Current Topics

Functional Changes Induced by Long-Term Stimulation of Nicotinic Acetylcholine Receptors

Nicotinic Receptor-Mediated Neuroprotection in Neurodegenerative Disease Models

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Multiple lines of evidence, from molecular and cellular to epidemiological, have implicated nicotinic transmission in the pathology of Alzheimer’s disease (AD) and Parkinson’s disease (PD). This review article presents evidence for nicotinic acetylcholine receptor (nAChR)-mediated protection and the signal transduction involved in this mechanism. The data is based mainly on our studies using rat-cultured primary neurons. Nicotine-induced protection was blocked by an α7 nAChR antagonist, a phosphatidylinositol 3-kinase (PI3K) inhibitor, and an Src inhibitor. Levels of phosphorylated Akt, an effector of PI3K, Bel-2 and Bel-x were increased by nicotine administration. From these experimental data, our hypothesis for the mechanism of nAChR-mediated survival signal transduction is that the α7 nAChR stimulates the Src family, which activates PI3K to phosphorylate Akt, which subsequently transmits the signal to up-regulate Bel-2 and Bel-x. Up-regulation of Bel-2 and Bel-x could prevent cells from neuronal death induced by β-amyloid (Aβ), glutamate and rotenone. These findings suggest that protective therapy with nAChR stimulation could delay the progress of neurodegenerative diseases such as AD and PD.

Key words nicotine; nicotinic acetylcholine receptor; neuroprotection; Alzheimer’s disease; Parkinson’s disease; amyotrophic lateral sclerosis

1. INTRODUCTION

Acetylcholine (ACh) is one of the major neurotransmitters in the central nervous system (CNS). ACh binds to nicotinic ACh receptors (nAChRs) and muscarinic ACh receptors (mAChRs). In the brain, nAChRs show additional complexity, as there are multiple receptor subtypes with differing properties and functions. 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.

Loss of neuronal nAChRs is increasingly associated with a number of disease states including Alzheimer’s disease (AD), Parkinson’s disease (PD), Lewy body disease (LBD), schizophrenia, autism, and attention deficit/hyperactivity disorder (ADHD). There is evidence that neuronal nAChRs are involved in neuronal survival and neuroprotection as well as in synaptic plasticity. Moreover, presynaptic nAChRs can modulate the release of many neurotransmitters, including dopamine, noradrenaline, serotonin, ACh, y-aminobutyric acid (GABA), and glutamate. These neurotransmitter systems play an important role in cognitive and non-cognitive functions such as learning, memory, attention, locomotion, motivation, reward, reinforcement, and anxiety. Thus, nAChRs are hopeful therapeutic targets for new treatments of these neurodegenerative disorders.

This review presents evidence for nAChR-mediated neuroprotection, based mainly on our studies.

2. NICOTINIC RECEPTOR-MEDIATED PROTECTION AGAINST GLUTAMATE CYTOTOXICITY

It is assumed that glutamate plays an important role in the neurodegeneration observed in hypoxic-ischemic brain injury. Several investigators have also suggested that cortical neurodegeneration in AD is attributable to glutamate. Moreover, brief glutamate exposure induces delayed cell death in cultured neurons from certain brain regions such as the cerebral cortex and hippocampus. In these brain regions, the N-methyl-D-aspartate (NMDA) glutamate receptor subtype plays a crucial role in glutamate neurotoxicity. Recent evidence has indicated the existence of nitric oxide (NO) synthase in the CNS, including the cerebral cortex. NMDA receptor stimulation induces Ca2+ influx into cells through ligand-gated ion channels, thereby triggering NO formation. NO is also thought to diffuse to the adjacent cells, resulting in the appropriate physiological response and/or glutamate-related cell death. However, there has been only limited information concerning the cholinergic interaction with glutamate neurotoxicity. Mattson and Olney et al. have demonstrated that stimulation of the muscarinic receptor potentiates neurodegeneration.

