Nicotine and Its Interaction with β-Amyloid Protein: A Short Review

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Two features of Alzheimer’s disease (AD) are β-amyloid protein (βAP) deposition and a severe cholinergic deficit. β-Amyloid protein is a 39–43-amino acid transmembrane fragment of a larger precursor molecule, amyloid precursor protein. It is a major constituent of senile plaque, a neuropathologic hallmark of AD, and has been shown to be neurotoxic in vivo and in vitro. The cholinergic neurotransmission system is seen as the primary target of AD. However, other systems are also found to show functional deficit. An association between cholinergic deficit and βAP is suggested by a negative correlation between cigarette smoking and AD. Evidence hitherto suggests that βAP causes neuronal death possibly via apoptosis by disrupting calcium homeostasis, which may involve direct activation or enhancement of ligand-gated or voltage-dependent calcium channels. Selective second messengers such as protein kinases are triggered that signal neuronal death. Nicotine or acetylcholinesterase inhibitors can partially prevent the neurotoxicity of βAP in vivo and in vitro. However, the exact mechanism by which nicotine provides its protective effects is not fully understood, but clearly there are protective roles for nicotine. Here, some aspects of βAP neurotoxicity and nicotinic intervention as a protective agent are discussed.


Key Words: β-Amyloid protein, neurotoxicity, neuroprotection, nicotine, acetylcholine receptor

Introduction

Earlier findings that cigarette smoking is negatively correlated with Parkinson’s disease (Bharucha et al 1986) and positively correlated with the delayed onset of Alzheimer’s disease (AD) (van Duijn and Hofman 1991) have encouraged studies investigating the neuroprotective role of nicotine (e.g., Knott et al 1999; Maggio et al 1998; Perry et al 1996; Ulrich et al 1997). So far, in vivo studies have been inconclusive (Behmand and Harik 1992; Janson and Møller 1993), but in vitro evidence has demonstrated more convincingly that nicotine inhibits cell loss induced by excitotoxic agents (Akaike et al 1994; Marin et al 1994).

Nicotinic receptor binding and choline acetyltransferase are positively correlated with smoking (Parks et al 1996). In this respect, it is pertinent that AD has long been associated with a cholinergic deficit, characterized by marked degeneration of cholinergic innervation from the nucleus basalis of Meynert to the cerebral cortex (Candy et al 1983), reduced levels of choline acetyltransferase and acetyl cholinesterase, and a much decreased density of nicotinic and M2 muscarinic receptors in the cerebral cortex and hippocampus (Davies and Maloney 1976; Flynn and Mash 1986; London et al 1989; for a review, see Roßner et al 1998). In this review aspects of the nicotinic acetylcholine system, β-amyloid protein (βAP) neurotoxicity, and the protective role of nicotine against βAP toxicity are presented.

Nicotinic Acetylcholine Receptors

Nicotinic acetylcholine receptors (nAChRs) are ligand-gated calcium-permeable receptors consisting of at least eight α-like subunit isoforms (α2–α9) and three β-like isoforms (β2–β4) (Kim et al 1997). These subunits, either as homomeric (α7–α9) or heteromeric (requiring β subunits), form receptors with distinct spatial and tissue-specific distributions (McGehee and Role 1995; Zarei et al 1999). In the neocortex the predominant subtype is α4β2, whereas others contain α4 and α3. The hippocampus, on the other hand, is rich in α7 subunits that could form the homomorphic receptor (Wonnacott 1997). γ-Aminobutyric acid (GABA)–ergic interneurons in the hippocampus possess nAChRs the activation of which leads to an increase in GABA release (Alkondon et al 1997; Lena and Changeux 1997; McMahon et al 1994; Radcliffe and Dani 1998). Enhanced GABA release by nicotine has been extensively studied at various drug concentrations (low μmol/L range equivalent to smokers’ intake and higher μmol/L range similar to synaptic cleft concentrations), and in all cases nicotine increases the release of the inhibitory
neurotransmitter (Radcliffe et al 1999). Recently it has been shown that these cholinergic receptors desensitize in response to nicotine when exposed with a time course and concentration equivalent to smokers’ intake, thereby reducing GABA release (Fisher et al 1998).

Relative to nonneuronal tissues, neuronal AChRs and the α7 receptor have higher Ca²⁺ permeability to the extent that calcium influx through these channels at presynaptic terminals may cause transmitter release (Castro and Albuquerque 1995; Decker and Dani 1990; Pugh and Berg 1994; Radcliffe et al 1999; Seguela et al 1993; Vemino et al 1992, 1994).

