Nicotine Enhances the Biosynthesis and Secretion of Transthyretin from the Choroid Plexus in Rats: Implications for β -Amyloid Formation

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Epidemiological studies indicated that cigarette smoking protects against the development of several neurodegenerative disorders, including Alzheimer's disease (AD). However, the molecular mechanism(s) underlying this is poorly understood. To gain insight into these protective effects, we used differential display PCR (DD-PCR) to amplify RNA from various brain regions of rats self-administering (SA) nicotine compared with yoked-saline controls. We found that the transthyretin (TTR) gene, whose product has been shown to bind to amyloid β (A β) protein and prevent A β aggregation, was more abundantly expressed (~1.5- to 2.0-fold) in the brainstem and hippocampus (areas containing choroid plexus) of nicotine SA rats. Subsequently, quantitative reverse transcription-PCR analysis confirmed these DD-PCR findings and demonstrated that nicotine increased TTR mRNA levels in these regions in a time- and

dose-dependent manner. Significantly higher TTR protein concentrations were also detected in the ventricular CSF of nicotine-treated rats. In contrast, no differences either in plasma TTR or in CSF and plasma retinol-binding protein were detected. Immunohistochemical analysis showed that immunoreactive TTR was 41.5% lower in the choroid plexus of nicotine-treated rats compared with the saline controls. On the basis of these data, we speculate that the protective effects of nicotine on the development of AD may be attributable, in part, to the increased biosynthesis and secretion of TTR from the choroid plexus. These findings also point toward new approaches that may take advantage of the potentially novel therapeutic effects of nicotinic agonists in patients with AD.

Key words: nicotine; transthyretin; β -amyloid; Alzheimer's disease; choroid plexus; differential display PCR

One of the central events in the pathogenesis of Alzheimer's disease (AD) is the deposition of amyloid β (A β) protein, a 4.3 kDa polypeptide derived from the A β precursor, that exists in both soluble and fibrillar forms. Soluble A β is a normal metabolic product detectable in the ventricular CSF and plasma of normal and AD subjects. *In vitro* studies with synthetic A β protein have shown that it aggregates readily, forming amyloid fibrils similar to the fibrils found in the brains of AD patients (Castano and Frangione, 1988). However, the molecular mechanism(s) by which soluble A β forms amyloid fibrils in the brains of AD patients is poorly understood. CSF contains several extracellular proteins that promote the solubility, transport, and clearance of A β , such as apolipoprotein E [apoE; derived from astrocytes (Carlsson et al., 1991)] and transthyretin [TTR; derived from the choroid plexus epithelium (Herbert et al., 1986)]; apoE seems to be important for A β accumulation, whereas TTR prevents A β aggregation.

TTR is a homotetrameric protein with a total molecular weight of 55 kDa that is found in the CSF and plasma. In the periphery, TTR plays a role in the transport of thyroxine and is indirectly involved in the transport of retinol by binding retinol-binding protein (RBP), the specific retinol carrier in blood (Kanai et al.,

1968). Within the CNS, TTR is the only known CSF protein synthesized solely by the choroid plexus (Herbert et al., 1986). *In vitro* studies have shown that purified TTR can bind A β and inhibit A β fibrillogenesis (Schwarzman et al., 1994; Golabek et al., 1995). Studies performed with *Caenorhabditis elegans* transgenic for either human A β or human TTR also have suggested that TTR can inhibit A β fibrillogenesis (Link, 1995). Declining levels of CSF TTR have been found to be associated with dementia of increasing severity in AD patients (Riisøen, 1988; Serot et al., 1997). On the basis of these findings, it was hypothesized that TTR may function to sequester A β peptide, thus preventing its aggregation and consequent amyloid fibril formation (Schwarzman et al., 1994).

Several large epidemiological studies have shown an inverse association between cigarette smoking and AD (Graves et al., 1991; van Duijn and Hofman, 1991; Brenner et al., 1993). Although it is known that tobacco smoking may have a protective role in the development of AD, the molecular mechanism underlying this is not understood. One hypothesis attributes the protective effects of smoking to an increased number of CNS nicotinic cholinergic receptors (NACHRs) that compensate for the usual reduction in NACHRs and choline acetyltransferase found in autopsy-confirmed AD (Whitehouse and Kalara, 1995; Whitehouse, 1997).

In this study, we show that nicotine, a major component in cigarette smoke (Le Houezec and Benowitz, 1991), can specifically enhance the biosynthesis and secretion of TTR from the choroid plexus. These results are consistent with our working hypothesis that nicotine protects against the development of AD

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by retarding the aggregation of amyloid, because of an increase in the biosynthesis and secretion of TTR from the choroid plexus.