We first examined the effects of nicotine on glutamate-induced neurotoxicity using primary cultures of rat cortical neurons. Cell viability was decreased by treatment with 1 mM glutamate for 10 min followed by incubation in glutamate-free medium for 1 h. Incubating the cultures with 10 μM nicotine for 24 h prior to 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 antago-
nists 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 S-nitrosocysteine (SNOC), an NO-generating agent. Incubating the cultures for 10 min in either 3 μM ionomycin- or 300 μM SNOC-containing medium markedly reduced cell viability. A 24 h pretreatment with nicotine significantly attenuated the ionomycin cytotoxicity, but did not affect the SNOC cytotoxicity19–22) (Fig. 1).

3. NICOTINIC RECEPTOR-MEDIATED PROTECTION AGAINST β-AMYLOID TOXICITY

AD is characterized by the presence of two types of abnormal deposits, senile plaques (SP) and neurofibrillary tangles (NFT), and by extensive neuronal loss (Giannakopoulos et al. 1996). β-Amyloid (Aβ) is a major element of SP 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 pathological changes and causes neurodegeneration or neuronal death in vivo.23)

We used the 25–35 fragment of the Aβ peptide because of the reported neurotoxic effects of this fragment.23) A 48 h exposure to 20 μM 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)30) on Aβ cytotoxicity was examined. Aβ cytotoxicity was significantly reduced when 10 μM cytisine or 1 μM DMXB was coadministered. These findings suggest that both α4β2 and α7 nicotinic receptor stimulation is protective against Aβ toxicity.25,26) In addition, MK-801, 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 (Fig. 1), although Aβ can kill many types of cells without NMDA receptors.25,28)

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,29) indicating that, to some extent, Aβ-induced cytotoxicity might be mediated via glutamate cytotoxicity.

In the next study, 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 nM) and Aβ 1–42 (100 pM) for 7 d did not induce cell death. These are the concentrations of Aβ in the cerebrospinal fluid (CSF) of AD patients.29) Although 20 μM glutamate alone did not significantly induce cell death, exposure to 20 μM glutamate for 24 h 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 μM for 7 d) and Aβ significantly reduced Aβ-enhanced glutamate cytotoxicity. We considered that the protective effect of nicotine against Aβ-enhanced glutamate cytotoxicity is mediated by its effect on glutamate toxicity.

4. 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 phosphatidylinositol 3-kinase (PI3K) cascade because PI3K has been shown to protect cells from apoptosis.30)

Long exposure to low concentrations of glutamate (50 μM for 24 h) induced cytotoxicity. Incubating the cultures with nicotine (10 μM for 24 h) prior to glutamate exposure significantly reduced glutamate cytotoxicity. Simultaneous application of LY294002, a PI3K inhibitor, with nicotine reduced the protective effect of nicotine. α-BTX, an α7 selective nicotinic receptor antagonist, blocked the protection provided both by nicotine and by DMXB, an α7 selective nicotinic receptor agonist. Furthermore, this DMXB-induced protection was also reduced by LY294002. Although α4β2 nicotine receptor stimulation also had a protective effect on Aβ- and glutamate-induced cytotoxicity, this effect was not inhibited by LY294002. PD98059, a mitogen-activated protein (MAP) kinase kinase (MAPKK, also known as MEK1) inhibitor, did not reduce the protective effect of nicotine, suggesting that the MAP kinase cascade is not directly involved in the protective effect of nicotine.

A non-receptor 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 which 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. It has been reported that Akt activation leads to the overexpression of Bcl-2. 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 h 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. These results suggest that nAChR stimulation protects neurons from glutamate-induced cytotoxicity by activating PI3K, which in turn activates Akt and upregulates Bcl-2 and Bcl-x.