Physiologically, nAChRs are distributed at presynaptic (terminal), postsynaptic (dendrites), and somatic sites (Wonnacott 1997). In the hippocampus, presynaptic activation of nAChRs is thought to dominate over the postsynaptic activation, which enhances neurotransmitter release at excitatory, inhibitory, and modulatory synapses (Fu et al 1999; Radcliffe et al 1999; Vidal 1996; Wonnacott 1997). For example, glutamatergic synapses contain α7 receptors, which respond to nicotine resulting in an increase in the frequency of miniature excitatory postsynaptic currents in a tetrodotoxin (TTX)-independent manner suggesting a presynaptic location (Araujo et al 1988; Gray et al 1996; McGeehe and Role 1995; Sacaan et al 1996). In studying the nAChR system in both the cortex and hippocampus, nicotine is seen to regulate its own release, implying the existence of self-reinforcing autoreceptors (Araujo et al 1988; Rowell and Winkler 1984; Wilkie et al 1996; for a review, see Wonnacott 1997). Furthermore, the rate of expression and distribution of nAChR subunits changes during development (Broido et al 1995; Court et al 1997; Zarei et al 1999; Zhang et al 1998) and in humans during aging (Marutle et al 1998).

The complexity of the nicotinic acetylcholine system underlines the importance of therapeutic strategies targeting specific brain regions and/or receptor subtypes. Below, how the neuronal cholinergic system is affected in AD is considered briefly.

Cholinergic System and Alzheimer’s Disease

Deficit or mutation in the cholinergic neurotransmission system is seen in a number of neurologic disorders, but in AD, the cholinergic system is believed to be the primary target (Bowen et al 1976; Davies and Maloney 1976; Perry et al 1977). This does not preclude the involvement of other neurotransmission systems, and in fact, since the report by Davies and Maloney (1976), other neurotransmitter systems have shown either functional deficit in different brain regions or differential downregulation of their receptor subtypes (for a review, see Kanfer et al 1999). Chronologically, cholinergic deficit in AD precedes the deficit in other neurotransmitter systems. This has led to the conclusion that the deficit in other neurotransmitter systems may be the consequence of cholinergic denervation reflecting transsynaptic function of this system (Kanfer et al 1999).

The role of nAChRs in cognitive function and development is well documented (e.g., Granon et al 1995; Levin 1992; Meyer et al 1998). The loss of cognitive performance in AD and Parkinson’s disease patients is positively correlated with the degree of central cholinergic deafferentation (Flynn and Mash 1986; Perry et al 1987a, 1987b; Rinne et al 1991; Sugaya et al 1990; for a review, see Roßner et al 1998). Furthermore, nicotine “replacement” reduces cognitive deficit and improves short-term, visual, and semantic memory in these patients and in animal models of AD (Hodges et al 1991a, 1991b; Jones et al 1992; McGurk et al 1991; Newhouse et al 1990; Perry et al 1996; Voytko et al 1994; for a review, see Vidal 1996).

To identify changes in nAChR subtypes, Martin-Ruiz et al (1999) studied AD brain tissues after autopsy and found a significant reduction in α4 receptor subunits but not α7 or α3 ones as compared with age-matched samples. The same subtype (α4) showed a significant binding elevation in a subgroup of control individuals who were habitual smokers. Similar findings are reported by Sugaya et al (1990) (see also Schroder and Wevers 1998). Although these results agree with others (Perry et al 1995) showing a significant drop in α4–β2 subunits, a greater loss of α7 subunits is seen in the midtemporal gyrus (but not in the frontal cortex or hippocampus) of the AD brain (Davies and Feisullin 1981; Sugaya et al 1990; Warpman and Nordberg 1995). Also, significant reduction in α3 and α4 subunit binding from AD brains with Swedish mutation is seen (Marutle et al 1999). Moreover, immunocytochemical studies on postmortem AD brains have revealed significant reduction in α3 and α4 subunits in the temporal cortex and, in addition to these subunits, a significant reduction in α7 subunit in the hippocampus (Guan et al 2000). It should be noted that, in contrast with the above reports, no changes in message expression for α3 and α4 are seen (Hellstrom-Lindahl et al 1999; Terzano et al 1998). In fact, an increase in the level of α7 messenger RNA (mRNA) is reported by Hellstrom-Lindahl et al (1999). Although variation in techniques and subject selection might be a factor in the apparent lack of concordance in these reports, they clearly signify changes in posttranscriptional stages of the nicotinic system in AD.

