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Neuroprotection via nAChRs: the role of nAChRs in neurodegenerative disorders such as Alzheimer's and Parkinson's disease

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1. ABSTRACT

Epidemiological studies have identified a negative correlation between smoking and the development of neurodegenerative disorders such as Parkinson's disease, and in some studies, Alzheimer's disease. These findings have been attributed to the ability of nicotine to act as a neuroprotective agent. A large number of studies demonstrate that nicotine can protect against neuronal death *in vitro* and *in vivo*, and the mechanisms underlying the ability of nicotine to protect against excitotoxicity and amyloid-□ toxicity are beginning to be elucidated. Despite the compelling evidence that nicotine is neuroprotective, it is clear that nicotine can be toxic under some circumstances. The balance between nicotine neuroprotection and toxicity depends on dose, developmental stage and regimen of administration. Therefore, a full understanding of the molecular and cellular effects of nicotine on signaling pathways relevant to neuronal survival is critical for informed drug discovery of nicotinic compounds to combat human neurodegeneration. This review summarizes recent studies related to the mechanisms underlying nicotine-mediated neuroprotection, and addresses issues that are relevant to use of nicotine as a neuroprotective agent *in vivo*.

2. INTRODUCTION

Smoking is negatively correlated with the development of Parkinson's Disease (PD) across a number of studies (1). Studies on the relationship between smoking and Alzheimer's Disease (AD) have been more variable, but there is some suggestion that smoking can be protective against the development of AD as well (1). While there are many constituents of tobacco smoke, these observations, coupled with the demonstration that nicotine can be neuroprotective in many cellular and animal models (reviewed in (2)), have led to the hypothesis that the nicotine in tobacco can protect against the development of these neurodegenerative diseases. In addition, both human and animal studies have shown that nicotine can improve cognitive function (reviewed in (3)) and nicotine has been investigated for its ability to improve symptoms in patients with PD and AD. It is also the case that in post-mortem and imaging studies, patients with many forms of dementia demonstrate decreased levels of nicotinic receptors (4). Taken together, these studies suggest that nicotinic receptors are important for the maintenance of both neuronal survival and cognitive function, and that

exogenously-administered nicotinic agents may preserve neurons in patients with neurodegenerative disease and increase performance in cognitive tasks in these individuals. This review will examine the evidence for and against a neuroprotective role for nicotine and will outline potential pathways that may underlie the ability of nicotine to prevent neuronal loss.

3. EPIDEMIOLOGICAL STUDIES OF SMOKING AND ALZHEIMER'S OR PARKINSON'S DISEASE

A negative correlation between the development of PD and smoking has been a very consistent finding across epidemiological studies (1, 5, 6), with the exception of one study with a small sample of PD patients that suggested smoking can increase the risk of PD in older subjects and those with a family history of PD (7). In general, non-smokers have an approximately two-fold increase in the risk of developing PD than smokers (1). Similarly, the incidence of smoking in control groups can be significantly higher than in PD patient groups in studies of disease progression (8). Despite the negative correlation, once the disease has been diagnosed, ongoing smoking does not appear to slow the progression of PD (8), although it may be that smokers who have developed PD are either insensitive to the protective effects or have already achieved as much neuroprotection from smoking as possible by the time the disease is diagnosed. More studies are necessary to confirm this finding.

The negative association between smoking and AD is less consistent. One factor that might contribute to this variability is that smoking can greatly increase the risk of ischemia. AD patients with multi-infarctual dementia have been included in several studies, perhaps resulting in a mixed population (1). Thus, smoking may be protective against AD in a subset of patients that are not at risk for ischemia, or there may be a balance across subjects between the protective effects of smoking on neurodegeneration and the greatly increased risk of infarct. A meta-analysis of the epidemiological studies of smoking and AD showed conflicting results between studies using a case-control (decreased AD risk) or cohort (increased AD risk) design (9).

