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The nicotinic acetylcholine receptor, smoking, and Alzheimer's disease

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Abstract. Cholinergic dysfunction is one of the cornerstones of Alzheimer's disease (AD) pathology. Reviewed here is evidence evaluating relationships between smoking, nicotine exposure, nicotinic cholinergic signaling, and AD. Epidemiological studies initially indicating a lower incidence of AD in smokers now suggest conflicting results. Clinicopathological findings also are mixed as to how smoking behavior affects manifestation of AD markers. Studies that show nicotine-induced increases in nicotinic acetylcholine receptors (nAChR) and protection against age-related nAChR decline contrast, perhaps in a functionally relevant way, to losses of nAChR in AD. Although epidemiological, clinicopathological, and functional studies in humans do not present a cohesive picture, much *in vitro* data suggests neuroprotective properties of nicotine when used in models of neurodegenerative disorders. Studies of nicotine and nicotinic agonist effects on cognitive function in the non-demented and in AD are not compelling. More work is needed to ascertain whether acute or repetitive activation of nAChR with acute or intermittent exposure to nicotine or the persistent inactivation of nAChR with chronic nicotine exposure is a therapeutic objective and/or explains any pro-cognitive effects of those drugs. Other studies show complex interactions between nAChR, nicotinic agonists, and agents implicated in AD etiology. Thus, while controversies still exist, ongoing research is illuminating how nicotinic receptor changes and functions may be relevant to clinical, pathological and neurochemical changes that occur in AD.

1. Introduction

Cholinergic dysfunction is one of the cornerstones of Alzheimer's disease (AD) pathology. Losses of acetylcholine production, decreased enzymatic activity (choline acetyltransferase (ChAT) and acetylcholinesterase (AChE), degeneration of cholinergic neurons, and cholinergic receptor loss characterize AD. Originally, because of the dominant notion that muscarinic acetylcholine receptors played much broader functional roles in the brain than nicotinic acetylcholine receptors (nAChR), many believed that the primary cholinergic receptor loss was of the muscarinic type. Subsequent studies, however, suggest that a relative sparing of this receptor population occur, especially the high-affinity M1 subtype. By contrast, although more work is needed to address many remaining questions regarding these measures and how they might be complicated by effects of chronic nicotine exposure [63], losses of nAChR protein and/or radioligand binding sites have been demonstrated in many studies [104]. Nevertheless, the clinical efficacy and utility of agonists developed to antagonists at either muscarinic or nicotinic receptors have been disappointing to date.

If nAChR loss, dysfunction or changes are involved in the etiology and/or symptomatology of AD, then it is important to determine whether and how drugs acting at nAChR alter initiation and/or progression of AD as well as any subsets of its symptoms. Because smoking is the most common method for exogenous nicotine delivery, we have an interest in understanding the relationship between smoking and AD. Initial studies showing a lower prevalence of AD among smokers sug-

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gested that smoking might be in some way protective against the development of AD. However several recent studies found similar or higher incidence of AD in smokers. Contrary to the conflicting epidemiological indications, much of the *in vitro* data suggests neuroprotective properties of nicotine when used in models of neurodegenerative disorders. This review discusses some of these controversies and attempts to illuminate how nicotinic receptor changes may be relevant to the clinical, pathological and neurochemical changes that occur in AD.

2. Smoking, AD, and cognition

2.1. Epidemiological studies of cigarette smoking effects on AD are mixed

Some epidemiological studies, after controlling for mortality stemming from cancer, emphysema, and coronary artery disease, have found that smokers have a lower risk for Alzheimer's disease (AD). In a population-based case-controlled study, Brenner et al. [8] reported an odds-ratio (OR: odds in the experimental group relative to the control group of developing the trait) of 0.61 for development of AD in smokers vs. nonsmokers. The odds-ratio for development of AD in smokers vs. non-smokers was 0.09 when hypertensive subjects with only higher education (college attendance) were included. Of smoking subjects, those with modest smoking exposure (< 40 pack-years) had the lowest risk (OR $= 0.18$) [8]. Lee [19] performed a meta-analysis of 19 case-controlled studies assessing the risk of AD and discovered a relative risk (RR) of 0.64 for smokers compared to nonsmokers. In familial AD, smoking has been reported to delay the onset of dementia by an average of 4.2 years [100]. Because of a concern about the possibility of sample bias due to increased mortality in smokers carrying the apolipoprotein $\varepsilon 4$ (Apo E4) allele, an indicator of increased susceptibility to AD, a follow-up study was completed and demonstrated no sample bias [99]. In another population-based study in Manitoba, Canada, smoking essentially had no effect on the relative risk for the development of AD [97]. Tyas et al. also looked at three other Canadian data sets and observed a protective effect of smoking when alcohol consumption was a covariate [96]. Smoking by itself was not protective.