βAP Is Neurotoxic

Abnormal production of the amyloidogenic protein (39 to 43 amino acid residues), which is cleaved from the amyloid precursor protein (APP) at β- and γ-secretase
sites of action, is closely linked to AD (for details, see Roßner et al 1998). β-Amyloid protein is a major constituent of senile plaque, a neuropathologic hallmark of AD, and has been shown to be neurotoxic both in vivo and in vitro. A shorter, hydrophobic fragment of the protein, βAP (25–35), though not present in biological systems, is widely used together with, or instead of, the endogenous fragment (βAP (1–42/43)) by a number of investigators and is found to be at least as toxic as the full-length fragment (Yankner et al 1989).

In a series of experiments βAP (25–35) (0.01 to 100 μmol/L, 5 days), given to 1-week-old hippocampal neuronal cultures, caused toxicity in a dose-dependent manner (Zamani et al 1997). The percentage of cells surviving was reduced from 85 ± 7% to 29 ± 4% at 0.1 μmol/L to 100 μmol/L βAP concentrations, respectively. The scramble fragment of the peptide did not induce neuronal loss. Kihara et al (1997) also saw some 60% cell loss with 20 μmol/L βAP (25–35) after a shorter, 48-hour incubation period. However, since their results show a linear time-dependent toxicity effect, more cell loss would be expected after 48 hours of incubation.

Both βAP (1–42) and (25–35) peptides aggregate in solution at different rates, with the former requiring some 24 hours for aggregation and the latter a few hours (Pike et al 1995). Both fragments, whether fresh (diffusible, nonfibrillar form) or “aged,” however, show neurotoxic effects (Pike et al 1993; Terzi et al 1994, 1997).

βAP Modification of Ion Channels

To study the mechanism of βAP neurotoxicity, several laboratories have looked at immediate to late physiologic events following exposure of neurons or neuronlike cells to various forms of βAP fragments. One early physiologic action of βAP is an increase in intracellular calcium concentration ([Ca^{2+}]i). In nerve growth factor–treated PC12 cells, the increase in [Ca^{2+}]i is immediate and dose dependent, which suggests a membrane effect (Joseph and Han 1992). Similarly, some doubling of [Ca^{2+}]i is reported in hippocampal primary cultures 24 hours after exposure to aged βAP (25–35) (Mattson et al 1993b; Mogensen et al 1998).

How βAP fragments induce increased [Ca^{2+}]i has been the subject of several studies. The early events seem to involve integration of these peptides with the membrane. In fact, using artificial membranes Terzi et al (1994) and Terzi et al (1997) found that both short and long fragments—βAP (25–35) and βAP (1–42), respectively—have a tendency to insert themselves into liposomes. Moreover, using the whole-cell patch clamp technique, Sanderson et al (1997) found that aggregated βAP (25–35) exerts its disruption of calcium homeostasis by forming membrane ionophores permeable to calcium. In support of channel formation, Arispe et al (1994a) suggest that the conductance is large enough to disrupt calcium homeostasis and possibly cause cell death in vivo.

Reports from other laboratories suggest an additional role for the βAP mechanism. Thus, βAP is found to enhance the activity of ligand-gated calcium channels or voltage-dependent calcium channels (VDCCs), with little or no effects on calcium homeostasis by itself. In the NIE-115 cell line, nimodipine (L-type channel selective) blocked the calcium current due to βAP (Davidson et al 1994). However, at the 100 μmol/L concentration used in their study, nimodipine could also have blocked N-type channels. Further support for the involvement of L-type VDCCs comes from Ueda et al (1997), who succeeded in preventing βAP (25–35) effect with L-type blockers, but not with blockers of N and P/Q channel. The protective effect of L-type channel blockers was not due to a reduction in free radicals, but to their ability to prevent an increase in [Ca^{2+}]i. However, the antioxidant vitamin E prevented the production of free radicals and rise in [Ca^{2+}]i by βAP. It is proposed, therefore, that chronic treatment with βAP may form free radicals, which then increase [Ca^{2+}]i via activation of VDCCs (Ueda et al 1997). This finding was supported in a study where the process of βAP (25–35) or βAP (1–40) toxicity consisted of an early, calcium-insensitive redox phase (0–12 hours posttreatment) and a late, calcium-sensitive cell death phase (3 days onwards) (Abe and Kimura 1996). This was contrary to glutamate-mediated neuronal loss, where the “early phase” redox period and “late phase” calcium-sensitive lactate dehydrogenase (LDH) production period were almost contemporaneous (Abe and Kimura 1996).

