorescence spectroscopy with ThT and electron microscopy to examine the effects of

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nicotine, pyridine, and N-methylpyrrolidine on the formation, extension, and disruption of fAβ(1-40) and fAβ(1-42) at pH 7.5 at 37°C in vitro.

Methods and Materials

Preparation of Aβ and fAβ Solutions

Aβ(1-40) (lot no. 519599, Bachem AG, Bubendorf, Switzerland; lot nos. 500520 and 501001, Peptide Institute, Osaka, Japan) and Aβ(1-42) (lot no. 510523, Peptide Institute) were dissolved by brief vortexing in 0.02% ammonia solution at a concentration of 500 μmol/L (2.2 mg/mL) and 250 μmol/L, respectively, in a 4°C room and immediately stored at −80°C before assaying [hereafter referred to as fresh Aβ(1-40) and Aβ(1-42) solutions]. fAβ(1-40) and fAβ(1-42) were formed from the fresh $Aβ(1-40)_{Bachem}$ and Aβ(1-42) solutions, respectively, sonicated, and stored at 4°C as described elsewhere (Hasegawa et al 1999).

Fresh, nonaggregated fAβ(1-40) and fAβ(1-42) were obtained by extending sonicated fAβ(1-40) or fAβ(1-42) with fresh $Aβ(1-40)_{Peptide\ Institute}$ or Aβ(1-42) solutions, respectively, just before the depolymerization reaction (Ono et al 2002). The reaction mixture was 600 μL and contained 10 μg/mL (2.3 μmol/L) fAβ(1-40) or fAβ(1-42); 50 μM $Aβ(1-40)_{Peptide\ Institute}$ or Aβ(1-42); 50 mmol/L phosphate buffer, pH 7.5; and 100 mmol/L NaCl. After incubation at 37°C for 3 to 6 hours under nonagitated conditions, the extension reaction proceeded to equilibrium as measured by the fluorescence of ThT. In the following experiment, the concentration of fAβ(1-40) and fAβ(1-42) in the final reaction mixture was considered to be 50 μmol/L.

Fluorescence Spectroscopy, Electron Microscopy, and Polarized Light Microscopy

Fluorescence spectroscopic study was performed as described elsewhere (Naiki and Nakakuki 1996) on a Hitachi F-3010 fluorescence spectrophotometer. Optimum fluorescence measurements of fAβ(1-40) and fAβ(1-42) were obtained at the excitation and emission wavelengths of 445 and 490 nm, respectively, with the reaction mixture containing 5 μmol/L ThT (Wako Pure Chemical Industries, Osaka, Japan) and 50 mmol/L of glycine-NaOH buffer, pH 8.5. Electron microscopic study, Congo red staining, and polarized light microscopic study of the reaction mixtures were performed as described elsewhere (Hasegawa et al 1999).

Polymerization Assay

Polymerization assay was performed as described elsewhere (Naiki et al 1998). Briefly, the reaction mixture contained 50 μmol/L $Aβ(1-40)_{Peptide\ Institute}$ or 25–50 μmol/L Aβ(1-42); 0 or 10 μg/mL sonicated fAβ(1-40) or fAβ(1-42), 0, 0.1, 1.0, 10, or 100 mmol/L or 1.0 mol/L nicotine (Sigma Chemical, St. Louis, MO), pyridine (Nacalai Tesque, Kyoto, Japan) or N-methylpyrrolidine (Tokyo Kasei Kogyo, Tokyo, Japan); 50 mmol/L phosphate buffer, pH 7.5; and 100 mmol/L NaCl.

Thirty-μL aliquots of the mixture were put into oil-free polymerase chain reaction (PCR) tubes (0.5 mL, no. 9046, Takara Shuzo, Otsu, Japan). The reaction tubes were then put into a DNA thermal cycler (no. PJ480, Perkin Elmer Cetus, Emeryville, CA). Starting at 4°C, the plate temperature was elevated at maximum speed to 37°C. Incubation times ranged between 0 and 12 days (as indicated in each figure), and the reaction was stopped by placing the tubes on ice. The reaction tubes were not agitated. From each reaction tube, three 5-μL aliquots were removed and then subjected to fluorescence spectroscopy; the mean of each of the three was determined. In the ThT solution, the concentration of nicotine, pyridine, and N-methylpyrrolidine examined in this study was diluted up to 1/200 of that in the reaction mixture. We confirmed that nicotine, pyridine, and N-methylpyrrolidine did not quench ThT fluorescence at the diluted concentration (data not shown).