Although the studies on the effect of smoking on the risk of AD are mixed, levels of amyloid-β (Aβ) and amyloid plaques were reduced in the brains of AD patients (10-12) and non-AD elderly patients (13) who smoked compared to their non-smoking counterparts in several studies. Although smoking appears to reduce Aβ, it may have opposite effects on neurofibrillary tangles induced by aberrant tau phosphorylation, since the smoking subjects with AD that had decreased numbers of amyloid plaques, also had increased numbers of tangles (10).

Taken together, the epidemiological studies suggest that some component of cigarette smoke may protect against the development of neurodegenerative diseases. Further, the lack of a consistent epidemiological effect of smoking on AD does not mean that nicotine will not be an effective therapeutic agent for either long-term neuroprotection or short-term cognitive enhancement, but simply that smoking may not be a good model for the actions of nicotine in this disease state. The ability of nicotine to protect against neuronal death from many types of insult both *in vivo* and *in vitro* has lead to the idea that it is the nicotine in tobacco smoke that is the critical agent resulting in protection against AD and PD. This is supported by the finding that nicotine administration in animal models of AD shows similar effects on levels of Aβ and tau-dependent neurofibrillary tangles to smoking in human subjects. In transgenic mice with a copy of the Swedish mutation of the amyloid precursor protein (APP), chronic oral nicotine administration can decrease Aβ levels and plaques and increase levels of α7* nAChRs (14, 15). However, in mice with both the Swedish mutation transgene and a human tau transgene (tau_{P301L}), chronic oral nicotine administration increased aggregation and phosphorylation of tau (16). Thus, the effect of nicotine and smoking on progression of AD is likely to represent a mix of protective and pathological consequences, which may contribute to the mixed outcome of the effect of smoking on AD in epidemiological studies.

Nicotine has also had both positive (17, 18) and negative (19, 20) effects on functional measures in PD patients, although very few studies have been done on the ability of nicotine to slow development or progression of PD. Similarly, nicotine can somewhat improve cognitive performance in AD patients, particularly in attentional measures (21), but it is not known whether nicotine will slow progression of the disease. Future studies will be necessary to make stronger links between the neuroprotective effects of nicotine and the possibility that nicotine treatment can be developed to combat the progression, and not just the symptoms, of PD and AD.

4. EXPRESSION AND FUNCTION OF NICOTINIC ACETYLCHOLINE RECEPTORS IN PARKINSON'S DISEASE MODELS

Nicotinic acetylcholine receptors (nAChRs) are the primary targets for nicotine in the brain and are composed of a combination of α– (α2-α10) and β– (β2-β4) subunits. The possibility that nicotine in tobacco smoke can protect against the development of PD has lead to the investigation of levels and function of nAChRs in animal models of PD. Dopaminergic neurons in the substantia nigra, that are lost selectively in PD, express multiple subtypes of nAChR, and are distinguished by a high level of expression of the α6 and β3 subunits in rodents (22, 23) and non-human primates (24, 25). These subunits are largely expressed in catecholaminergic neurons of the brain (26, 27). The α6 and β3 subunits combine with other subunits, in particular the β2 subunit, which is necessary for the assembly of these receptors (22, 23, 28). The α4 and α5 subunits are also important for nAChRs in the substantia nigra, and the α4/β2/α5 and α6/β2/β3 combinations make up a majority of the nAChRs in this region (23). Finally, the α7 subunit, which can form functional, homomeric nAChRs, is also expressed in dopaminergic neurons and contributes to some nicotine-

stimulated currents (29, 30). This broad diversity of nAChR subtypes suggests that nicotine can modulate the function of dopaminergic neurons and that each subtype may play a different role in nicotine-mediated modulation.