In rat membrane preparation, N-methyl-D-aspartate receptor (NMDAR) involvement is not clear, with some reporting an enhancement and others suggesting a reduction of binding of MK-801, glutamate, or glycine by βAP (Calligaro et al 1993; Cowburn et al 1994, 1997). In hippocampal neuronal cultures, however, Brorson et al (1995) found βAP (25–35)– or (1–40)–mediated increase in [Ca^{2+}]i leading to an increase in spontaneous bursts that were inhibited by TTX, AMPA receptor, NMDAR, or L-type VDCC blockers. But, no direct enhancement of NMDAR- or kainate receptor-mediated calcium influx was observed.

Despite a strong support from a number of groups, the role of VDCCs and glutamatergic receptors in βAP toxicity has not been resolved completely. Whitson and Appel (1995), for example, found no VDCC or NMDAR involvement in their hippocampal neurons exposed to βAP (1–40). Furthermore, in PC12 cells, βAP (25–35) neurotoxicity and calcium homeostasis were insensitive to calcium channel blockers but sensi-
tive to antioxidants such as vitamin E. This is attributed to the ability of βAP (25–35) to produce free radicals shortly after its preparation in solution (Hensley et al 1994). It is noteworthy that the elevated free radical formation has been reported in postmortem tissues from AD patients (Zhou et al 1995).

An intracellular signal for the release of neurotransmitters is indicated by an increase in presynaptic Ca\(^{2+}\). It would therefore be expected that βAP should enhance neurotransmitter release. This was the case in both young and aged rats, for excitatory but not inhibitory neurotransmitters under K\(^{+}\)-induced depolarization in vitro (Arias et al 1995). However, if this is followed by depolarization mediated by high K\(^{+}\) concentration, then the neurotoxicity is prevented (Pike et al 1996). This phenomenon is attributed to the depolarization-mediated activation of VDCCs, since pretreatment with a VDCC activator, bay-K, under a “mild” depolarization (10 mmol/L K\(^{+}\)), was also found to be protective against βAP toxicity (Pike et al 1996).

In addition to calcium channel activity, βAP seems to require active K\(^{+}\) channels. For instance, tetrodoylammonium-sensitive delayed-rectifier K\(^{+}\) channels were involved in the βAP (1–40)-mediated neurotoxicity in the septal/neuronal blastoma hybrid cell line SN56 (Colom et al 1998). The sister hybrid cell line, SN48, devoid of tetrodoylammonium-sensitive K\(^{+}\) channels, resisted βAP (1–40) neurotoxicity. Noticeably, SN56 cells do not seem to express high threshold calcium channels and possess low threshold Ni\(^{2+}\)-sensitive calcium channels instead that are not involved in βAP (1–40) neurotoxicity (Colom et al 1998). The mechanism for this increased K\(^{+}\) current by βAP and ensuing neurotoxicity is unknown, but oxidative stress may be a factor (Colom et al 1998). The protective effect of tetrodoylammonium is seen as its ability to raise [Ca\(^{2+}\)], which is mimicked by increased depolarization induced by high extracellular K\(^{+}\) concentration [K\(^{+}\)]. And as seen above, high [K\(^{+}\)] significantly protects against βAP assault in hippocampal neurons (Pike et al 1996).

The above reports suggest that βAP exerts its neurotoxic effect via different routes, which are probably tissue or cell type specific and occur at various developmental stages. In support of this notion, Scorzziello et al (1996) show that βAP (25–35) causes significantly more neural toxicity in immature cerebellar granule cell cultures but only slightly enhanced glutamate-mediated cell loss in mature (differentiated) cultures. βAP (25–35) alone does not affect the basal levels of [Ca\(^{2+}\)] but enhances glutamate-mediated neurotoxicity. This β-amyloid-enhanced neurotoxicity is not mediated by NMDAR activity, suggesting a different route for Ca\(^{2+}\) influx (Scorzziello et al 1996). Furthermore, due to its high inhibitory physiology, cerebellar tissue shows a distinctly different response to βAP neurotoxicity. According to Price et al (1998), cerebellar granule neurons show an increased βAP-mediated [Ca\(^{2+}\)] rise through mainly N-type and not L-type calcium channels. No nonspecific ionophore formation, due to βAP integration with the membrane, was observed in their study. Recently, we also found some cell type specificity for βAP (25–35) toxicity. In young organotypic hippocampal slices, βAP was found to be toxic both to dentate gyrus granule cells and CA1/CA3 pyramidal cells. However, in 6-month-old slices neuronal loss was mainly confined to the pyramidal cell layer (Y.S. Allen and M.R. Zamani, unpublished data) (Figure 1). These observations point to possible developmentally regulated mechanisms that render pyramidal neurons more vulnerable to βAP toxicity than granule cells.