Disruption Assay

Disruption assay was performed as described elsewhere (Ono et al 2002). Briefly, the reaction mixture contained 25 μmol/L fresh fAβ(1-40) or fAβ(1-42); 0, 10, or 100 mmol/L or 1.0 mol/L nicotine, pyridine, or N-methylpyrrolidine; 50 mM phosphate buffer, pH 7.5; 100 mmol/L NaCl; and 1% (wt/vol) polyvinyl alcohol (Wako Pure Chemical Industries, Osaka, Japan) to avoid the aggregation of fAβ and the adsorption of fAβ onto the inner wall of the reaction tube during the reaction.

After mixing by pipette, three 5-μL aliquots were subjected to fluorescence spectroscopy, and 30-μL aliquots were put into PCR tubes. The reaction tubes were then transferred into a DNA thermal cycler. Starting at 4°C, the plate temperature was elevated at maximum speed to 37°C. Incubation times ranged between 0 and 72 hours (as indicated in each figure), and the reaction was stopped by placing the tubes on ice. The reaction tubes were not agitated during the reaction. From each reaction tube, three 5-μL aliquots were subjected to fluorescence spectroscopy, and the mean of the three measurements was determined.

Other Analytical Procedures

Protein concentrations of the supernatants of the reaction mixtures after centrifugation were determined according to the method described by Bradford (1976) with a protein assay kit (Bio-Rad Laboratories, Hercules, CA). The Aβ(1-40) solution quantified by amino acid analysis was used as the standard. Paired Student's *t* test was used for statistical analysis.

Results

Effect of Nicotine on the Kinetics of fAβ Polymerization

As shown in Figure 1A, when fresh Aβ(1-40) was incubated at 37°C, the fluorescence of ThT followed a characteristic sigmoidal curve. This curve is consistent with a nucleation-dependent polymerization model (Jarrett and Lansbury 1993; Naiki et al 1997). fAβ(1-40) stained

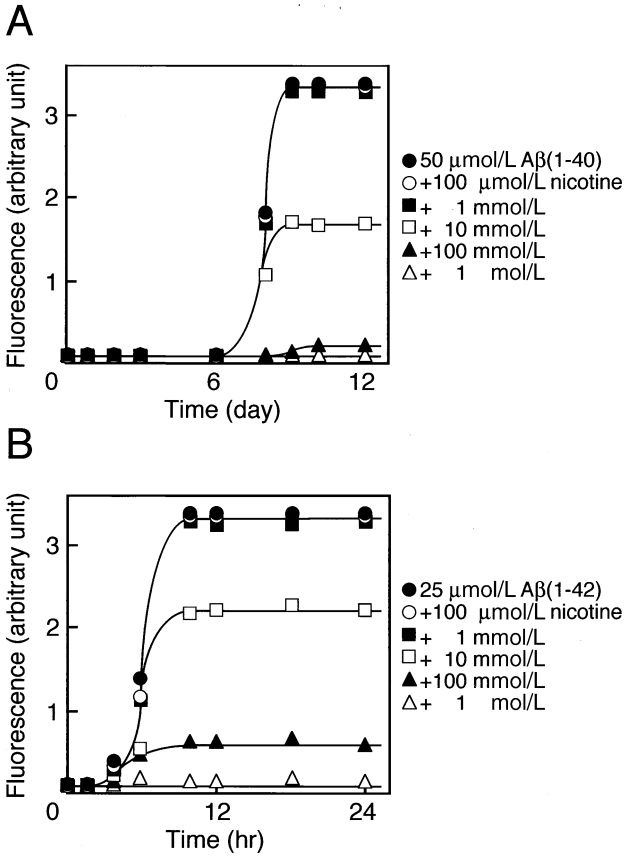

Figure 1. Effect of nicotine on the kinetics of fAβ(1-40) (**A**) and fAβ(1-42) (**B**) formation from fresh Aβ(1-40) and Aβ(1-42), respectively. The reaction mixtures containing 50 μmol/L Aβ(1-40) (**A**) or 25 μmol/L Aβ(1-42) (**B**); 50 mmol/L phosphate buffer, pH 7.5; 100 mmol/L NaCl; and 0 (●), .1 (○), 1.0 (■), 10 (□), or 100 mmol/L (▲) or 1.0 mol/L (△) nicotine were incubated at 37°C for the indicated times. Each figure is a representative pattern of three independent experiments.