Some nicotinic ligand binding studies have shown that postmortem brain tissue from patients with PD shows a loss of nAChRs in dopaminergic regions, consistent with the death of dopamine neurons (31, 32). In addition, loss of nicotinic binding sites has been observed in the basal forebrain and cortex of PD patients, particularly those with dementia (31, 33-35). Experiments in both rodent (36) and non-human primate models of PD (24, 37) have suggested that there is a selective loss of α6/β2/β3 nAChRs following treatment with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Since MPTP results in selective loss of dopaminergic terminals (36), this suggests that the α6 and β3 subunits are selectively trafficked to the nerve terminals of dopaminergic neurons, whereas α4/β2/α5 nAChRs may be more concentrated at the cell soma. Thus, these two nAChR subtypes are likely to have a differential ability to increase release of dopamine from the nerve terminals and increase firing rate at the cell soma, respectively, and perhaps would have differing effects on survival of dopaminergic neurons. In agreement with the idea that α6/β2/β3 nAChRs may be particularly important for maintained dopaminergic function in PD, L-DOPA exposure preferentially rescues this subtype in parallel with rescue of the behavioral phenotype following MPTP treatment in non-human primates (38). These studies have lead to the suggestion that α6-containing (α6*) nAChRs could be important targets for drug development to treat PD and other neurodegenerative disorders (39). Paradoxically, although α6/β2/β3 nAChRs were decreased in both the caudate and putamen of non-human primates after MPTP treatment, nicotine-mediated dopamine release was reduced in caudate but not putamen (40). In fact, nicotine-elicited dopamine release was largely spared in ventromedial striatum after MPTP treatment (41). This suggests that nAChR-mediated dopamine release is modulated by other factors in addition to nAChR number.

5. LOSS OF NICOTINE BINDING SITES IN ALZHEIMER'S DISEASE

Just as a loss of nicotine binding sites has been observed in PD (31-35), there is a much more extensive literature demonstrating loss of high affinity nicotine binding sites in postmortem brain tissue from patients with AD (42-44) and in functional imaging studies (45). These high affinity nicotine binding sites likely represent receptors containing the β2 subunit (23, 46). Receptors containing the α7 subunit, which are identified by α-bungarotoxin binding, do not seem to be as profoundly affected as the high affinity sites in the cerebral cortex of AD patients (47, 48); however, there is a progressive loss of α7 nAChRs in transgenice mice with the Swedish mutation of APP and human tau that correlates with Aβ accumulation (16).

The decrease in nicotinic binding in the brains of AD patients appears to be due to decreased levels of the nAChR subunit proteins but not the mRNA encoding these proteins. No change has been seen in nAChR mRNAs in AD brain (49, 50), but α3, α4, α7 and β2 subunit protein levels are decreased in cortex and hippocampus of AD patients (48, 49, 51). Interestingly, levels of nAChR subunit proteins are upregulated in AD patients who smoke (49), as might be expected given the increase in nicotine binding sites are increased by smoking in postmortem brain from healthy controls (52); however, levels of nAChR proteins in AD patients who smoke are still lower than normal controls of any smoking status (49). Taken together, these results suggest that there is a loss of several subtypes of nAChR in the brains of patients with AD, with little effect at the transcriptional level.

6. NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS AND NEUROPROTECTION

The neuroprotective effects of nicotine have been well-characterized both in cultured neurons and in the intact animal in a number of neurodegeneration models related to excitotoxicity (reviewed in (2)). *In vitro*, nicotine and nicotinic agonists protect against glutamate excitotoxicity in primary hippocampal and cortical neurons (53-59), and *in vivo*, nicotine can prevent cell death from a number of neurotoxic insults including excitotoxicity, ischemia and even knife cut (58, 60-63). Of particular interest for AD, nicotine can protect against toxicity induced by β-amyloid in hippocampal (64, 65) and cortical (66, 67) neurons. These effects are blocked by both α4/β2*- and α7*-selective nicotinic antagonists, suggesting that they are mediated through activation of multiple neuronal nAChRs (64-67); however, nicotine may also bind to Aβ directly, inhibiting formation of Aβ fibrils and disrupting already formed fibrils (68). Activation of nAChRs increases secretion of non-amyloidogenic fragments of APP *in vitro* (69, 70) and chronic nicotine administration can also block APP processing into Aβ fragments *in vivo* through an nAChR-dependent mechanism (71). Thus, the ability of nicotine to protect against Aβ neurotoxicity may involve multiple mechanisms and nAChR subtypes.