In summary, it is evident that βAP is neurotoxic, but its mode of action may vary depending on several factors. These may be summarized as 1) neuronal cell types (e.g., hippocampal vs. cerebellar), 2) uniformity of culture (whether or not cultures contain glial “scaffolding”), 3) type of culture (acute brain slice, dissociated culture), 4) developmental stage of the culture (mature vs. immature, differentiated vs. undifferentiated), 5) age of the animal from which the cultures or brain slices are obtained (embryonic, postnatal, adult), 6) density of cultured cells (Mattson et al 1993a), 7) the β-amyloid fragment type (25–35 vs. 1–40) (Kihara et al 1997, 1998), 8) “age” and concentration of the fragment (Mattson et al 1993a), and 9) duration of exposure of cells to the βAP fragments.

**Cellular Events following Ca\(^{2+}\) Mobilization**

Since the accumulation of βAP in the brain is gradual and progressive, the induction of neuronal death should take a
more "physiologic" course rather than the large and sudden rises in \( [\text{Ca}^{2+}] \) seen in most in vitro preparations. Specific signal transduction cascades may become involved during βAP accumulation in the affected brain, which compromise cell membrane integrity by reconfiguring membrane-associated proteins, modulate the activity of cation channels, cause conformational changes in proteins, induce protein turnover, and eventually cause cell death.

Support for the above scenarios is accumulating. For example, long-term modification of VDCCs by βAP may be sensitive to functional metabotropic glutamate receptors (mGluRs). According to Copani et al (1995), mGluR agonists prevented βAP (2–35)-mediated cell loss in both cerebellar granule cells and mixed cortical cultures. Their results were consistent with the use of agonists for specific mGluR subtypes for different cultures, since cerebellar granule cells express mGluR subtypes in different proportions than cortical cultures (Tanabe et al 1992). The data are interpreted in terms of VDCC inhibition mediated by mGluR activation. Consistent with this interpretation, Copani et al (1995) found that direct inhibition of VDCCs was as "neuroprotective" against βAP toxicity. No G-protein analysis was done in this work, but Sorrentino et al (1996) and Singh et al (1997) support the notion of a pertussis toxin-sensitive βAP-mediated signal transduction pathway.

Eikici et al (1999), on the other hand, support the notion of protein modification by βAP. They found that βAP imposes neurotoxicity by increasing tau phosphorylation. Tau is the major component of neurofibrillary tangles in the brains of AD patients. Abnormal phosphorylation of tau can lead to the formation of paired helical filaments of the neurofibrillary tangles. It has therefore been suggested that βAP-mediated tau phosphorylation is achieved through activation of the mitogen-activated protein signal transduction pathway leading to phosphorylation of \( L \)-type calcium channels with a consequence of increased VDCC activity and \( [\text{Ca}^{2+}] \) elevation. This triggers calcium-dependent kinases such as protein kinase C and calcium-calmodulin-dependent kinase that phosphorylate tau (Eikici et al 1999).

Activation of transduction pathways can lead to increased genomic activity and protein turnover. Thus, βAP is shown to induce cycloheximide-sensitive protein synthesis (Pike et al 1996).

The end point of βAP-induced toxicity is cell death through necrosis and/or apoptosis. In vitro, this seems to depend on the excitation state of the neural cultures. Thus, concomitant with elevated neural excitation, membrane fragmentation and somatic swelling leading to LDH release ensues (Koh et al 1990; Mattson et al 1992). However, in the absence of elevated neural excitation, βAP seems to cause an apoptosis-path neuronal death (Forloni et al 1993; Loo et al 1993).

