Nicotinic Receptor–Mediated Protection against 
β-Amyloid Neurotoxicity

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Multiple lines of evidence, from molecular and cellular to epidemiologic, have implicated nicotinic transmission in the pathology of Alzheimer’s disease. In this review we present evidence for nicotinic receptor–mediated protection against β-amyloid and glutamate neurotoxicity, and the signal transduction involved in this mechanism. The data are based mainly on our studies using rat-cultured primary neurons. Nicotine-induced protection was blocked by an α7 nicotinic receptor antagonist, a phosphatidylinositol 3-kinase inhibitor, and an Src inhibitor. Levels of phosphorylated Akt, an effector of phosphatidylinositol 3-kinase; Bcl-2; and Bcl-x were increased by nicotine administration. From these experimental data, our hypothesis for the mechanism of nicotinic receptor–mediated survival signal transduction is that the α7 nicotinic receptor stimulates the Src family, which activates phosphatidylinositol 3-kinase to phosphorylate Akt, which subsequently transmits the signal to upregulate Bcl-2 and Bcl-x. Upregulation of Bcl-2 and Bcl-x could prevent cells from neuronal death induced by β-amyloid and glutamate. These findings suggest that an early diagnosis of Alzheimer’s disease and protective therapy with nicotinic receptor stimulation could delay the progress of Alzheimer’s disease. Biol Psychiatry 2001; 49:233–239 © 2001 Society of Biological Psychiatry

Key Words: Alzheimer’s disease, nicotine, nicotinic receptor, β-amyloid, glutamate, protection

Introduction

A cetylcholine (ACh) is one of the major neurotransmitters in the central nervous system (CNS). The cerebral cortex contains a dense plexus of cholinergic axon terminals that arise from the cells of the basal forebrain including the nucleus basalis of Meynert (Bigl et al 1982; Mesulam et al 1983). Degeneration of this cholinergic projection is recognized as one of the most prominent pathologic changes in Alzheimer’s disease (AD) (Rosser et al 1982; Whitehouse et al 1981).

Administration of nicotine to animals and normal humans facilitates attention and memory-related tasks (Levin 1992; Wesnes and Warburton 1983) and improves perceptual and visual attention deficits (Jones et al 1992) as well as semantic memory performance in AD patients (Parks et al 1996). Elderly people are more sensitive than young adults to nicotinic ACh receptor blockade by nicotinic antagonists (Newhouse et al 1994). Therefore, attenuation of nicotinic ACh receptor function in cortical and hippocampal regions may contribute to memory impairment in AD. In the brain, nicotinic receptors show additional complexity, as there are multiple receptor subtypes with differing properties and functions (Clarke et al 1985; Lindstrom et al 1995). At least six α subunits (α2–α7, α9 in mammals; α8 in chicks) and three β subunits (β2–β4) have been identified in the brain. Both α and β subunits are required to form functional receptors in Xenopus oocyte expression systems, with the exception of α7 subunits, which apparently form functional homo-oligomeric receptors.

In this review we present evidence for nicotinic receptor–mediated protection against glutamate- and β-amyloid (Aβ)–induced neurotoxicity, based mainly on our studies.
adjacent cells, resulting in the appropriate physiologic response and/or glutamate-related cell death (Choi et al 1989; Dawson et al 1991; Hartley and Choi 1989). However, there has been only limited information concerning the cholinergic interaction with glutamate neurotoxicity. Mattson (1989) and Olney et al (1991) have demonstrated that stimulation of the muscarinic receptor potentiates neurodegeneration.

We examined the effects of nicotine on glutamate-induced neurotoxicity using primary cultures of rat cortical neurons. Cell viability was decreased by treatment with 1 mmol/L glutamate for 10 min followed by incubation in glutamate-free medium for 1 hour. Incubating the cultures with 10 μmol/L nicotine for 24 hours before glutamate exposure significantly reduced glutamate cytotoxicity. To investigate whether nicotine-induced neuroprotection is due to a specific effect mediated by nicotinic receptors, the effects of cholinergic antagonists were examined. Addition of dihydro-β-erythroidine (DHβE), an α4β2 nicotinic receptor antagonist, or α-bungarotoxin (α-BTX), an α7 selective nicotinic receptor antagonist, to the medium containing nicotine reduced the protective effect of nicotine.

We also examined the protection of nicotine against the effects of ionomycin, a calcium ionophore, and SNOC, an NO-generating agent. Incubating the cultures for 10 min in either 3 μmol/L ionomycin- or 300 μmol/L SNOC-containing medium markedly reduced cell viability. A 24-hour pretreatment with nicotine significantly attenuated the ionomycin cytotoxicity but did not affect the SNOC cytotoxicity (Akaike et al 1994; Kaneko et al 1997; Shimohama et al 1996, 1998).