5. GALANTAMINE MODULATES nAChR AND BLOCKS α7-ENHANCED GLUTAMATE TOXICITY

Galantamine is a plant alkaloid that is used in the treatment of AD. We have studied the effects of galantamine on α7-enhanced glutamate toxicity using primary rat cultured cortical neurons. Nicotine and galantamine alone, and in combination, protected neurons against this neurotoxicity. The protection was not blocked by α4β2 nAChR antagonists, but was partially blocked by α7 nAChR antagonists. Galantamine induced phosphorylation of Akt, an effector of PI3K, while PI3K inhibitors blocked the protective effect and Akt phosphorylation. The antibody FK1, which selectively blocks while PI3K inhibitors blocked the protective effect and Akt phosphorylation induced by galantamine. Our data suggest that neuroprotection by galantamine is mediated, at least in part, by α7 nAChR-PI3K cascade.

6. STIMULATION OF nAChRs PROTECTS SPINAL MOTOR NEURONS

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by the rapidly progressive degeneration of motor neurons resulting in paralysis and, within a few years, death. In some families of familial ALS (FALS), mutations of the superoxide dismutase 1 (SOD1) gene have been demonstrated. However, the cause of sporadic ALS is still unknown and with the exception of the recently introduced glutamate-release inhibitor, riluzole, no effective treatments have been identified. Therefore, it is important to develop a treatment for ALS. Although the pathogenesis of sporadic ALS is unclear, several clinical studies suggest the role of glutamate-induced excitotoxicity.

Our previous study demonstrated that rat spinal cord cultures exposed to long-term (24 h) low-dose glutamate exhibit selective motor neuronal death and proposed this paradigm as an in vitro model for ALS. There is a study which demonstrates an early decrease in cholinergic input on motor neurons in the spinal cords of patients with ALS. Therefore, we investigated the neuroprotective effect of nicotine and galantamine, an AChE-I with allosteric nAChR-potentiating properties, against spinal motor neuronal death induced by glutamate using dissociated cultures of fetal rat spinal cord. The study demonstrated that administration of nicotine prevented glutamate-induced motor neuronal death in primary cultures of the rat spinal cord. The nicotine-induced neuroprotection was inhibited by either dihydro-β-erythroidin (DHβE) or α-bungarotoxin (αBT), suggesting that it is mediated through both α4β2 and α7 nicotinic acetylcholine receptors (nAChRs). Both α4β2 and α7 nAChRs were identified on rat spinal motor neurons by immunohistochemical methods. We also demonstrated that galantamine, an acetylcholinesterase inhibitor with allosteric nAChR-potentiating ligand properties, prevented glutamate-induced motor neuronal death. Aberration in the PI3-kinase-Akt signaling system has been reported in both ALS patients and ALS transgenic mice. Thus, it is possible that the PI3-kinase-Akt cascade is also involved in nAChR-mediated neuroprotection against glutamate-induced spinal motor neuronal death. These results suggest that stimulation of nAChR may be used as a treatment for ALS.

7. NICOTINIC RECEPTOR-MEDIATED NEUROPROTECTION IN ROTENONE-INDUCED PARKINSON’S DISEASE MODELS

Parkinson’s disease (PD) is the second most common progressive neurodegenerative disorder. It is characterized by relatively selective degeneration of dopaminergic neurons in the substantia nigra and loss of dopamine in the striatum resulting in resting tremor, rigidity, bradykinesia and postural instability. Although the pathogenesis of PD is still unclear, it is thought that both environmental and genetic factors cause neurodegeneration. Rural residency, pesticides and intrinsic toxic agents were reported as environmental risk factors for sporadic PD. The use of pesticides increases the risk of PD, possibly via reduced activity of complex I in the mitochondrial respiratory chain in the substantia nigra and result in the pathogenesis of PD. 6-Hydroxydopamine (6-OHDA), a H2O2 pro-oxidant and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a mitochondrial complex I inhibitor, have been widely used to produce toxin models of sporadic PD. Chronic exposure to rotenone, a nature-derived pesticide, could be a more appropriate animal PD model be-
cause rotenone-treated animals show slowly progressive dopamine (DA) neuronal loss, and Lewy body-like particles, which are primarily aggregations of \( \alpha \)-synuclein.46,47)