Finally, at low concentrations, early in the course of its synthesis, βAP may have a different biological function than later on when its local concentration in the affected brain is high. Thus, at low (nanomolar or picomolar) concentrations where no direct cell death can be detected in vitro, βAP disrupts cholinergic system by 1) reducing the concentration, release, or synthesis of acetylcholine and 2) reducing choline acetyltransferase and the activity of pyruvate dehydrogenase (involved in the production of acetylcholine) (Auld et al 1998; Hoshi et al 1997; Kar et al 1996; Roßner et al 1998). If AD in general and βAP in specific compromise the cholinergic system, intervention techniques such as "replacement" of cholinergic nuclei or raising the brain’s cholinergic “output” through pharmacologic means may help to alleviate some of the symptoms of the disease.

The Role of Nicotine against βAP Toxicity

Recently, several laboratories have addressed the interaction between βAP and the cholinergic system (Cheung et al 1993; Itoh et al 1996; Kihara et al 1997, 1998; Kim et al 1997; Maurice et al 1996; Montegghia et al 1994; Singh et al 1998a, 1998b; Zamani et al 1997). In most cases βAP-induced toxicity can be significantly, or even completely, prevented by activating the nicotinic system.

In our hands (Zamani et al 1997), toxicity mediated by βAP (25–35) (100 \( \mu \text{mol/L} \), 5 days) was partially, but significantly, prevented in hippocampal neurons by nicotine in a dose-dependent manner and with maximum inhibition at 10 \( \mu \text{mol/L} \) (Figure 2). The degree of protection was significantly larger when samples were pre-exposed to nicotine for 5 days (chronic) rather than 15 min (acute). At all concentrations, the protective effect of nicotine differed significantly between the two pretreatment time courses.

The protective effect of nicotine was receptor mediated. Thus, we showed that coadministration of mecamylamine (0.1–10 \( \mu \text{mol/L} \)) and nicotine (10 \( \mu \text{mol/L} \)) to hippocampal cultures for 5 days significantly diminished the neuroprotection by nicotine (Figure 2). Mecamylamine (10 \( \mu \text{mol/L} \), 5 days) pretreatment alone did not affect neuronal viability or prevent βAP (25–35) toxicity (Figure 2). Similar results have been reported (Kihara et al 1997, 1998). The muscarinic system does not seem to be involved in neuroprotection, since the application of muscarine itself did not offer any protection against βAP toxicity (Kihara et al 1997). Further investigation by Kihara et al (1998) unveiled the role of specific nAChR subtypes in neuroprotection.
Thus, simultaneous application of dihydro-β-erythroidine, which targets the α4–β2 nicotinic receptor, prevented the protective effect of nicotine. Cytisine, a specific agonist of the α4–β2 receptor, however, showed a dose-dependent neuroprotective effect. In contrast, some studies have shown protective effects against βAP toxicity with an α7 nAChR partial agonist [3-(2,4-dimethoxybenzylidene)anabaseine] in human cell lines (Meyer et al. 1998). In the same way as with Kihara et al. (1998), the partial agonist in this study was added simultaneously with βAP (25–35). The apparent discrepancy in these reports is most likely the result of tissue specificity in terms of nAChR subtype distribution and function, but the underlying biological pathways involved in nicotinic protection could be very similar. This point is further elucidated when one compares the protective role of nicotine with other neurotoxins such as glutamate. Here, Minana et al. (1998) proposed an α7-sensitive path for glutamate- or NMDA-mediated toxicity, as opposed to an α4–β2-mediated path for βAP, as found by others (Abe and Kimura 1996; Kihara et al. 1998) and by us (M.R. Zamani and Y.S. Allen, unpublished data).

The “chronic” exposure of neurons to nicotine in our study (Zamani et al. 1997) was an attempt to mimic the condition where nicotine intake through cigarette smoking may precede the onset of AD by many years. The lack of protection against βAP toxicity by nicotine after acute exposure was significant and contrasted with studies where concomitant application of the amyloid fragments and nicotine resulted in significant protection (Kihara et al. 1997, 1998). Explanations for this discrepancy may be found in βAP concentrations, its “aging” state, and the length of exposure of neurons to the amyloid fragment. Aggregation of βAP is not an issue because the solutions were made several hours before treatment, during which aggregation would have occurred (Pike et al. 1995). We therefore anticipated that our experimental regimen would be more detrimental to the neurons than the protocol used by others (e.g., Kihara et al. 1997, 1998) and that our results pointed to nicotine-induced long-term cellular events, possibly involving genomic activation, that rendered neurons resistant to βAP toxicity.