Of relevance to PD, nicotine can also protect striatal and dopamine neurons from excitotoxic cell death *in vitro*, and knife cut lesions or dopaminergic agents such as methamphetamine and 6-hydroxy-dopamine *in vivo* (72-77), through activation of α4/β2* nAChRs (77). In models of PD, nicotine also protects primary cultured neurons from MPTP toxicity through action at heteromeric nAChRs (78). Further, acute and chronic nicotine administration prevents dopamine neuron loss *in vivo* following lesion with MPTP (75, 79-81). Acute administration of nicotine is known to activate nAChRs, whereas chronic administration can desensitize and inactivate nAChRs (82). Therefore, it may not be surprising that infusion of nicotine through osmotic minipumps, a treatment that is thought to lead to significant nAChR desensitization, does not protect against, and actually increases, neurotoxicity by MPTP (83-85). Another suggestion is that nicotine's ability to increase oxidative stress may contribute to the toxicity of high doses of nicotine (86, 87).

Nicotine can also be neurotoxic to developing neurons (88-91), suggesting that there are differential signaling pathways activated in adult neurons that lead to neuroprotection and immature neurons that may favor neurotoxicity of nicotine. The neurotoxic effects may be related to activation of α7* nAChRs, since cultured neurons from knockout mice lacking this subunit do not show developmental neurotoxicity to nicotine (91) and knockin of a hyperactive α7 subunit results in wide-spread neuronal death in mice (92). In addition, stimulation of α7* nAChRs can increase markers of apoptosis in developing neurons and adult hippocampal progenitor cells (93-97). Developing neurons may be less able to buffer calcium (98) or induce expression of calcium binding proteins (99) than mature neurons; thus, the neurotoxic effects of nAChR activation may be due to the ability of nAChRs to increase calcium entry (100), coupled with incomplete calcium buffering in immature neurons (98). In support of this possibility, increasing the expression of the calcium buffering protein calbindin in immature hippocampal progenitor cells can counteract the neurotoxic effects of nicotine (96). Treatment with chronic, high-dose nicotine can be toxic to adult neurons as well (83, 85), suggesting that even in mature neurons, high doses of nicotine may allow too much calcium into the cell and exacerbate, rather than protect against, cell death.

In general, continuous exposure to nicotine is less effective in neuroprotection than either acute or chronic-intermittent exposure to nicotine (83-85), although chronic exposure through the minipump can be effective, at least at low doses (77, 101). Consistent with the effect on neuronal survival, chronic-intermittent nicotine administration can increase levels of basic fibroblast growth factor-2 (FGF-2), a neuroprotective protein, in dopaminergic brain regions (102-105), while continuous infusion of nicotine can decrease FGF-2 levels (106). Since growth factor stimulation may be one of the mechanisms underlying the neuroprotective effects of nicotine (102), these data provide strong evidence that regimens that provide low doses of nicotine intermittently, similar to smoking, are more effective than continuous infusion of high dose nicotine in promoting neuronal survival.

Multiple nAChR subtypes contribute to nicotine-induced neuroprotection *in vitro* and *in vivo*. Both homomeric, α7* (54, 56, 57, 63, 66, 107) and heteromeric, α4/β2* (and potentially α3/β4*) nAChRs (56, 64, 67, 77, 91, 108) can contribute to the neuroprotective actions of nicotine. There appears to be a greater contribution of α4/β2* nAChRs to neuroprotection in striatal and cortical neurons (77, 91, 108) and a more prominent role for α7* nAChRs in hippocampal neurons (107, 109), however, suggesting that either differential expression of nAChR subtypes, or differential expression of downstream signaling molecules can modulate the neuroprotective effects of nicotine in various cell types. In addition to their ability to mediate some of the neuroprotective effects of acute nicotine treatment (77, 91, 108), α4/β2* nAChRs in mice are necessary for survival of hippocampal and cortical neurons (110) and maintained cognitive function (110, 111) during aging. Thus, the ability of nicotine to protect neurons from toxic insults may reflect an important role for ongoing nAChR signaling against neuronal injury *in vivo*.