However, other studies do not find a reduced incidence of AD in smokers. Using a population-based, case-controlled study of 258 AD cases and 535 age- and community-matched controls, the Canadian Health Study of Aging demonstrated no lower incidence of AD in smokers [14]. In fact, the OR was increased in heavy smokers (> 37 pack-years, OR $= 2.8$). Likewise, the United Kingdom Medical Research Council (MRC) performed a survey for detecting cases of dementia from 1545 subjects recruited into a hypertension treatment trial. 50 demented patients were detected and an additional 223 unmatched controls were used. This group concluded that smoking increases the risk of AD for mild smokers (< 10 cigarettes/day, OR $= 1.4$) and for moderate to heavy smokers (> 10 cigarettes/day, OR $= 2.6$) [72]. Doll et al. prospectively studied a cohort of male British doctors and found that smoking had no protective effect [17]. In a community-based prospective cohort study of 6870 elderly in the Netherlands, Ott et al. [62] found that the risk of dementia doubled in smokers compared to nonsmokers, and the risk of AD was even greater. This effect appeared to be influenced by apo-E4 status such that smoking apo-E4 (-) subjects had a higher risk of developing AD (RR $=$ 4.6) than smoking apo-E4 (+) subjects (RR $= 0.6$) [62]. In another prospective community-based, longitudinal study of the elderly, smoking was shown to increase the risk of development of AD [52]. However, an editorial comment of the Merchant study is that it reflects differential survival between smokers and nonsmokers with nonsmokers outliving smokers and higher survival rates of Apo-E4(-) compared to Apo-E4(+) [76]. In a meta-analysis of several European databases, "current smoking" status increased the incidence of AD (RR $= 1.74$) [40]. Thus, several prospective community-based studies of the elderly do not find that smoking is protective against the development of AD. Many of the initial case control studies suggesting a protective effect of smoking may have been driven by sample bias [37] since cohort studies now show perhaps even an increased risk of AD development. Wang and colleagues [101] support this viewpoint in a population based study that demonstrates a decreased prevalence of AD in smokers but not a decrease in incidence. Further, 5-year mortality of AD smokers was increased. They concluded that smokers were not protected against AD [101].

Demonstrations of reduced risk for other neurodegenerative diseases in smokers are clearer. For example, in a review of 46 published reports, Morens et al. [53,54] concluded that cigarette smoking is associated with approximately a 50% reduction in the risk of developing Parkinson's disease. This may be because nicotine exposure upregulates dopaminergic neu-

rotransmission or because tobacco smoke may inhibit monoamine oxidase activity. However, more work is needed to evaluate reasons why data on smoking behavior and AD risk does not corroborate the protection that smoking appears to have against PD.

2.2. Clinical-pathological studies of cigarette smoking effects on AD are mixed

Clinical-pathological studies have suggested that nicotine exposure may have some effect on the neuropathological changes in AD. In an autopsy series of 301 consecutive, unselected AD brains aged 65 years and older, Ulrich et al. [98] demonstrated that smoking may have mixed effects on the phenotypic expression of AD, causing a reduction of senile plaques (SPs) in female smokers with AD, but an overall increase in neurofibrillary tangles (NFTs) and Braak stage in all smokers with AD. However, this study did not differentiate between active and former smokers, nor did it quantify tobacco consumption.

We performed a clinical-pathological analysis to compare the phenotype of AD in 52 nonsmokers, 58 former smokers, and 16 active smokers matched for education and age at death. Without stratifying by apo-E, no differences were observed between active, former, and nonsmokers regarding disease duration, dementia severity at death, ChAT activity, or counts of synapses, total plaques, or neuritic plaques. These data suggest that tobacco consumption does not alter the clinical course of AD. Active smokers were phenotypically indistinguishable from nonsmokers. Tobacco exposure, perhaps a surrogate for nicotine exposure, may influence the degree of neurofibrillary change in AD only in the presence of apo-E4 (Sabbagh et al. submitted).