In addition to the in vitro studies, many experiments have been carried out in intact animals to demonstrate βAP toxicity and its prevention by nicotine treatment. For example, rodents infused cerebrally with either fresh or aged βAP (25–35 or 1–40) show significantly reduced acetylcholine and dopamine release, and choline acetyltransferase activity (Itoh et al. 1996; Maurice et al. 1996). Cognitive functions were also impaired. However, if mice are treated with nicotine or the cholinesterase inhibitor tacrine, the βAP effect is no longer detected (Maurice et al. 1996; Nitta et al. 1994).

Although convincing evidence by us and others (Kihara et al. 1997, 1998) demonstrates the involvement of nAChRs in nicotine-mediated protection, data from several laboratories suggest a more direct role for nicotine that bypasses its putative receptors. For instance, Singh et al. (1998a, 1998b) show that nicotine prevents the activation by βAP (25–35) of phospholipase A2 and phospholipase D, but not phospholipase C. This may occur through an “atypical” acetylcholine pathway, which is not sensitive to known nAChR antagonists such as hexamethonium and D-tubocurarine. This is similar to data published by Marin et al. (1997) involving elevation of phospholipase A2 activity by excitatory amino acids. The nicotine inhibition of βAP toxicity is not likely to be due to the ability of nicotine to prevent aggregation of the peptide (Salomon et al. 1996), since in similar preparations nicotine failed to affect βAP-induced elevation in phospholipase C activity (Singh et al. 1998b).

Although the exact mechanism of nicotine protection against βAP neurotoxicity is yet to be elucidated, reports examining nicotine and cholinesterase inhibitors point to a variety of biological events. These range from stimulation of neurotransmitter release to production of trophic factors.
and inhibition of β-sheet formation. A few scenarios are considered here. Nicotine replenishment of the central nervous system (CNS) may substitute for the downregulated cholinergic system of AD patients leading to increased neurotransmitter release at cholinergic sites. This should result in improved cognitive functions. Whether this nicotinic replacement results in slowing down of the progression of AD neuropathology is hard to prove, but epidemiologic studies from smokers, who on average show a delayed onset of AD, would support this conclusion. However, no evidence suggests that the course of the disease can be reversed by current therapeutic approaches.

Nicotine may also induce the production of trophic factors. In support of this view, Pike et al (1996) found a resistance to toxicity by βAPP (1–42) and (25–35) in hippocampal cultures that were pretreated with depolarizing concentrations of K⁺. This process was dependent on VDCC activity and protein synthesis. Pre-exposure to high K⁺ concentrations may trigger synthesis of certain proteins in neurons that render them resistant to βAPP toxicity (Pike et al 1996). Similarly, cells more prone to βAPP toxicity, when cultured at low density, were rescued by basic fibroblast growth factor pretreatment (Mattson et al 1993b).

Our data (Zamani et al 1997) and those of others suggest that inhibition of β-sheet formation by nicotine (as reported by Salomon et al 1996) does not play a major role in neuroprotection, since chronic nicotine pretreatment significantly prevented βAPP toxicity, whereas concomitant exposure with βAPP did not. Nicotinic receptor modulation and/or trophic factor production may play a more significant role in this process.

Finally, recent data suggest that nicotine could protect against βAPP by inducing the release of secreted forms of βAPP. This has been shown by Kim et al (1997) to occur in PC12 cells without affecting APP mRNA expression. The secreted βAPP, produced by α-secretase, is known to have neurotrophic effects and regulate [Ca²⁺] levels (Mattson et al 1993b). The release of secreted βAPP is induced by nicotine and requires functional nAChRs. Furthermore, these soluble fragments can activate fast inactivating K⁺ channels (Furukawa et al 1996). Nicotine may, therefore, use the same amyloid protein processing machinery to induce a reduction in neural excitation, which then counters the toxic effects of βAPP. Whether nicotine can modulate neurotransmitter release in the CNS of smokers in a similar manner or whether comparable mechanisms are responsible for neuroprotection against βAPP and possibly in the late onset of AD and Parkinson’s disease is yet uncertain.