MATERIALS AND METHODS

Animals and nicotine administration

Animals. Male Holtzman rats (225–250 gm; Harlan Sprague Dawley, Madison, WI) were used for all experiments. Rats were housed individually in wire-bottomed cages at 22°C and maintained on a 12:12 hr light/dark cycle (lights off at 10 A.M. and on at 10 P.M.). Standard laboratory rat chow and water were available *ad libitum* throughout the experiments. All procedures were conducted in accordance with the *National Institutes of Health Guidelines for the Care and Use of Laboratory Animals* with the approval of our Institutional Animal Care and Use Committee.

Nicotine self-administration. The self-administration procedure was essentially that described previously (Valentine et al., 1997). Briefly, male Holtzman rats were anesthetized, implanted with jugular catheters and tethers, and placed into operant chambers (Coulbourn, Allentown, PA) for the duration of the experiment. During recovery, daily injections of gentamycin (4 mg/kg, i.v.) and hourly injections of saline (50 μ l containing 200 U/ml heparin, i.v.) were administered. On the third day of recovery, the light over each lever was turned on, and intravenous nicotine (0.03 mg/kg per injection, pH 7.0; expressed as the free base of nicotine sulfate; Sigma, St. Louis, MO) was made available contingent on one press of one of the two levers (the rats do not receive any previous training). A 7 sec time-out, during which the light over the active lever was off, followed each injection. Nicotine was always available except for a 1–2 hr period at the end of the light portion of the light cycle, when the nicotine solutions were changed and the animals were cared for; the cue lights over the levers were not on during this period.

To identify alterations in gene expression related to the effects of nicotine use per se, in the initial experiment, we divided the rats from each of four full-sibling families equally among the four treatment groups. Rats self-administered nicotine for 3, 10, or 20 d, and one group received saline for 20 d. These three intervals were based on results obtained previously (Valentine et al., 1997); they represent the acquisition, early stabilization, and late stabilization of nicotine self-administration in our model.

Intraperitoneal nicotine administration. Rats received nicotine dihydrochloride or saline by intraperitoneal injection at doses of 2.0–6.0 mg/kg per d given in five equally divided doses at 2 hr intervals from 9:00 A.M. to 5:00 P.M. for 14 d.

CSF collections

Rats were anesthetized with xylazine–ketamine (5.35 mg/kg of body weight, i.m.; Parke-Davis, Morris Plains, NJ), and then a 23 gauge hypodermic tube was stereotactically implanted into the cerebral aqueduct for use as a guide cannula. The coordinates were as follows: anteroposterior, +0.8 mm; dorsoventral, –5.2 mm; and mediolateral, 0.0 mm, relative to the interaural line with the flat skull (Fu et al., 1998). After brain surgery, a 30 gauge hypodermic cannula connected to a polyethylene-20 tube was used to withdraw 5 μ l of CSF from the cerebral aqueduct.

Brain punches and RNA isolation

At each time point, after receiving a lethal overdose of sodium pentobarbital (125 mg/kg, i.p.), rats were decapitated, and brains were removed immediately. Two millimeter slices were made with a Stoelting tissue slicer, using the midoptic chiasm as the standardized landmark for each rat; sections were cut both anterior (cortex and striatum) and posterior (all others) to this point. Each coronal 2 mm section was placed on an ice-cold dish, and specific brain regions were isolated; punches and landmarks used were minor modifications of our previously described method (Sharp and Matta, 1993). Because we did not know a priori that the choroid plexus would be a site of interest, specific punches of choroid plexus alone were not performed. The areas included within the brainstem and hippocampal punches incorporated most of the choroid plexus contained within these regions. Total RNA was isolated from individual frozen brain regions by guanidine isothiocyanate extraction and CsCl centrifugation (Chirgwin et al., 1979). Before use, RNA was treated with RNase-free DNase I at 37°C for 30 min.