Taken together, the studies reviewed here support the idea that nicotine is neuroprotective against many types of toxic stimuli in many neuronal subtypes. Despite these consistent findings, it is clear that nicotine can be neurotoxic under some circumstances, with particular vulnerability in developing neurons and with continuous infusion and higher doses most likely to cause neuronal death. In addition, it is clear that multiple nAChR subtypes, particularly α7* and α4/β2* nAChRs, can mediate the neuroprotective effects of nicotine, with different cell types preferentially sensitive to one or both of these nAChR subtypes.

7. POTENTIAL MECHANISMS UNDERLYING NICOTINE-MEDIATED NEUROPROTECTION

Although the neuroprotective effects of nicotine are well established, the mechanisms of nAChR-mediated neuroprotection are still not well defined. Hints offered by available studies point to a diverse range of potential mechanisms. This is in part due to the multiplicity of *in vitro* or *in vivo* systems analyzed. Moreover, even within the same system, nicotinic ligands may act on multiple nAChRs and modulate multiple post-receptor signaling pathways. Defining which of these mechanisms is essential for the neuroprotective effects of nicotine has remained, in most cases, elusive.

Before discussing potential receptor-mediated mechanisms, it must be mentioned that some protective effects of nicotine may be independent from binding to nAChRs. Nicotine is a lipophilic drug and can enter target cells and interact with as yet undefined molecular targets. In recent years, a few papers have shown protective effects of nicotine that cannot be blocked (or mimicked) by nicotinic antagonists (see table 3 in (2) and (68, 112)). For instance, nicotine-mediated protection against MPP+-induced cytochrome c release and mitochondrial swelling in an *in vitro* model of PD does not seem to be mediated through nAChRs (112), and nicotine has been shown to bind to Aβ directly (68).

The neuroprotective effects of nicotine mediated through nAChRs can be either direct (i.e., nAChRs are expressed by degenerating cells) or indirect (through release of other neurotransmitters or trophic agents; see Figure 1). It is clear that nicotine can increase the release of a large number of neurotransmitters and neuromodulators (113); therefore, an indirect protective action of nicotinic agents very likely occurs through release of other neurotransmitters in several instances *in vivo*. *In vitro* nicotine protection against glutamate toxicity could be blocked by D1 antagonists in retinal neurons, suggesting that this neuroprotection is mediated by stimulation of dopamine release (114). Similarly, some of the toxic effects of nicotine could be mediated through release of excitatory amino acids. For example, exacerbation of methamphetamine toxicity by nicotine in rat striatum appears to be mediated through α7* nAChR-induced