Summary

The pathogenesis of AD involves abnormal production or processing of βAPP, especially since Down’s syndrome (trisomy 21) invariably results in AD (the gene for APP is located on chromosome 21) (Kola and Hertzog 1997). Alzheimer’s disease is also associated with impairment of the cholinergic system, particularly the nicotinic component, with a loss of acetylcholinesterase and choline acetyltransferase. Early findings by Bowen et al (1976), Davies and Maloney (1976), and Perry et al (1977) have yielded much research. However, in this brief review attention was focused on the neural nicotinic system, βAPP toxicity, and nicotine “replacement” for protection against βAPP toxicity.

How βAPP production and its gradual deposition lead to neuronal loss is not fully understood, but growing evidence supports the following model:

1. β-Amyloid protein membrane insertion destabilizes the membrane, affecting its fluidity (Avdulov et al 1997; Muller et al 1995; for a review, see Kanfer et al 1999).
2. There is an increase in [Ca²⁺] (Mogensen et al 1998) through either production of cation ionophores or activation of ligand-gated calcium channels and/or VDCCs, directly, or through G protein-sensitive signals (Arispe et al 1994b; Sanderson et al 1997).
3. Calcium-sensitive cascades are then triggered, leading to enhanced transmitter release, increased tau protein phosphorylation resulting in formation of neurofibrillary tangles, and protein turnover.
4. Neuronal death ensues either by apoptosis or necrosis of primarily the cholinergic system.

In considering therapeutic approaches to AD and the use of cholinergic drugs, one is reminded of the great complexity of the nicotinic cholinergic system in the CNS, which is due to the fact that 1) nAChR subunits change during development and aging and are present in most areas of the brain that may or may not be affected by the disease; 2) nAChR subunits can form functional receptors in various combinations with potentially different pharmacologic properties; 3) the neuronal cholinergic system comprises two different forms of local circuitry and projection fibers emanating from specific brain nuclei/regions; 4) these fibers may form presynaptic, postsynaptic, or somatic innervation; 5) presynaptic nAChRs enhance the release of neurotransmitters including acetylcholine; and 6) the presence of axo-axonic synapses with sensitivity to low concentrations of nicotine suggests that at least under some conditions the nicotinic system may enhance transmission in a diffuse manner rather than take part in the activity of a specific neural network for information processing (Sivilotti and Colquhoun 1995; Wonnacott 1997). The muscarinic system may also be influenced by therapeutic drugs such as acetylcholinester-
Cholinesterase inhibitors, resulting in adverse effects such as receptor downregulation or desensitization in areas of the brain unaffected by AD (however, for an alternative suggestion, see Abdallah and el-Fakahany 1991). To minimize the undesirable side effects associated with nonspecific drugs, recent developments have resulted in the production of pharmacologically more selective cholinergic drugs. An example of such a drug specifically targeting α7 nicotinic receptor subtypes is the partial agonist 3-(2,4-dimethoxybenzylidene)anabaseine, which is currently in clinical trials (Meyer et al 1998) and is shown to have positive therapeutic effects.

An alternative approach for the elevation of cholinergic output in the brain of AD patients is the use of cholinesterase inhibitors. Noticeably, their effect is indiscriminate, resulting in enhanced excitatory, inhibitory, modulatory, or even acetylcholine release at presynaptic sites where nicotinic receptors are found but may be relatively spared by AD. However, if the “soup theory of the brain” is to be favored (Sivilotti and Colquhoun 1995), minimal side effects would be expected.

Cholinesterase inhibitors (some reversible and some irreversible) such as tacrine, donepezil, physostigmine, eptastigmine, metrifonate, rivastigmine, and galantamine have now been used in several studies in patients with Alzheimer-type syndrome and have generally resulted in some improvement, sometimes lasting several years (Eagger et al 1991; Krall et al 1999; Nordberg 1993; Nordberg and Svensson 1998; Summers et al 1986). However, a degree of “habituation” to some of these drugs is apparent.

In summary, drugs targeting specific nicotinic (or muscarinic) subtypes with preferential central activity will be of future interest, but the cholinesterase inhibitors may still prove to be the most promising in alleviating symptoms of AD.

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The authors thank The Wellcome Trust for financially supporting this work, and very much appreciate the intellectual involvement of Professor Jeffery Gray in the initial stages of this project.

Aspects of this work were presented at the symposium “Nicotine Mechanisms in Alzheimer’s Disease,” March 16–19, 2000, Fajardo, Puerto Rico. The conference was sponsored by the Society of Biological Psychiatry through an unrestricted educational grant provided by Janssen Pharmaceutica LP.
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Nicotine and βAP Toxicity