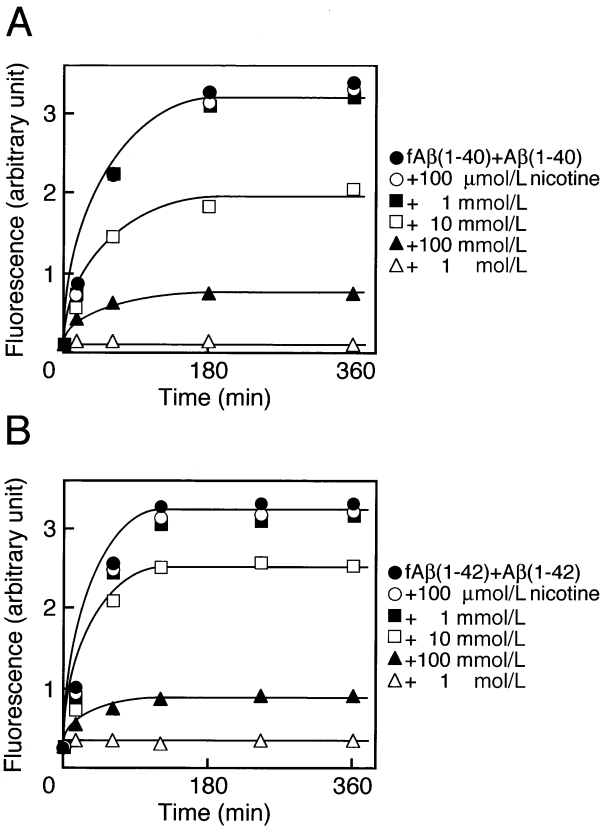

Figure 2. Effect of nicotine on the kinetics of fAβ(1-40) (**A**) and fAβ(1-42) (**B**) extension. The reaction mixtures containing 10 μg/mL (2.3 μmol/L) sonicated fAβ(1-40) (**A**) or fAβ(1-42) (**B**); 50 μmol/L Aβ(1-40) (**A**) or Aβ(1-42) (**B**); 50 mmol/L phosphate buffer, pH 7.5; 100 mmol/L NaCl; and 0 (●), .1 (○), 1.0 (■), 10 (□), or 100 mmol/L (▲) or 1.0 mol/L (△) nicotine were incubated at 37°C for the indicated times. Each figure is a representative pattern of three independent experiments.

with Congo red showed typical orange-green birefringence under polarized light (data not shown). When Aβ(1-40) was incubated with increasing concentrations of nicotine, the final equilibrium level decreased dose dependently. A significant inhibitory effect of nicotine was observed at concentrations above 10 mmol/L. Similar data (Figure 1B) were obtained with Aβ(1-42).

As shown in Figure 2A, when fresh Aβ(1-40) was incubated with fAβ(1-40) at 37°C, the fluorescence increased hyperbolically without a lag phase and proceeded to equilibrium much more rapidly than without seeds (compare Figures 1A and 2A). This curve is consistent with a first-order kinetic model (Naiki and Nakakuki 1996). When Aβ(1-40) and fAβ(1-40) were incubated with increasing concentrations of nicotine, the final equilibrium level decreased dose dependently. A significant inhibitory effect of nicotine was observed at concentrations above 10 mmol/L. Similar data (Figure 2B) were obtained for the extension of fAβ(1-42). The data in Figures 1 and 2 may indicate that nicotine could inhibit both the nucleation reaction of Aβ and the extension reaction of fAβ.

When fAβ(1-40) were extensively sonicated, they sheared, yet the typical structure of the amyloid fibrils was preserved (Figure 3A). When fresh Aβ(1-40) was incubated with sonicated fAβ(1-40) at 37°C, clear fibril extension was observed via electron microscope (Figure 3B); however, 1.0 mmol/L nicotine completely inhibited the extension of sonicated fAβ(1-40), as shown in Figure 3C. One-hundred mmol/L nicotine also inhibited the extension of fAβ(1-40) similarly (data not shown). Similar data (not shown) were obtained for the extension of fAβ(1-42).