Differential display PCR

Total RNA (0.2 μ g) from each brain region was reverse-transcribed, as described by Liang and Pardee (1992), except that T3(dT₁₂)MA,

T3(dT₁₂)MT, T3(dT₁₂)MG, and T3(dT₁₂)MC primers were used (M is a degenerate mixture of dA, dC, and dG). Then, 2 μ l of cDNA was amplified in 1 $\times$ PCR buffer containing 1.25 mM MgCl₂, 2.0 mM dNTP, 0.1 μ l of [α -³²P]dATP, 2.5 U of *AmpliTaq* DNA polymerase, and the appropriate T3(dT₁₂)MN primers in combination with 1 of 10 SP6-arbitrary primers. The PCR mixtures were then subjected to 40 cycles of denaturation (92°C; 30 sec), annealing (42°C; 2 min), and extension (72°C; 90 sec), followed by 72°C for 5 min. The amplified cDNAs were separated on 4.5% polyacrylamide gels by the Genomix LR DNA Sequencer (Genomix, Foster City, CA) and exposed to x-ray film for 1–2 d. Interesting cDNA bands were cut from the gel and reamplified with T3 and SP6 primers, under modified PCR conditions. The reamplified PCR products were then subjected to sequence analysis with the appropriate primers used in the differential display PCR (DD-PCR) reaction, using a Thermal Sequenase sequencing kit (Amersham, Cleveland, OH).

Semiquantitative reverse transcription-PCR

The strategy used to optimize the reaction conditions (i.e., amplification cycles and the input volume of cDNA mixtures) for the semiquantitative reverse transcription-PCR (SQ-RT-PCR) was essentially the same as that described previously (Li et al., 1997).

Radioimmunoassay and immunohistochemistry

Plasma and CSF TTR and RBP concentrations were measured by radioimmunoassay (RIA) as described previously (Blaner, 1990). For immunohistochemistry, rats were cardiac perfused with 4% paraformaldehyde (0.05 M phosphate buffer, pH 6.8), and brains were infused with 20% sucrose in PBS, cryosectioned at 25 μ m, stored in cryoprotectant, and processed as described previously (Matta et al., 1997). Briefly, sections were rinsed in PBS, blocked with normal rabbit serum in PBS, and incubated overnight at 4°C with sheep anti-human TTR antibody (1:750; ICN Biochemicals, Aurora, OH). After multiple PBS rinses, sections were incubated with biotinylated rabbit anti-sheep antibody (1:500; Vector Laboratories, Burlingame, CA) followed by Elite ABC complex (1:500; Vector Laboratories), visualized with diaminobenzidine in Tris buffer, rinsed multiple times in water, dehydrated through graded alcohol into xylene, and then coverslipped using Permount. Sections were not counterstained. Controls for specificity of staining included preabsorption of the primary antiserum with the antigen (10 μ g/ml diluted antiserum for 18–24 hr at 4°C) and replacing the primary antiserum with an antiserum made in the sheep against an irrelevant peptide.

Statistical analysis

Data (mean $\pm$ SEM) for SQ-RT-PCR, plasma and CSF TTR, and RBP concentrations were analyzed using ANOVA or unpaired Student's *t* test (Systat 6.0; SPSS Inc, Chicago, IL). Significant *F* tests were followed by comparisons using the Bonferroni procedure. Semiquantitative area density analysis of the choroid plexus was performed with NIH Image 1.6.1 (W. Rasband, National Institutes of Health); a minimum of six sections per rat was analyzed at 20 $\times$ magnification, using coded slides. Data are presented as optical density units (O.D.) per square pixel, standardized for background.

RESULTS

Identification of TTR mRNA by DD-PCR

A total of 40 primer combinations, derived from 10 arbitrary (SP6-OPA-1, -2, -4, -6, -8, -10, -16, -17, -18, -19, and -20) and 4 anchor [T3(dT₁₂)MN] primers, was used for DD-PCR analysis of tissue punches from the brainstem (BS), hippocampus (Hp), striatum (ST), amygdala (Amyg), hypothalamus (MBH), cortex (CTX), and ventral tegmental area (VTA) of self-administering (SA) nicotine and yoked-saline rats. Several bands were differentially expressed in various brain regions of nicotine SA compared with saline rats. Subsequent cloning and sequence analysis indicated that these DD-PCR clones represented different gene products.

In this report, we focus on the identification and characterization of the TTR gene, whose expression was significantly increased in the Hp (Fig. 1A) and BS (Fig. 1B) of nicotine SA rats. Different TTR mRNA regions were amplified by DD-PCR. For

Figure 1. Differential display of Hp6 and BS2 cDNA fragments in the Hp and BS. Total RNA (0.2 μ g) pooled from the Hp or BS punches of three rats self-administering nicotine (0.03 mg/kg per injection; free base) for 3, 10, or 20 d or saline for 20 d were differentially displayed with either T_3 (dT₁₂)MG and SP6-OPA-2 (A; Hp) or T_3 (dT₁₂)MA and SP6-OPA-4 (B; BS) primers. Signals demonstrating altered expression of gene(s) by differential display are labeled *Hp6* or *BS2*. Subcloning and sequence analysis indicated that DD-PCR clones Hp6 and BS2 are identical to the various regions of TTR mRNA (as described in the text).