Thus, clinicopathological studies do not find clear effects of smoking on hallmarks of AD pathology. There are no published data on other types of tobacco products (i.e., snuff, or chewing tobacco) and AD.

2.3. Effects of smoking or nicotine administration reveal modest clinical benefit in cognition

Cigarette smoking has been associated with reduced risk of visuospatial and attention decline in nondemented elderly although there was no protection from the development of AD [41]. In contrast, in studies of brain function, smoking has also been shown to adversely affect cognitive function in nondemented elderly men, an effect reduced by the presence of apo-E4 [9]. Cigarette smoking has been shown to increase nAChR activity on cortex, hippocampus and thalamus [6,7,63]. These studies suggest that a clinical and biological effect from smoking can be observed.

Support for the potential cognitive benefits of nicotine comes from prospective studies of nicotine and nicotine agonist therapy in the treatment of AD. Small trials involving the subcutaneous and intravenous administration of nicotine demonstrated mild benefits in cognition in AD subjects; specifically in areas of attention, information processing, and short-term memory [28,55,63,81]. Recent studies with the nicotine patch showed beneficial effects on attention in AD [42, 47,105,107]. Large prospective trials of nicotinic agonists such as ABT-418 have been undertaken [106]. A pilot trial on 6 AD patients administered ABT-418 demonstrated improvement on recall tasks, spatial learning, and memory [71]. Several nicotinic receptor agonists for the treatment of AD are in development [63].

Thus, although not systematically examined, studies of brain function are mixed with regard to effects of smoking and nicotine administration.

3. The nicotinic acetylcholine receptor, nicotine, and its *in vivo* and *in vitro* effects

3.1. The Nicotinic Receptor in the CNS

The nAChRs of the CNS exist as members of a diverse family of yet-to-be-fully-defined subtypes [29, 59]. Each subtype consists of subunits, and the specific kinds and numbers of subunits per nAChR subtype differ for each subtype. Nine nicotinic receptor α subunits (α2–α10) and three β subunits (β2–β4), each encoded by a different gene, are candidate components of nAChR in the brain [104]. Heterologous expression studies indicate that α2, α3, α4, or α6 subunits are able to combine with β2 or β4 subunits to form "binary" nAChR complexes, apparently in an $(\alpha)_2(\beta)_3$ stoichiometry. α5 and β3 subunits can act as "wild-cards" and integrate into binary complexes to form "ternary" complexes in ways that alter receptor functional and ligand recognition properties. Some heterologously expressed nAChR can contain more than one of the α2, α3, α4 or α6 kind of subunit and/or both β2 and β4 subunits as well. In heterologous expression systems, α7, α8 (identified so far only in chick), α9, and α10 subunits can form homomeric receptors, although α9 and α10 subunit-containing and α7 and α8 subunit-containing heteromeric receptors can also form. Less

well understood are the subunit combinations and stoichiometries of naturally expressed nAChR.

An $\alpha4\beta2$-nAChR subtype appears to be the most commonly expressed in the human brain, has the $(\alpha4)_2(\alpha2)_3$ stoichiometry, both pre- and post-synaptic dispositions, and a high affinity for nicotine [47,56]. The $\alpha7$-nAChR is naturally expressed as a homomer, has lower affinity for nicotine, and is also commonly expressed in the human brain pre-synaptically and post-synaptically [1,2,47]. NAChR containing other subunits have been shown to exist, localized in important brain regions, but they are less abundant and less well studied and understood.