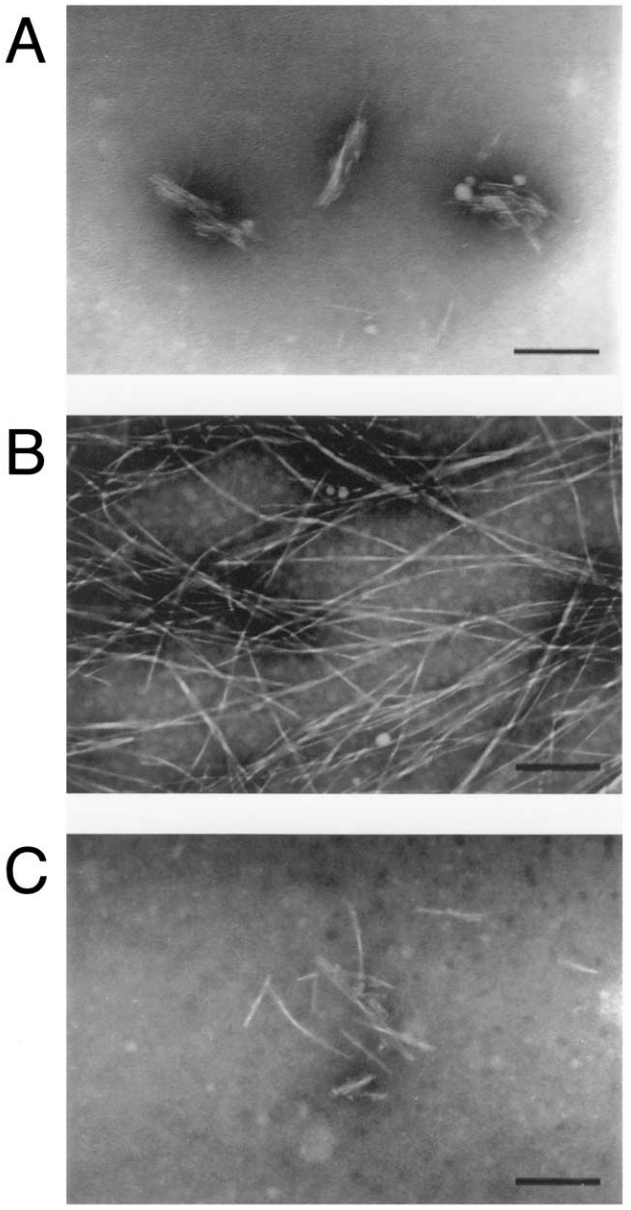

Figure 3. Electron micrographs of extended fAβ(1-40). The reaction mixtures containing 10 μg/mL (2.3 μmol/L) sonicated fAβ(1-40); 50 μmol/L Aβ(1-40); 50 mmol/L phosphate buffer, pH 7.5; 100 mmol/L NaCl; and 0 (**B**) or 1.0 mol/L nicotine (**A, C**) were incubated at 37°C for 0 (**A**) or 6 hours (**B, C**). Scale bars indicate a length of 250 nm.

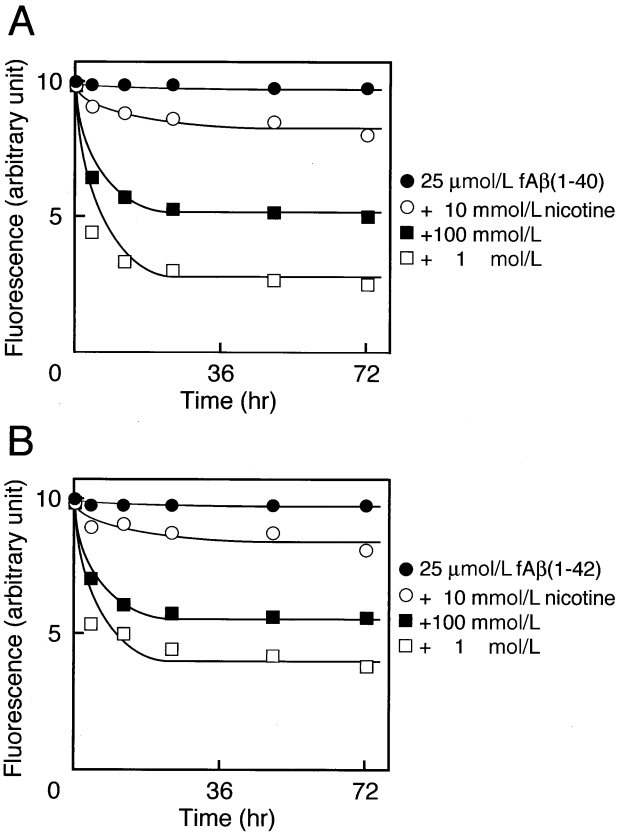

Figure 4. Effect of nicotine on the disruption of fAβ(1-40) (**A**) and fAβ(1-42) (**B**). The reaction mixtures containing 25 μmol/L fAβ(1-40) (**A**) or fAβ(1-42) (**B**); 50 mmol/L phosphate buffer, pH 7.5; 100 mmol/L NaCl; and 0 (●), 10 (○), or 100 mmol/L (■) or 1.0 mol/L (□) nicotine were incubated at 37°C for the indicated times. Each figure is a representative pattern of three independent experiments.