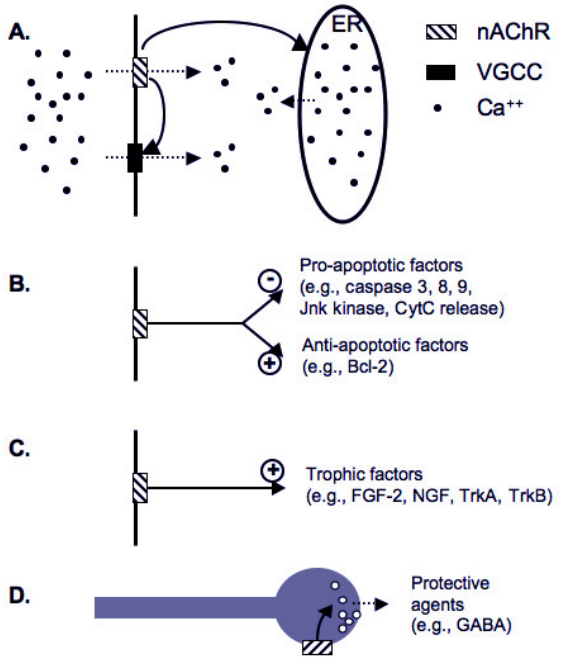

Figure 1. Potential mechanisms underlying nicotine-mediated neuroprotection – Nicotine may have both direct, or cell autonomous, effects on neurons that result in increased survival or may have effects on neighboring neurons to result in more indirect neuroprotective effects. Examples of potential direct neuroprotective effects of nicotine are shown in A, B and C. A. Nicotine can activate different classes of nAChRs resulting in either direct entry of calcium, or depolarization leading to calcium entry through voltage-gated calcium channels and thus decrease the ability of glutamate or other excitotoxic agents to increase calcium levels through voltage-gated calcium channels or from intracellular stores in the endoplasmic reticulum. B. Nicotine can decrease the levels or activity of pro-apoptotic factors such as caspases, jnk kinase or cytochrome c and can increase the levels or activity of anti-apoptotic factors such as Bcl-2. C. Nicotine can increase the synthesis and release of growth factors such as NGF and FGF-2 and increase the levels of neurotrophic receptors such as TrkA and TrkB. D. Nicotine may have more indirect effects on neuronal survival by causing the release of neurotransmitters that decrease excitability, such as GABA, from populations of neighboring cells. CytC, cytochrome c, ER, endoplasmic reticulum; nAChR, nicotinic acetylcholine receptor, VGCC, voltage-gated calcium channel.

elease of glutamate (115). Interestingly, blockade of α7* nAChRs by MLA can be neuroprotective in hippocampal slices (116).

The various subtypes of nAChR are permeable to both sodium and (to different extents depending on the subtype) calcium, leading to cell membrane depolarization and increase in cytoplasmic calcium levels. Moreover, in certain cell types nAChRs are associated with other proteins such as such as phosphatidylinositol 3-kinase (PI3K) in cortical neurons, (117), dopamine D2 receptors in midbrain dopamine neurons (118), or voltage-gated calcium channels in striatal synaptosomes (119), whose activity nAChRs can influence directly.

Among the molecular events that follow receptor activation, changes in cytoplasmic calcium dynamics have a major role in mediating several nAChR effects (108, 120-122). Accordingly, in many instances, nAChR-mediated neuroprotection seems to require increase of cytoplasmic calcium levels in target cells (54, 56, 108, 123, 124). Calcium entry can occur directly through nAChRs (100, 125), particularly α7* nAChRs which have a high calcium conductance (126), and calcium levels can be increased indirectly through activation of voltage-gated calcium channels or release of calcium from intracellular stores (107-109, 127). Calcium can, in turn, affect a wide variety of calcium-dependent molecular cascades and cell processes (127) ultimately leading to cell survival or neuroprotection.

Nicotine does not appear to affect calcium entry due to stimulation of glutamate receptors in hippocampal (107) or cerebellar neurons (128), but does decrease influx through L-type calcium channels in cortical neurons (108) and increase expression of the calcium buffer calbindin in hippocampal cells (59). Therefore, at least in hippocampal neurons, nicotine may regulate levels of calcium indirectly through an increase in calcium binding proteins, rather than through direct regulation of calcium entry. In addition, these effects of nicotine appear to require initial calcium entry through nAChRs (59, 108). Thus, it is possible that a modest increase in calcium due to nAChR activation can lead to intracellular signaling events that protect against larger calcium influxes as a result of excitotoxic insults. This balance between protective effects of small calcium signals and toxic effects of large calcium signals may explain the differential protective and neurotoxic effects of low-dose and high-dose nicotine administration. The apparently contradictory involvement of calcium in nicotine-mediated neuroprotection and excitoxic cell death may also result from different kinetics of intracellular calcium elevation by these agents, as well as different subcellular sites of calcium entry.

While changes in calcium dynamics appear to be important for nicotine-mediated neuroprotection, subsequent effectors are in large part unknown. Low levels of calcium can activate the calcium-dependent phosphatase calcineurin in cortical neurons, and this appears to be important for inactivation of L-type calcium channels and nicotine effects on neuroprotection in these cells (108); however, this mechanism does not appear to be critical for hippocampal neurons (107).