3.2. Chronic nicotine exposure induces changes in nAChR

Numerous studies now demonstrate that chronic nicotine exposure induces increased numbers ("upregulation") of CNS nAChR in animals and in human smokers *in vivo* (although there may be some regional and strain-specific exceptions) [6,10,45,46,108]. Upregulation of naturally or heterologously expressed $\alpha4\beta2$-nAChR sites also occurs in response to chronic nicotine treatment *in vitro* [5,65]. NAChR corresponding to native muscle-type αl-nAChR, autonomic $\alpha3\beta4$-nAChR, or naturally or heterologously expressed $\alpha7$-nAChR also increase upon chronic exposure of cells expressing those nAChR subtypes to nicotine [29,46, 64,90]. These effects appear to be post-transcriptional, because changes in levels of messenger RNA coding for nAChR subunits are absent or very small and because upregulation occurs regardless of whether subunit genes are under natural or artificial (heterologous expression vector) promoter control [104]. Because upregulatory effects of nicotine exposure on nAChRs are opposite of the effects on nAChR seen in AD, it is logical to speculate whether these findings can be rationalized with a lower incidence of neurodegenerative disorders in smokers. To investigate this, aged (24–28 month) mice, which show regionally-specific age-related declines in nAChR numbers, were administered nicotine orally for six weeks. Such exposure to nicotine did not prevent or reverse age-related nAChR declines, although longer-term (11 month) nicotine delivery to younger mice (beginning at 14 months) did preserve nAChR at levels higher than in non-nicotine treated controls [78].

In regard to functional effects, acute exposure to nicotine or the endogenous neurotransmitter, acetylcholine (ACh) activates nAChR function. Several fail-safe mechanisms ensure that nAChRs are not chronically exposed to ACh and are able to be intermittently rather than persistently activated by pulse trains of ACh release. However, habitual use of tobacco products produces chronic exposure to nicotine. Work in several laboratories show that chronic nicotine exposure produces a loss of nicotine-sensitive nAChR functional activity in the brain that can persist for days *in vivo* or even in reduced preparations [24,38,39,49,50,86,87]. Function of nAChR in several model systems is also persistently lost on chronic nicotine exposure in dose- and time-dependent ways that are nAChR subtype-specific through a process called "persistent inactivation". This process is distinct from the more reversible process classically described as nAChR "desensitization" [10, 29,75].

3.3. Nicotine is neuroprotective in vitro

From the studies in Parkinson's disease and some of the results in AD studies, the notion began to evolve that nicotine may have neuroprotective effects. Neuroprotective effects of nicotine have been demonstrated *in vitro*. Nicotine has recently been shown to inhibit Aβ aggregation by preventing the conversion from α-helix to β-sheet conformations [82]. However, in another study, nicotine did not influence formation of β-sheet structure [32]. Nicotine and nicotinic agonists has been shown to be protective against NMDA-induced but not AMPA-induced excitotoxicity [18,48, 66]. Nicotine does not seem to protect against toxicity from nitric oxide (NO) formation in cell culture [31, 88,89]. Nicotine protects against dexamethasone potentiation of kainic acid-induced neurotoxicity in cultured hippocampal neurons [85]. A possible protective mechanism may be to prevent the process that NMDA-receptor activation induces Ca^{2+} entry into the neuron, which activates NO synthase (NOS), which then exerts free-radical damage. Thus, nicotine may inhibit NMDA-induced cytotoxicity mediated through NOS stimulation by acting at NMDA receptors or effectors leading to NOS activation. It cannot protect against stimulation of NOS through other pathways or at steps downstream from NOS activation [31]. Nicotine enhances α-secretase activity, resulting in increased soluble APP release in PC12 and hamster ovary cells *in vitro* [20,36].

Nicotine may also be protective against Aβ toxicity as well [34]. In cultures where Aβ25-35 was neurotoxic, nicotine blocked Aβ induced neuronal loss [109]. The attenuation of Aβ induced neurotoxicity by nico-

tine is receptor specific. The $\alpha 7$ and the $\alpha 4\beta 2$ nicotinic receptors have been proposed to mediate this attenuation [33,35,102]. Nicotine also inhibits Aβ induced phospholipase A2 activation [91]. Moreover, nicotine appears to have antioxidant effects *in vitro* [43]. Thus, nicotine appears to have potent neuroprotective effects *in vitro* [39].

4. Nicotinic receptor changes and interactions between nicotinic receptors and suspected etiological factors in AD

Pathologically, AD is characterized by a variety of changes including the presence of amyloid SPs and NFTs. Structurally, losses of synapses are seen in many areas have been correlated to dementia severity [15, 95]. Neurochemically, significant loss of ChAT is well known [13]. Losses in the cholinergic, noradrenergic, serotonergic, and glutaminergic systems are seen as well [27].