Disruption Assay

As shown in Figure 4, the fluorescence of ThT was almost unchanged during the incubation of fresh fAβ(1-40) or fAβ(1-42) at 37°C without additional molecules; however, the ThT fluorescence decreased immediately after addition of nicotine in a concentration-dependent manner. At 72 hours, the fluorescence of fAβ(1-40) incubated with 10 and 100 mmol/L and 1.0 mol/L nicotine was 79%, 49%, and 27% of the initial fluorescence, respectively (Figure 4A). Similarly, the fluorescence of fAβ(1-42) incubated with 10 or 100 mmol/L and 1.0 mol/L nicotine for 72 hours was 82%, 55%, and 40% of the initial fluorescence, respectively (Figure 4B).

As shown in Figure 5A, fresh fAβ(1-40) assumed a nonbranched, helical filament structure of approximately 10 nm width and exhibited a helical periodicity of approximately 220 nm, as described previously (Naiki and Nakakuki 1996). After incubation of 25 μmol/L of fresh fAβ(1-40) with 1.0 mol/L nicotine for 24 hours, many short, sheared fibrils were observed (Figure 5B). At 72 hours, the number of fibrils was reduced markedly, and small amorphous aggregates were occasionally observed (Figure 5C); 100 mmol/L nicotine also disrupted the preformed fAβ(1-40) similarly (data not shown). Similar data (not shown) were obtained with fAβ(1-42).

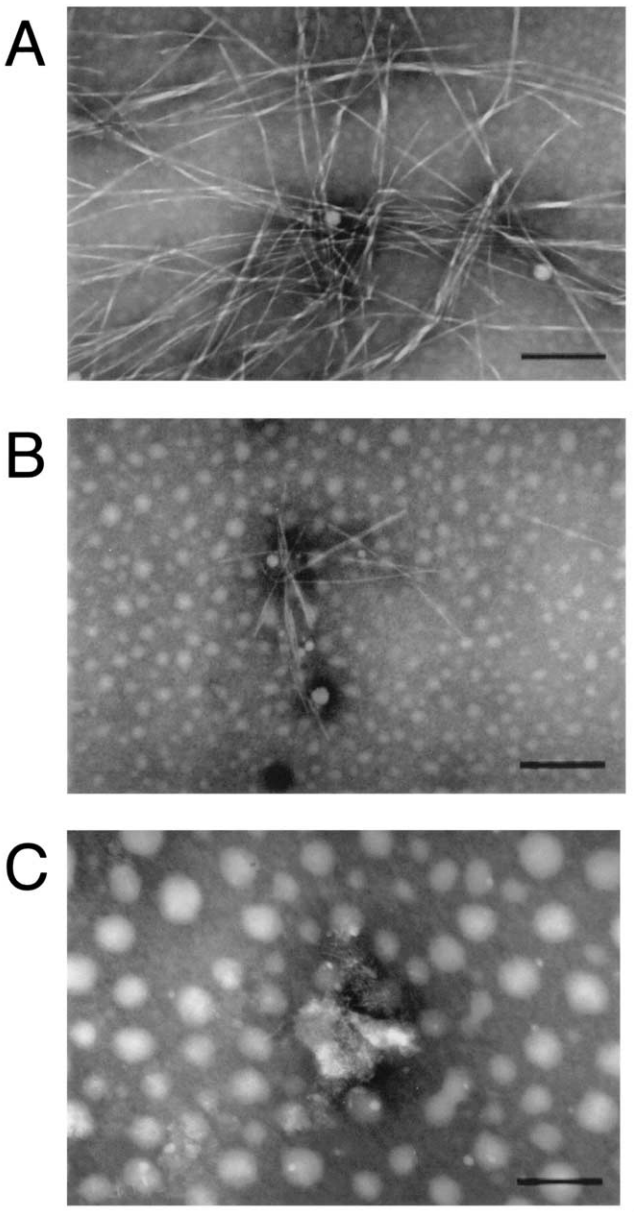

Figure 5. Electron micrographs of disrupted fAβ(1-40). The reaction mixture containing 25 μmol/L fAβ(1-40); 50 mmol/L phosphate buffer, pH 7.5; 100 mmol/L NaCl; and 1.0 mol/L nicotine was incubated at 37°C for 0 (**A**), 24 (**B**), or 72 hours (**C**). Scale bars indicate a length of 250 nm.