Figure 2. Direct comparison of the amounts of DNA amplified from TTR mRNA isolated from the Hp (A, C) and BS (B, D) punches of rats self-administering nicotine for 3, 10, or 20 d or saline for 20 d. Total RNA was pooled from the corresponding tissue punches of three rats and used for semiquantitative RT-PCR amplification. A, B, The amplified PCR product was separated by electrophoresis. C, D, The expected product bands on an ethidium bromide-staining gel were excised, and the radioactivity of each band was determined. Amounts of DNA amplified from glyceraldehyde-3-phosphate dehydrogenase (G3PDH) RNA were used to normalize RNA concentrations of each sample. Amplification cycles were 35 for TTR and 25 for G3PDH (same as used below; see Figs. 3, 4). d3, Day 3.

example, DD-PCR clone Hp6, amplified by SP6-OPA-2 and T_3 (dT₁₂)MG primers in the Hp, is identical to nucleotides 98–399 of TTR, whereas BS2, amplified by SP6-OPA-4 and T_3 (dT₁₂)MA primers in the BS, is identical to nucleotides 289–584 of TTR (Dickson et al., 1985).

Nicotine increased TTR mRNA levels in a time- and dose-dependent manner

To confirm the DD-PCR results, we synthesized a pair of primers corresponding to nucleotide positions 108–132 (5'-GTCCTGGATGCTGTCCGAGGCAGCC-3') and 552–528 (5'-GCATCTTCCCGAGTTGCTAACACGG-3') of the TTR mRNA sequence. The expected PCR product is 445 bp in both the Hp (Fig. 2A) and BS (Fig. 2B). SQ-RT-PCR of samples from the Hp and BS showed that TTR mRNA levels increased with the duration of exposure to self-administered nicotine (Fig. 2C,D for these regions, respectively).

Figure 3. Effects of various nicotine doses on TTR mRNA levels in the BS. The expression level of TTR mRNA was measured by SQ-RT-PCR. Total RNA was used for PCR amplification, and the RNA concentration of each sample was normalized by G3PDH. Nicotine was administered by intraperitoneal injection at doses of 2.0–6.0 mg/kg per d in five equally divided doses at 2 hr intervals for 14 d. The incorporated radioactivity (mean $\pm$ SEM) shown in A is illustrated in B. Means that do not share a common letter (a–c) differ significantly ($p < 0.05$).

Figure 4. Effects of various nicotine doses on TTR mRNA levels in the Hp. The expression level of TTR mRNA was measured by SQ-RT-PCR; total RNA was used for amplification. The amounts of DNA amplified from G3PDH RNA were used to normalize the results. The incorporated radioactivity (mean $\pm$ SEM) shown in A is illustrated in B. Means that do not share a common letter (a–c) differ significantly ($p < 0.05$).

A linear increase of TTR mRNA expression was also observed in the BS (Fig. 3) and Hp (Fig. 4) of rats receiving nicotine intraperitoneally (2.0–6.0 mg/kg per d for 14 d). However, 6 mg/kg nicotine per day induced less of an increase in TTR mRNA levels than did 4 mg/kg nicotine per day in the Hp. No TTR mRNA was detectable after PCR amplification of the other five regions (e.g., ST, Amyg, MBH, CTX, and VTA) (data not shown). These data confirmed the DD-PCR results. Moreover, they indicate that nicotine dose- and time-dependently elevated

Table 1. Comparisons of TTR and RBP concentrations between nicotine-treated and yoked-saline rats

Group/protein	Nicotine (<i>n</i> = 8)	Saline (<i>n</i> = 6)	Probability
Plasma TTR	14.18 ± 1.83	12.43 ± 1.65	0.711
CSF TTR	0.266 ± 0.009	0.238 ± 0.008	0.040
Plasma RBP	1.391 ± 0.068	1.417 ± 0.092	0.962
CSF RBP	0.067 ± 0.003	0.072 ± 0.005	0.410

TTR mRNA levels selectively in tissue punches from only two of seven brain regions.