**Nicotinic Receptor-Mediated Protection against β-Amyloid Toxicity**

Alzheimer’s disease is characterized by the presence of two types of abnormal deposits, senile plaques (SPs) and neurofibrillary tangles, and by extensive neuronal loss (Giannakopoulos et al 1996). β-Amyloid is a major element of SPs and one of the candidates for the cause of the neurodegeneration found in AD. It has been shown that the accumulation of Aβ precedes other pathologic changes and causes neurodegeneration or neuronal death in vivo (Yankner et al 1990). Several mutations of the Aβ precursor protein are found in familial AD, and these mutations are involved in amyloidogenesis (Citron et al 1992). Also, familial AD mutations of presenilin 1 (PS-1) enhance the generation of Aβ 1–42 (Tomita et al 1997).

We used the 25–35 fragment of the Aβ peptide because of the reported neurotoxic effects of this fragment (Yankner et al 1990). A 48-hour exposure to 20 μmol/L Aβ caused a significant reduction in the neuronal cells.

Simultaneous incubation of the cultures with nicotine and Aβ significantly reduced the Aβ-induced cytotoxicity. The protective effect of nicotine was reduced by both DHβE and α-BTX. The effect of a selective α4β2 nicotinic receptor agonist, cytisine, and a selective α7 nicotinic receptor agonist, 3-(2,4)-dimethoxybenzylidene anabaseine (DMXB) (Hunter et al 1994), on Aβ cytotoxicity was examined. β-Amyloid cytotoxicity was significantly reduced when 10 μmol/L cytisine or 1 μmol/L DMXB was coadministered. These findings suggest that both α4β2 and α7 nicotinic receptor stimulation are protective against Aβ cytotoxicity (Kihara et al 1997, 1998). In addition, MK801, an NMDA receptor antagonist, inhibited Aβ cytotoxicity when administrated simultaneously with Aβ, suggesting that Aβ cytotoxicity is mediated via the NMDA receptor or via glutamate in cultured cortical neurons (Figure 1), although Aβ can kill many types of cells without NMDA receptors (Gridley et al 1997; McLaurin et al 1999).

**Nicotinic Receptor-Mediated Protection against β-Amyloid-Enhanced Glutamate Toxicity**

Although it is thought that PS-1 mutations enhance the generation of Aβ 1–42, it is controversial whether Aβ is directly toxic to neurons. We found that Aβ 25–35 is toxic and that this neurotoxicity is inhibited by MK801. It can therefore be hypothesized that Aβ might modulate or enhance glutamate-induced cytotoxicity. Indeed, Aβ causes a reduction in glutamate uptake in cultured astrocytes (Harris et al 1996), indicating that, to some extent,
Aβ-induced cytotoxicity might be mediated via glutamate cytotoxicity.

In a current study (Kihara et al 2000), the 1–40 and 1–42 fragments of Aβ were used because they are fragments found in the brains of AD patients. Incubation of the cortical neurons with both Aβ 1–40 (1 nmol/L) and Aβ 1–42 (100 pmol/L) for 7 days did not induce cell death. These are the concentrations of Aβ in the cerebrospinal fluid of AD patients (Jensen et al 1999). Although 20 μmol/L glutamate alone did not significantly induce cell death, exposure to 20 μmol/L glutamate for 24 hours caused a significant reduction in the neuronal cells in the Aβ-treated group, showing that Aβ itself is not toxic at low concentrations, but makes neurons vulnerable to glutamate. Conversely, coincubation of the cultures with nicotine (50 μmol/L for 7 days) and Aβ significantly reduced Aβ-enhanced glutamate cytotoxicity. We have already shown that nicotine protects neurons from glutamate-induced cytotoxicity (Akaike et al 1994; Kaneko et al 1997; Shimohama et al 1996) and believe that the protective effect of nicotine against Aβ-enhanced glutamate cytotoxicity is mediated by its effect on glutamate toxicity.

Involvement of the Phosphatidylinositol 3-Kinase (PI3K) Cascade in Nicotinic Receptor-Mediated Neuroprotection

To investigate the mechanism of the protective effect of nicotine, we focused on the PI3K cascade because PI3K is involved in the mechanism of the protective effect. A nonreceptor tyrosine kinase inhibitor, PP2, did reduce the protective effect of nicotine, suggesting that Src is involved in the mechanism of the protective effect. Cycloheximide also inhibited the protection, implying that some protein synthesis is necessary for this effect.

Akt is a serine/threonine protein kinase and a putative effector of PI3K, for when PI3K is activated, it phosphorylates Akt. To investigate the activation of Akt by nicotine through PI3K, we examined the level of phosphorylated Akt using an antiphospho-specific Akt antibody.

The phosphorylated form of Akt appeared just after the application of nicotine. Nicotine-induced Akt phosphorylation was blocked by simultaneous application of LY294002, but not of PD98059, indicating that PI3K and not MAPK is involved. The Akt phosphorylation is blocked by α-BTX, but not by DHβE, implying that nicotine-induced Akt phosphorylation is mediated by α7 but not by α4β2 nicotinic receptors. PP2 also blocked Akt phosphorylation, which suggests involvement of tyrosine kinase. The level of total Akt protein that was detected with anti-Akt antibody remained unchanged.