After incubation with 1.0 mol/L nicotine for 72 hours, fAβ(1-40) and fAβ(1-42) were stained with Congo red much more weakly (data not shown) than fresh fAβ(1-40) and fAβ(1-42); however, they all showed orange-green birefringence under polarized light (data not shown). This may indicate that a significant amount of intact fAβ(1-40) and fAβ(1-42) still remains in the mixture after the reaction. When the protein concentration of the supernatant after centrifugation at 4°C for 2 hours at 1.6×10^4 *g* was measured by the Bradford assay, no proteins were detected in the supernatant in any condition (data not shown). This may indicate that although nicotine could break down fAβ(1-40) and fAβ(1-42) to visible aggregates (Figure 5C), it could not depolymerize fAβ(1-40) and fAβ(1-42) to monomers or oligomers of Aβ(1-40) and Aβ(1-42).

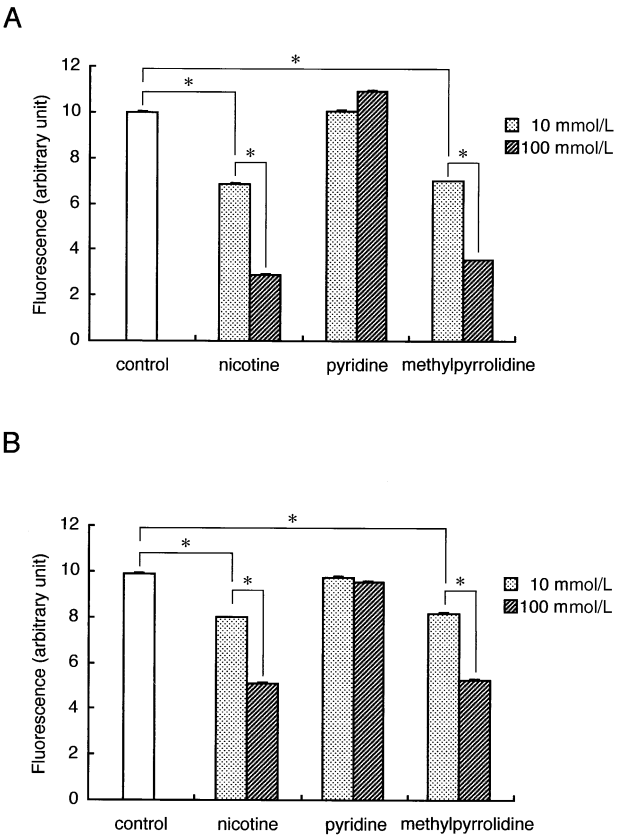

Figure 6. Effects of nicotine, pyridine, and N-methylpyrrolidine on fAβ(1-42) formation from fresh Aβ(1-42) (**A**) and the disruption of fAβ(1-40) (**B**). The reaction mixture containing 25 μmol/L Aβ(1-42) (**A**) or 25 μmol/L fAβ(1-40) (**B**); 50 mmol/L phosphate buffer, pH 7.5; 100 mmol/L NaCl; and 0, 10, or 100 mmol/L nicotine, pyridine, or N-methylpyrrolidine was incubated at 37°C for 12 (**A**) or 24 hours (**B**). Each column represents mean + SD of the three independent experiments. *$p < .01$ (paired Student's *t* test).

Comparison of the Effects of Nicotine, Pyridine, and N-Methylpyrrolidine on the Formation and Disruption of fAβs

As shown in Figure 6A, nicotine and N-methylpyrrolidine similarly inhibited fAβ(1-42) formation from fresh Aβ(1-42) in a dose-dependent manner. Moreover, they disrupted fAβ(1-40) similarly (Figure 6B). On the other hand,

pyridine neither inhibited fAβ(1-42) formation nor disrupted fAβ(1-40), as shown in Figures 6A and 6B.