Increased CSF TTR concentrations in nicotine-treated rats

Enhanced TTR mRNA expression could be expected to increase CSF TTR levels, if nicotine stimulated the synthesis and secretion of TTR from the choroid plexus (included in the tissue punches from the Hp and BS). Therefore, rats were given intraperitoneal nicotine (4.0 mg/kg per d, in divided doses) or saline five times a day for 14 d; blood and CSF were collected for TTR and RBP radioimmunoassays. In agreement with the TTR mRNA expression data, significantly higher TTR concentrations (~12%; *p* = 0.04) were present in the CSF of nicotine-treated rats (Table 1). In contrast, no differences were detected in plasma TTR concentrations. Moreover, no differences were detected in the RBP levels in plasma and CSF obtained from the nicotine and saline groups. These experiments indicate that the induction of TTR mRNA and protein expression by nicotine are restricted to the CNS and result in elevated CSF TTR levels.

Elevated TTR secretion from the choroid plexus of nicotine-treated rats

Rats (*n* = 4/group) were administered intraperitoneal nicotine (4.0 mg/kg per d) or saline for 2 weeks, and immunohistochemical analysis was performed on the Hp choroid plexus. As expected, TTR was detectable in the choroid plexus obtained from the nicotine- and saline-treated rats, but the relative density of the TTR immunostaining was less in the nicotine groups (Fig. 5*B,A* for nicotine vs saline, respectively). We found that immunoreactive TTR was ~41.5% lower in the nicotine-treated rats (9398 ± 632 O.D./pixel²) than that in the yoked-saline controls (16,069 ± 1159 O.D./pixel²; *p* < 0.01), suggesting that nicotine specifically enhances TTR secretion from the choroid plexus. These results agree well with the findings obtained by DD-PCR and semiquantitative RT-PCR of mRNA levels in the brainstem and hippocampus.

DISCUSSION

A significant inverse association between cigarette smoking and Alzheimer's disease indicates that tobacco smoking may have protective effects on the development of the disease (Graves et al., 1991). The therapeutic use of nicotine products in patients with AD has demonstrated short-term improvements in learning, attention, and information processing (Jones et al., 1992; Wilson et al., 1995; Newhouse et al., 1997). However, the mechanism(s) underlying these pharmacological effects of nicotine is primarily unknown. These ameliorative effects of short-term therapy are possibly caused by enhanced neuronal function, rather than by alterations in the pathogenesis of AD. To gain insight into the mechanism by which chronic exposure to nicotine affects CNS gene expression, we used DD-PCR to amplify regional brain

Figure 5. Immunoreactive TTR in the choroid plexus. Rats (*n* = 4/group) received saline (*A*) or nicotine (4.0 mg/kg per d; *B*) by intraperitoneal injection for 14 d and then were cardiac perfused with 4% paraformaldehyde; cryosections of brain tissue were processed for immunohistochemical localization with TTR antibody. Transthyretin immunoreactivity was significantly reduced (by 41.5%; *p* < 0.01) in the choroid plexus of nicotine-treated rats compared with saline controls. Scale bar, 1 mm.

mRNAs from nicotine SA rats compared with saline controls. We found that nicotine significantly increased the expression of TTR mRNA in the choroid plexus obtained from the two brain regions known to contain choroid plexus. We then focused on TTR protein, which would be the operative agent conferring the neuroprotective properties of nicotine. We demonstrated that significantly higher TTR concentrations were detected in the CSF of the nicotine-treated rats, indicating that increased TTR mRNA expression was associated with enhanced secretion into the CSF. In contrast, no significant differences in plasma TTR concentrations were detected. Immunoreactive-TTR protein levels also were significantly reduced in the choroid plexus of nicotine-treated rats. This is most consistent with enhanced secretion exceeding the enhanced synthesis of TTR. In summary, our results indicate that nicotine not only increases TTR mRNA and protein expression but also increases its secretion by the choroid plexus. Therefore, we propose that the protective effects of nicotine on the development of AD may, in part, result from the increased biosynthesis and secretion of TTR by nicotine.

Transthyretin, the transporter of thyroid hormones and vitamin A *in vivo*, represents 20% of the protein synthesized and 50% of the protein secreted by the choroid plexus (Dickson et al., 1986). Transthyretin mRNA has been found to be highly expressed in the choroid plexus and liver and expressed at significantly lower levels in the meninges of the rat brain (Soprano et al., 1985; Blay et al., 1993). However, the synthesis and secretion of transthyretin are regulated independently in liver and choroid plexus (Dickson et al., 1986). In addition to serving as a transport protein for thyroxine and retinol, in association with retinol-binding protein, transthyretin may play an important role in the pathogenesis of AD (Soprano et al., 1985; Makover et al., 1988). A significant reduction of TTR in CSF has been detected in AD patients compared with age-matched healthy controls (Riisøen, 1988; Davidsson et al., 1997; Serot et al., 1997). This may reflect the absorption of transthyretin by the amyloid deposited in senile plaques. Recently, Merched et al. (1998) reported that there exists a negative correlation between the abundance of senile plaque, a major hallmark of the AD brain, and the mean level of TTR in CSF from the same patients. Thus, TTR levels were significantly higher in the CSF of AD patients with fewer senile plaques.