On the other hand, current drug therapy is limited to supplementing DA or enhancing dopaminergic effect. Some may have neuroprotective effects, but their effects remain controversial.48—50) It has also been reported that smokers have a lower risk for PD,51,52) and nAChRs were decreased in the brains of PD patients53) and PD model animals.54) Nicotine may upregulate DA release at striatum from nigral dopaminergic neurons,55) followed by stimulation of \( \alpha 4 \beta 2 \) nAChRs.56) Furthermore, nicotine could protect mitochondria and had protective effect from oxidative stress.57,58) In studies made in vivo, stimulation of nAChRs resulted in neuroprotection in cerebral ischemia and PD model animals.22,59,60) In this study, we investigated the neuroprotective effect of nicotine against nigral DA neuronal death induced by rotenone using a chronic rotenone-treated PD mouse model, and analyzed molecular mechanisms of the protection in dissociated cultures of the fetal rat ventral mesencephalon.

We observed that simultaneous subcutaneous administration of nicotine inhibited both motor deficits and DA neuronal cell loss in the substantia nigra of rotenone-treated mice. Next we analyzed the molecular mechanisms of DA neuroprotective effect of nicotine against rotenone-induced toxicity using primary DA neuronal culture. We found that DA neuroprotective effects of nicotine were inhibited by DH\( \beta E \), \( \epsilon 8 \)BuTx, and/or PI3K-Akt/protein kinase B (PKB) inhibitors, demonstrating that rotenone-toxicity on DA neurons are inhibited via activation of \( \alpha 4 \beta 2 \) or \( \alpha 7 \) nAChRs- PI3K-Akt/PKB pathways. These results suggest that nAChR stimulation can protect DA neurons against degeneration.61)

8. SYNERGISTIC EFFECT OF GALANTAMINE ON NICOTINE-INDUCED NEUROPROTECTION IN 6-OHDA-INDUCED HEMIPARKINSONIAN RAT MODEL

Galanitamine is an acetylcholinesterase inhibitor and an allosteric potentiating ligand for nAChRs. However, the effects of galantamine and nicotine on dopaminergic neurons remain unclear. This study evaluated the neuroprotective effects of galantamine and nicotine in a rat 6-hydroxydopamine (6-OHDA)-induced hemiparkinsonian model. 6-OHDA with or without galantamine and/or nicotine were injected into unilateral substantia nigra of rats. Although methamphetamine-stimulated rotational behavior and dopaminergic neuronal loss induced by 6-OHDA were not inhibited by galantamine alone, those were moderately inhibited by nicotine alone. In addition, 6-OHDA-induced neuronal loss and rotational behavior were synergistically inhibited by co-injection of galantamine and nicotine. These protective effects were abolished by mecamylamine, an nAChR antagonist. We further found that \( \alpha 7 \) nAChR was expressed on both tyrosine hydroxylase (TH)-immunopositive and TH-immunonegative neurons in the SNpc. A combination of galantamine and nicotine greatly suppressed 6-OHDA-induced reduction of TH-immunopositive/\( \alpha 7 \) nAChR-immunopositive neurons. These results suggest that galantamine synergistically enhances the neuroprotective effect of nicotine against 6-OHDA-induced dopaminergic neuronal loss through an allosteric modulation of \( \alpha 7 \) nAChR activation.62)

9. CONCLUSION

Our studies showed that nAChR stimulation protected neurons from \( \alpha \beta \) and glutamate-induced neurotoxicity and rotenone- and 6-OHDA-induced neurotoxicity. This allowed us to hypothesize that nicotinic receptors are involved in a neuroprotective cascade. 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. nAChR stimulation transduces these survival signals in addition to its role as a neurotransmitter. From the experimental data, our hypothesis for the mechanism of nAChR-mediated survival signal transduction is as follows: \( \alpha 7 \) nicotinic receptors stimulate the Src family, which in turn activates PI3K. PI3K 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. It is promising that protective therapy with nAChR stimulation could delay the progress of neurodegenerative diseases such as AD, PD and ALS.

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