Discussion

Salomon et al (1996) showed that 50 μmol/L nicotine inhibits fAβ(1-42) formation from 50 μmol/L Aβ(1-42) using centrifugation and ultraviolet spectroscopy. They indirectly measured the amount of fAβ(1-42) formed, by quantifying the concentration of soluble Aβ(1-42) remaining in the supernatant after centrifugation. On the other hand, we monitored the formation and disruption of fAβ(1-40) and fAβ(1-42) directly using fluorescence spectroscopy with ThT and electron microscopy. As shown in Figures 1, 2, and 4, the antiamyloidogenic effects of nicotine were observed at concentrations above 10 mmol/L, about 200 times higher than the effective concentration reported by Salomon et al (50 μmol/L). Although we have no clear interpretation for this discrepancy, the methods to monitor the formation and disruption of fAβs may cause this difference. Our systematic in vitro study indicated that the overall activity of the antiamyloidogenic molecules may be on the order of NDGA ≫ RIF = tetracycline > poly(vinylsulfonic acid, sodium salt) = 1,3-propanedisulfonic acid, disodium salt > β-sheet breaker peptide (iAβ5) > nicotine (Figures 1, 2, and 4; Ono et al 2002). This comparison may indicate that the antiamyloidogenic effects of nicotine in vitro may be too weak to exert a pharmacological activity in vivo; however, we cannot rule out the possibility that low concentrations of nicotine may exhibit antiamyloidogenic activities in vivo when administered in high doses and for a long time.

As for the antiamyloidogenic mechanisms of nicotine, Zeng et al (2001) suggested that nicotine inhibits fAβ formation probably by binding to a small, soluble β-sheet aggregate. Tomiyama et al (1994, 1996) suggested that RIF binds to Aβ by hydrophobic interactions between its lipophilic ansa chain and the hydrophobic region of Aβ, thus blocking associations between Aβ molecules leading to fAβ formation. The antiamyloidogenic and fibril-breaking activity of tetracyclines, small-molecule anionic sulphonates or sulphates, melatonin, and β-sheet breaker peptides may also be related to the propensity to bind to the specific sites of Aβ (Forloni et al 2001; Kisilevsky et al 1995; Pappolla et al 1998; Soto et al 1996). Interestingly, NDGA, RIF, melatonin, and nicotine have all been reported to have antioxidant activity (Goodman et al 1994; Linert et al 1999; Pappolla et al 1998; Tomiyama et al 1996). Thus, it may be reasonable to consider that nicotine and other organic compounds with antioxidant effects could bind specifically to Aβ or fAβ, inhibit fAβ formation, or disrupt preformed fAβ through mechanisms yet unknown.

The antiamyloidogenic effects of nicotine were similar to those of N-methylpyrrolidine, indicating that the effects of nicotine could be attributed to N-methylpyrrolidine moieties of nicotine (Figure 6). These results are consistent with the report from Salomon et al (1996) that the binding of nicotine to Aβ(1-28) primarily involves the N-CH_3 and 5′CH_2 pyrrolidine moieties of nicotine and the histidine residues of Aβ(1-28). On the other hand, Salomon et al (1996) reported that both N-methylpyrrolidine and pyridine at a concentration of 50 μmol/L weakly accelerate the aggregation of 50 μmol/L Aβ(1-42). They suggested that both the pyrrolidine and pyridine ring must be connected at the C3–C2′ bond to inhibit the aggregation of Aβ. Although we have no clear interpretation for this discrepancy, the methods to monitor the formation of fAβs may again cause this difference.

In conclusion, the neuroprotective effect of nicotine may be exerted not only by the upregulation of nicotinic receptors, but also by antiamyloidogenic effects, including the disruption of preformed fAβ. Further study is essential for the development of more effective nicotinelike compounds to interfere directly with the neurodegenerative process in AD, especially the accumulation of Aβ in the CNS.

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References

Bradford MM (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248–254.

Doody RS, Stevens JC, Beck C, Dubinsky RM, Kaye JA, Gwyther L, et al (2001): Practice parameter: Management of dementia (an evidence-based review). Report of the quality standards subcommittee of the American Academy of Neurology. *Neurology* 56:1154–1166.

Emilien G, Beyreuther K, Masters CL, Maloteaux JM (2000): Prospects for pharmacological intervention in Alzheimer disease. *Arch Neurol* 57:454–459.

Forloni G, Colombo L, Girola L, Tagliavini F, Salmona M (2001): Anti-amyloidogenic activity of tetracyclines: Studies in vitro. *FEBS Lett* 487:404–407.

Goodman Y, Steiner MR, Steiner SM, Mattson MP (1994): Nordihydroguaiaretic acid protects hippocampal neurons against amyloid β-peptide toxicity, and attenuates free radical and calcium accumulation. *Brain Res* 654:171–176.

Graves AB, Mortimer JA (1994): Does smoking reduce the risks of Parkinson's and Alzheimer's disease? *J Smoking-Related Dis* 5:79–90.

Hasegawa K, Yamaguchi I, Omata S, Gejyo F, Naiki H (1999): Interaction between Aβ(1-42) and Aβ(1-40) in Alzheimer's

β-amyloid fibril formation in vitro. *Biochemistry* 38:15514–15521.