Our radioimmunoassay data indicated that TTR concentrations in CSF were ~12% higher in the nicotine-treated rats compared with the saline controls. In contrast, no differences were evident in the plasma TTR concentrations, suggesting that the stimulatory effects of nicotine on TTR expression are restricted to the brain. Davidsson et al. (1997) reported a 10.1 and 8.0% reduction of TTR concentrations in the CSF from early- and late-onset patients, respectively, with AD. It is a plausible hypothesis that the increased biosynthesis and secretion of TTR induced by nicotine would compensate for the reduction of TTR reported in the CSF of AD patients.

Although the precise mechanism by which TTR may be involved in AD is unknown, evidence supports a significant role of TTR in the pathogenesis of AD (Merched et al., 1998). TTR has been shown to be the carrier of A β in CSF and to prevent formation of amyloid fibrils (Schwarzman et al., 1994; Link, 1995). Furthermore, Mazur-Kolecka et al. (1995) showed that TTR prevents apoE-induced accumulation of A β in cultured smooth muscle cells. The fate of A β in a biological system may depend on competition between the different A β carriers present in body fluids, e.g., apoE and TTR. apoE, deposited within cells along with A β , may enhance A β accumulation (Merched et al., 1998), whereas TTR appears to prevent A β aggregation. Thus, alterations in the concentrations and binding affinities of A β carriers in body fluids and brain parenchyma could be a major factor regulating A β deposition within brain parenchyma. Therefore, the enhanced biosynthesis and secretion of TTR by nicotine reported herein may reduce the concentration of free A β that is available to form fibrils in brain tissue.

As indicated previously, the TTR gene was first identified by DD-PCR amplification from rats in which nicotine was administered using a newly developed self-administration model (Valentine et al., 1997). Relative to other nicotine self-administration rodent models, this model is more relevant to human smokers. For example, rats consumed ~0.18–1.38 mg/kg nicotine per day (Valentine et al., 1997), an amount comparable with that consumed by human smokers [range, 0.14–1.14 mg/kg per d (Benowitz and Jacob, 1990)]. Therefore, using a model in which rats have unlimited access to doses and conditions of nicotine self-administration that more closely approximate the conditions of human nicotine use than are seen in existing models, we have

observed the potentially beneficial effects of nicotine on TTR production and secretion.

Epidemiological studies of AD have shown that smokers are at a lower risk of developing AD than are nonsmokers. Meta-analysis of 19 published studies on the relationship between smoking and AD indicated that the relative risk of developing AD is 0.64 for smokers compared with nonsmokers (Lee, 1994). Furthermore, a recent study (van Duijn et al., 1995) found that the protective effect of smoking was even larger, especially for individuals who were positive for apoE4 and who had a positive family history of early-onset of AD (odds ratio = 0.10). Explanations of the protective effects of nicotine on the pathogenesis of AD have been focused on the "cholinergic hypothesis." Evidence supporting this hypothesis includes the significant reductions in choline acetyltransferase (Corkin, 1981) and nicotinic cholinergic receptor number in subjects with autopsy-confirmed AD (Whitehouse et al., 1982). Another possible explanation for the protective effects of nicotine on the development of AD is that nicotine has been shown to have direct effects on the processing and secretion of the β amyloid precursor protein (BAPP) (Efthimiopoulos et al., 1996; Kim et al., 1997). For example, in pheochromocytoma-12 cells transfected with a full-length APP cDNA, Kim et al. (1997) found that nicotine increased the release of a secreted form of β APP into the conditioned medium without affecting the expression level of β APP mRNA. Herein, we provide further insight into the protective effects of nicotine on the development of AD that are realized by increasing TTR biosynthesis and secretion from the choroid plexus. Increased CSF TTR concentrations would change the equilibrium for bound versus free A β peptide and its interaction with other carrier proteins, thus reducing A β aggregation in brain parenchyma. Thus, these observations may have significant implications for the development of nicotinic agonists that inhibit the pathogenesis of AD.

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