Nicotine-mediated neuroprotection appears to involve modulation of apoptotic signaling pathways in some cells types. For example, nicotine decreases caspase 3 activation induced by Aβ in hippocampal neurons (65), decreases activation of the downstream cell death effector jnk kinase in tumor cells (129) and decreases cytochrome C release, activation of caspases 3, 8 and 9, DNA laddering

and neuronal nitric oxide synthase activity in spinal cord cultures exposed to arachidonic acid (130-132). Paradoxically, the ability of nicotine to decrease activity of caspase 8 in cortical neurons antagonizes the ability of TNFa to protect these neurons from cell death, since caspase 8 cleaves the GluR1 subtype of glutamate receptor in these neurons (133). Thus, the ability of nicotine to modulate apoptotic pathways has different effects on neuronal survival depending on the cell type.

Studies of the mechanistic aspects of nicotine neuroprotection are typically *in vitro* studies with short-term exposure to the drug. While these studies are certainly of interest, it should be emphasized that effects of prolonged or repeated exposure to nicotinic agonists in *in vivo* models may be more relevant to understand the contribution of nAChRs in disease states and to assess the possible efficacy of nicotinic therapies. As mentioned above, prolonged exposure to nicotine can induce expression of proteins that will alter subsequent responses to calcium, such as calcium transporters (134) and calcium binding proteins (59, 135). Growth factors are also putative effectors of nAChR-related neuroprotection and can result in long-term changes in neuronal signaling. Nicotine treatment can increase expression and release of the growth factor FGF-2, but not several other members of the FGF family or receptors for FGF-2. FGF-2 is induced as a result of stimulation of α4β2* nAChRs in cortex, hippocampus, substantia nigra and striatum of rats, supporting a role for these receptors in neuroprotection in both PD and AD models (75, 102-104). In addition to the FGF family, the neurotrophins are also upregulated by nicotine treatment. The neurotrophin receptor trk A can be upregulated both in PC12 cells (136) and in the hippocampus (137) through activation of α7* nAChRs. In addition, NGF and its receptor trkB can be upregulated in the hippocampus, while NT3 is downregulated (138). Finally, several transcription factors downstream of growth factor receptors are also upregulated following repeated nicotine administration, including Nurr77, Egr-1 and Egr-2 (105). Taken together, these expression data suggest that both FGF-2 and NGF are candidates for effectors of nicotine-mediated neuroprotection.

While modulation of calcium signaling and growth factor expression are likely to be general mechanisms underlying nicotine neuroprotection, the connections between AD and nAChRs has attracted much interest to the specific mechanisms underlying nicotine-mediated neuroprotection against Aβ neurotoxicity. In primary cortical neurons, nicotine-induced protection against Aβ involves α7* nAChRs and PI3K that are physically associated in these cells, and nicotine can increase the activity of Akt, which is an effector of PI3K (117). Expression of Bcl-2, a cell survival protein, was also increased by nicotine in that study (117). Nicotine-induced protection against Aβ is also α7* nAChR-dependent in hippocampal neurons (65) and Aβ and the α7* subunit can be co-immunoprecipitated (139); however, a contribution of heteromeric nAChRs to protection against Aβ toxicity has also been observed (66). Indeed, in cortical neurons from a transgenic mouse expressing a dominant-negative form of the α7 subunit, nicotine loses its protective effects against NMDA toxicity, but maintains protection against Aβ25-35 in cortical neurons, suggesting that α7* nAChRs are critical for protection against NMDA-mediated excitotoxicity, but not essential for protection against Aβ-induced cortical cell death (140). In addition, α7* nAChRs mediate an Aβ-induced decrease of surface NMDA receptors, which may contribute to AD synaptic dysfunction (141). Consistent with the idea that α7* nAChRs are necessary for protection against NMDA-mediated toxicity, the α7 blocker MLA, but not the heteromeric nAChR antagonist DhβE, blocks nicotine's effects on NMDA toxicity in hippocampal slice culture (142).