Jarrett JT, Lansbury PT Jr (1993): Seeding "one-dimensional crystallization" of amyloid: A pathogenic mechanism in Alzheimer's disease and scrapie? *Cell* 73:1055–1058.

Jarvick ME (1991): Beneficial effects of nicotine. *Br J Addict* 86:571–575.

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

Kisilevsky R, Lemieux LJ, Fraser PE, Kong X, Hultin PG, Szarek WA (1995): Arresting amyloidosis in vivo using small-molecule anionic sulphonates or sulphates: Implications for Alzheimer's disease. *Nat Med* 1:143–148.

Lerner A, Koss E, Debanne S, Rowland D, Smyth K, Friedland R (1997): Smoking and oestrogen-replacement therapy as protective factors for Alzheimer's disease. *Lancet* 349:403–404.

Levin ED, Simon BB (1998): Nicotinic acetylcholine involvement in cognitive function in animals. *Psychopharmacology* 138:217–230.

Linert W, Bridge MH, Huber M, Bjugstad KB, Grossman S, Arendash GW (1999): In vitro and in vivo studies investigating possible antioxidant actions of nicotine: Relevance to Parkinson's and Alzheimer's diseases. *Biochim Biophys Acta* 1454:143–152.

Lomakin A, Teplow DB, Kirschner DA, Benedek GB (1997): Kinetic theory of fibrillogenesis of amyloid β-protein. *Proc Natl Acad Sci USA* 94:7942–7947.

Naiki H, Gejyo F (1999): Kinetic analysis of amyloid fibril formation. *Methods Enzymol* 309:305–318.

Naiki H, Gejyo F, Nakakuki K (1997): Concentration-dependent inhibitory effects of apolipoprotein E on Alzheimer's β-amyloid fibril formation in vitro. *Biochemistry* 36:6243–6250.

Naiki H, Hasegawa K, Yamaguchi I, Nakamura H, Gejyo F, Nakakuki K (1998): Apolipoprotein E and antioxidants have different mechanisms of inhibiting Alzheimer's β-amyloid fibril formation in vitro. *Biochemistry* 37:17882–17889.

Naiki H, Nakakuki K (1996): First-order kinetic model of Alzheimer's β-amyloid fibril extension in vitro. *Lab Invest* 74:374–383.

Ono K, Hasegawa K, Yoshiike Y, Takashima A, Yamada M, Naiki H (2002): Nordihydroguaiaretic acid potently breaks down preformed Alzheimer's β-amyloid fibrils in vitro. *J Neurochem* 81:434–440.

Pappolla M, Bozner P, Soto C, Shao H, Robakis NK, Zagorski M, et al (1998): Inhibition of Alzheimer β-fibrillogenesis by melatonin. *J Biol Chem* 273:7185–7188.

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

Selkoe DJ (2000): The origins of Alzheimer's disease: A is for amyloid. *JAMA* 283:1615–1617.

Soto C, Kindy MS, Baumann M, Frangione B (1996): Inhibition of Alzheimer's amyloidosis by peptides that prevent β-sheet conformation. *Biochem Biophys Res Commun* 226:672–680.

Tomiyama T, Asano S, Suwa Y, Morita T, Kataoka K, Mori H, et al (1994): Rifampicin prevents the aggregation and neurotoxicity of amyloid β protein in vitro. *Biochem Biophys Res Commun* 204:76–83.

Tomiyama T, Shoji A, Kataoka K, Suwa Y, Asano S, Kaneko H, et al (1996): Inhibition of amyloid β protein aggregation and neurotoxicity by rifampicin. *J Biol Chem* 271:6839–6844.

Ulrich J, Johannson-Locher G, Seiler WO, Stähelin HB (1997): Does smoking protect from Alzheimer's disease? Alzheimer-type changes in 301 unselected brains from patients with known smoking history. *Acta Neuropathol (Berl)* 94:450–454.

White HK, Levin ED (1999): Four week nicotine skin patch treatment effects on cognitive performance in Alzheimer's disease. *Psychopharmacology* 143:158–165.

Wilson AL, Langley LK, Monley J, Bauer T, Rottunda S, Mcfalls E, et al (1995): Nicotine patches in Alzheimer's disease: Pilot study on learning, memory, and safety. *Pharmacol Biochem Behav* 51:509–514.

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

Zeng H, Zhang Y, Peng L-J, Shao H, Menon NK, Yang J, et al (2001): Nicotine and amyloid formation. *Biol Psychiatry* 49:248–257.