Alterations of the cholinergic systems have been under investigation for decades. Initially it was thought that high affinity M1 muscarinic receptors were affected in AD [13,55,61]. Subsequent studies refute this, and the consensus is that muscarinic receptors are not selectively affected in AD [3,30,57,60,69].

High affinity nAChR, likely to be of the $\alpha 4\beta 2$ subtype, are significantly lost in AD [3,21,22,60,68,69,77, 79,83,84,93,103]. This loss likely occurs early in the disease [58]. Further, the $\alpha 4$ subunit seems to be selectively lost in AD [11,66] and the nAChR loss seems to be regionally specific in AD [11]. However, it is not yet clear whether receptor loss is due to death of neurons expressing receptors, loss of synapses bearing receptors, loss (down-regulation) of receptors from surviving neurons, or some combination of these effects. No changes are observed at the level of transcription of subunit mRNAs. New evidence suggests protein level expression of the $\alpha 4$ subunit is reduced [104]. In contrast, we found, like others, that the $\alpha 7$-nAChR appears to be spared in AD [12,51,74,93] although some groups have reported a loss of $\alpha 7$-nAChRs in AD [4].

Other features of the interaction between the nAChR and AD have been investigated [58]. Loss of nAChR is not affected by apolipoprotein E genotype [73,94]. NAChR losses seem to parallel plaque and tangle counts in the entorhinal and subicular areas in AD [68] but not in frontal cortex [80]. Recently, nAChR loss has been found to correlate more specifically with Aβ_{42} levels rather than senile plaques [66]. NAChR loss does not correlate with cognitive decline in AD or DLB [80], but may correlate to dementia severity [66]. We found that loss of synapses correlates well with loss of nAChR binding in AD but loss of ChAT activity did not [79]. In contrast, loss of nAChR correlated well with loss of ChAT activity in DLB patients [74]. Hippocampal ChAT and nAChR losses are less reduced in smokers with AD versus non-smokers with AD [67].

In experimental models, amyloid infusion into rats was found to produce a loss of nAChR [26]. Several recent studies find evidence of interactions between β-amyloid and different nAChR subtypes, and some of these interactions affect nAChR function [16, 44,70,102]. Immunohistochemical studies co-localize nAChR subunit-like immunoreactive material identified on neurons and fibers in normal controls to plaques and tangles in human AD subjects [92]. Correlations between nAChR loss and plaques and tangles in the APP 670/671 double Swedish mutation did not suggest a direct relationship [58]. These studies suggest possible links between β-amyloid and nAChR in AD.

5. Conclusions

This summary attempts to clarify associations, which have drawn considerable attention, between smoking, nicotine, nicotinic receptors, and AD. Some general principles and critical questions emerge that might guide continuing work to elucidate these relationships. One principle is that tobacco smoking is certainly not equivalent to direct administration of nicotine. Tobacco contains thousands of compounds, many of which are known to be toxic, that could negate any neuroprotective or acute cognition-enhancing effects of nicotine. Thus, it would be surprising if smoking behavior affected the incidence or progression of symptoms of AD in the same way as nicotine or nicotinic drugs. Nevertheless, an important lead would be indicated if smoking behavior indeed is shown to produce lower incidence of AD. Another is that smoking behavior and nicotinic drugs may have beneficial effects on only a subset of the population, just as smoking behavior itself is a feature of only a subset of individuals. More work is needed to determine more precisely the therapeutic objectives in potential nicotinic drug treatment of dementia and to establish the mechanism(s) involved in any cognition-enhancing effects of such treatment or of smoking behavior.

A critical question is whether neuroprotective and cognitive enhancements are mediated via acute activa-

tion, repetitive activation, or more chronic inactivation of nAChR function or by some combination of these actions. It also is important to determine how such actions are integrated across the diverse nAChR subtypes implicated in brain function and whether changes in receptor number have any significance for these effects. Also deserving more attention is the issue of whether neuroprotective qualities of nicotine exemplified *in vitro* also are present *in vivo* and whether such actions of nicotine can affect any of the pathophysiological hallmarks of AD. This is perhaps the most promising line of inquiry that would provide renewed impetus to explore further whether nicotinic receptors are desirable therapeutic targets.

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