Bcl-2 and Bcl-x proteins are antiapoptotic proteins that can prevent cell death induced by a variety of toxic attacks (Zhong et al 1993). It has been reported that Akt activation leads to the overexpression of Bcl-2 (Matsuzaki et al 1999). Because nicotine can activate Akt via PI3K, we examined the protein levels of Bcl-2 and Bcl-x. We found that treatment with nicotine for 24 hours increased the levels of Bcl-2 and Bcl-x, and this was inhibited by LY294002, which indicates involvement of the PI3K cascade in nicotine-induced Bcl-2 and Bcl-x upregulation (Figure 3).

These results suggest that nicotinic receptor stimulation protects neurons from glutamate-induced cytotoxicity by
activating PI3K, which in turn activates Akt and upregulates Bcl-2 and Bcl-x. Although nicotinic receptors are ionotropic, we considered that nicotinic receptors may directly transmit signals to PI3K.

Discussion

In dementia diseases such as AD, the cholinergic system is affected and a reduction in the number of nicotinic receptors has been reported (Shimohama et al 1986; Whitehouse and Kalaria 1995). This, in conjunction with the memory-enhancing activity of nicotine and selective nicotinic receptor agonists such as the α7 nicotinic receptor agonist DMXB (Meyer et al 1997), suggests a significant role for nicotinic receptors in learning and memory. Therefore, it is generally recognized that the downregulation of nicotinic receptors is involved in the intellectual dysfunction in AD. In contrast, our studies showed that nicotinic receptor stimulation protected neurons from Aβ- and glutamate-induced neurotoxicity. This allowed us to hypothesize that nicotinic receptors are involved in a neuroprotective cascade (Akaike et al 1994; Kaneko et al 1997; Kihara et al 1997, 1998; Shimohama et al 1996). Other studies have demonstrated that nicotine, acting through nicotinic receptors, can enhance neuronal survival against a variety of neurotoxic attacks (Chen et al 1995; Marin et al 1994; Nanri et al 1998; Zamani et al 1997) and reduce disruption of ascending cholinergic and nigrostriatal dopaminergic pathways (Fuxe et al 1990; Janson et al 1989, 1991; Nanri et al 1997; Sjak-Shie and Meyer 1993; Socci and Arendash 1996). In addition, α7 nicotinic agonists prevent Aβ-induced toxicity as well as toxicity from nerve growth factor (NGF)-containing serum deprivation and ethanol-induced oxidative stress in PC12 cells or human SK-N-SH cells (Li et al 1999, 2000; Meyer et al 1998), and activation of α7 nicotinic receptor promoted survival of spinal cord motor neurons (Messi et al 1997). Furthermore, it has been reported that mutant mice lacking the β2 nicotinic receptor subunit are affected by neuronal cell death or neurodegeneration during aging (Zoli et al 1999).

Recently we also clarified that the PI3K–Akt cascade contributes to the neuroprotective effect of nicotine and that the Bcl-2 family is activated downstream of the PI3K–Akt cascade and works as an antineuronal death factor. It is thought that PI3K–Akt activation promotes cell survival and that upregulation of Bcl-2 is a major component of this cell survival mechanism (Eves et al 1998; Matsuzaki et al 1999). Nicotinic receptor stimulation transduces these survival signals in addition to its role as a neurotransmitter. From the experimental data, our hypothesis for the mechanism of nicotinic receptor–mediated survival signal transduction is as follows: α7 nicotinic receptors stimulate the Src family, which in turn activates PI3K. Phosphatidylinositol 3-kinase phosphorylates Akt, which causes upregulation of Bcl-2 and Bcl-x. We have shown that an inhibitor of Src tyrosine kinase reduces Akt phosphorylation. Therefore, nicotinic receptor stimulation might phosphorylate Akt via a signal through Src to PI3K. Phosphorylated Akt may in turn upregulate Bcl-2. Upregulation of Bcl-2 and Bcl-x could prevent cells from neuronal death induced by Aβ and glutamate (Figure 4). However, nicotine can prevent apoptosis in human lung cancer cells through the extracellular signal-regulated protein kinase/mitogen-activated protein kinase pathway (Heusch and Maneckjee 1998), and α7 nicotinic receptor–mediated neuroprotection against NGF-containing serum deprivation requires protein kinase C activation in PC12 cells (Li et al 1999).

Cholinotherapy is currently being applied with clinically symptomatic benefits in terms of acetylcholinester-
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Figure 4. Proposed hypothesis for the mechanism of nicotinic receptor-mediated survival signal transduction. α7 nicotinic receptor stimulation might phosphorylate Akt via a signal through Src to phosphatidylinositol (PI) 3-kinase. Phosphorylated Akt may in turn upregulate Bcl-2 and Bcl-x. Upregulation of Bcl-2 and Bcl-x could prevent cells from neuronal death induced by β-amyloid and glutamate. AChR, acetylcholine receptor; PKB, protein kinase B.

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