While nicotine and nAChRs have the ability to counteract some of the effects of Aβ toxicity, Aβ has reciprocal effects on nAChRs, suggesting that nAChR function may be blocked by release of Aβ in the AD brain. Treatment with Aβ can significantly decrease both nicotinic binding sites and the protein and mRNA levels of nAChR α3, α7 and β2 subunits in PC12 cells (51); although elevation of Aβ *in vivo* results in upregulation of α7* nAChRs, downregulation of mitogen activated protein kinase and decreased cAMP-regulated element binding protein phosphorylation in the hippocampus (143, 144). β-amyloid peptides can bind with high affinity to both α7- (139) and heteromeric nAChRs (145); however, there is no consensus on the functional effect of that interaction, since Aβ has been reported to activate, competitively antagonize or noncompetitively antagonize nAChR activity (143, 145-150). Binding to α7-type nAChRs facilitates internalization and accumulation of Aβ (151), providing a possible explanation for the vulnerability of α7 subunit-expressing cells to degeneration in AD.

8. PERSPECTIVES

The studies reviewed here provide strong evidence, both *in vitro* in many cell types, and *in vivo* in brain areas that degenerate in diseases such as PD and AD, that nicotine can protect against neuronal death. It seems likely that the ability of nicotine to be neuroprotective underlies the epidemiological finding that smoking is negatively correlated with the development of PD.

The link between smoking, nicotine and AD is much more complex. At each level (epidemiological effects of smoking on AD, *in vivo* studies of nicotine in transgenic AD models and cellular or molecular studies of Aβ and nAChRs), there are both positive and negative effects of smoking and nicotine on markers of AD. Despite the large number of studies on nicotine-mediated neuroprotection, it is clear that nicotine can be toxic as well as protective. Either prolonged activation of nAChRs or their desensitization by long-term nicotine administration can be neurotoxic to developing neurons, or at high doses, to adult neurons. Nicotine can increase oxidative stress at high concentrations in a receptor-independent manner, and calcium entry through nAChRs can contribute to excitoxicity in some cases.

At the molecular level, Aβ can both activate and inhibit nAChRs, although there does not seem to be a consistent principle with respect to nAChR subtype or neuronal cell type that can explain the variability in the effects of Aβ on nAChR function. More studies on the subunit combinations and kinetics of the interaction between nAChRs and Aβ will be necessary to identify the critical elements responsible for the functional interaction between these proteins.

At the organismal level, the opposing ability of nicotine administration to decrease Aβ load but increase tau aggregation suggests that systemic nicotine administration may not be able to halt the progression of AD until some method to protect against neurofibrillary tangle formation is identified. In addition, the ability of smoking to increase risk of cerebral ischemia, a predisposing factor for the development of dementia, reinforces the idea that more research is necessary on safer ways to deliver nicotine. Along those lines, the classical drug-delivery paradigm of maintaining stable blood levels of nicotine is not likely to be the most effective way of delivering nicotine for therapeutic efficacy. Regimens in animals that deliver nicotine in a pulsatile fashion are more effective in neuroprotection than regimens, such as osmotic minipumps, that maintain stable blood levels, likely because nAChRs are desensitized in the continuing presence of nicotine. Thus, intermittent dosing that leads to multiple peaks of nicotine in the brain over the course of the day may be necessary for therapeutic applications of nicotine. Another caveat may be to avoid excessive calcium entry through α7* receptors, either by using nicotinic agonists with selectivity for α4/β2* receptors or α7 partial agonists.

It is clear that nicotine has long-lasting effects on neuronal signaling that contribute to its neuroprotective effects. Anti-apoptotic pathways, calcium signaling and growth factor signaling have all been shown to respond to nicotine administration, leading to the possibility that nicotine is neuroprotective because it stimulates the convergence of multiple pathways that prevent cell death. These effects may also be magnified because nicotine activates multiple nAChR subtypes, with different roles in promoting intracellular signaling in neurons. Thus, both α7* and α4/β2* nAChRs are likely to contribute to the neuroprotective properties of nicotine, although the effects of one or more of these subtypes may predominate in individual neuronal populations. Overall, these studies provide ample support for a continuing drug discovery effort to identify nicotinic compounds that are protective against human neurodegenerative disease.

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Abbreviations: AD: Alzheimer's disease, PD: Parkinson's disease, nAChR: nicotinic acetylcholine receptor, Aβ: amyloid-beta-peptide, APP: amyloid precursor protein, MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, FGF-2: basic fibroblast growth factor-2, PI3K: phosphatidylinositol 3-kinase

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