Multiple lines of evidence, from molecular and cellular to epidemiological, have implicated nicotinic transmission in the pathogenesis of Alzheimer’s disease (AD). Here we show the signal transduction mechanism involved in nicotinic receptor-mediated protection against β-amyloid-enhanced glutamate neurotoxicity. Nicotine-induced protection was suppressed by an α7 nicotinic receptor antagonist (α-bungarotoxin), a phosphatidylinositol 3-kinase (PI3K) inhibitor (LY294002 and wortmannin), and a Src inhibitor (PP2). Levels of phosphorylated Akt, an effector of PI3K, and Bcl-2 were increased by nicotine. The α7 nicotinic receptor was physically associated with the PI3K p85 subunit and Fyn. These findings indicate that the α7 nicotinic receptor transduces signals to PI3K in a cascade, which ultimately contributes to a neuroprotective effect. This might form the basis of a new treatment for AD.

Alzheimer’s disease (AD) is one of the common diseases presenting dementia. There are no definitive treatments or prophylactic agents for this neurodegenerative disease. AD is characterized by the presence of two types of abnormal deposit, senile plaques and neurofibrillary tangles, and by extensive neuronal loss (1). β-Amyloid (Aβ) is a major constituent of senile plaques and one of the candidates for the cause of the neurodegeneration found in AD, because a negative correlation was found between senile plaques and neuron density (1). It has been hypothesized that accumulation of Aβ precedes other pathological changes and causes neurodegeneration or neuronal death in vivo (2). Several mutations of the Aβ precursor protein are found in familial AD, and these mutations are involved in amyloidogenesis (3). It has also been shown that familial AD mutations of presenilin 1 enhance the generation of Aβ 1–42 (4). However, presenilin 1 transgenic mice do not have amyloid plaques in their brains, possibly because presenilin 1 mutations facilitate apoptotic neuronal death without plaque formation (5). In addition, it is controversial whether Aβ is directly toxic to neurons or not.

We have found that Aβ 25–35 is toxic to neurons and that this cytotoxicity is inhibited by MK801, an N-methyl-D-aspartate receptor antagonist. Therefore, we hypothesized that Aβ might modulate or enhance glutamate-induced cytotoxicity. Glutamate, one of the excitotoxic neurotransmitters in the CNS, can cause intracellular Ca2+ influx, activation of Ca2+-dependent enzymes such as nitric oxide (NO) synthase, and production of toxic oxygen radicals leading to cell death (6). In addition, some reports have shown that Aβ causes a reduction in glutamate uptake in cultured astrocytes (7), indicating that Aβ-induced cytotoxicity might be mediated via glutamate cytoxicity to some extent.

In our previous reports, we showed that nicotinic acetylcholine receptor agonists exert a protective effect against glutamate- and Aβ-induced neurotoxicity (8–12). Recently, it has been reported that activated phosphatidylinositol 3-kinase (PI3K) and Akt kinase promote neuron survival (13). Anti-apoptotic proteins such as Bcl-2, Bcl-x, and Bad were thought to be involved in this survival system. Nicotinic receptors are ionotropic receptors, which allow Ca2+ to enter cells and function physiologically. It has been shown that the PI3K cascade is activated by tyrosine kinase or G protein-mediated signals in neuronal cells (14). Conversely, there is no evidence that nicotinic receptors contain a G protein or tyrosine kinase. However, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors are also ionotropic receptors, and it was recently shown that a member of the Src family, Lyn, is physically associated with AMPA receptors and mediates signals to PI3K (15). Thus, there is a possibility that ionotropic receptors such as nicotinic receptors could be associated with a tyrosine kinase such as Src.

In the present study, we showed that, at physiological concentrations, Aβ itself is not neurotoxic but enhances the cell death induced by glutamate. The neuroprotective effect of nicotine was examined, focusing on the involvement of the PI3K cascade. In addition, we investigated whether nicotinic receptors function as metabotropic receptors through any kinase families.

EXPERIMENTAL PROCEDURES

Materials—The sources of drugs and materials used in this study were as follows: Eagle’s minimum essential medium (EMEM) (Nissui Pharmaceutical Co.); β-amyloid protein fragments 1–40, 1–42, 40–1, 42–1, 1–16, 12–28 and amyloid P component (27–38) (Bachem); (-)-nicotine, MK801, and monoclonal anti-α7 nicotinic acetylcholine antibody (Research Biochemicals International); α-bungarotoxin (Wako); LY294002 (Biomol Research Laboratories, Inc.); PP2 (Calbiochem); PD98059, anti-phospho- and nonphospho-specific p44/p42 mitogen-acti

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† To whom correspondence should be addressed: Dept. of Neurology, Graduate School of Medicine, Kyoto University, Kyoto 606-8507, Japan. Tel.: 81-75-751-3767; Fax: 81-75-751-9541; E-mail: i53367@sakura.kudpc.kyoto-u.ac.jp.

‡ From the Department of Pharmacology, Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto 606-8501, Japan.

§ The abbreviations used are: AD, Alzheimer’s disease; Aβ, β-amyloid; NO, nitric oxide; PI3K, phosphatidylinositol 3-kinase; AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; EMEM, Eagle’s minimum essential medium; MAP2, microtubule-associated protein 2; αBTX, α-bungarotoxin; DMXB, 3-(2,4)-dimethoxybenzylidene anabaseine; MAPK, mitogen-activated protein kinase; IP, immunoprecipitate(s).

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tivated protein (MAP) kinase and Akt antibodies (New England Biolabs); anti-microtubule-associated protein 2 (MAP2) antibody (Sigma); polyclonal anti-Fyn antibody (Upstate Biotechnology Inc.); polyclonal anti-PI3K p85 subunit antibody and anti-α4 nicotinic acetylcholine antibody (Santa Cruz); monoclonal anti-PI3K p85 subunit antibody and anti-Bcl-2 antibody (Transduction Laboratories).

Cell Cultures—Primary cultures were obtained from the cerebral cortex of fetal rats (17–19 days gestation) by procedures described previously (11, 16). Briefly, single cells dissociated from the cerebral cortex of fetal rats were plated out onto plastic coverslips placed in Falcon dishes. Cultures were incubated in EMEM supplemented with 10% fetal calf serum (1 to 5 days after plating out) or 10% horse serum (6 to 12 days after plating out), glutamine (2 mM), glucose (total 11 mM), NaHCO₃ (24 mM), and HEPES (10 mM). Cultures were maintained at 37 °C in a humidified atmosphere of 5% CO₂. Only mature cultures (10 to 14 days in vitro) were used for the experiments. The animals were treated in accordance with the guidelines published in the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Treatment of the Cultures—All experiments were carried out in EMEM at 37 °C. Cultured neurons were exposed to glutamate for 24 h followed by incubation with EMEM for a further 24 h. Aβ and nicotine were added to the medium before exposure to glutamate. Antagonists were simultaneously added with nicotine. When cells were exposed to glutamate, other drugs were removed.

Assessment of Neurotoxicity—The number of neurons was evaluated by immunostaining with anti-MAP2 antibody. Neurotoxicity in each experiment was defined as a reduction in the survival rate, which was expressed as percentage survival relative to the survival observed in control cultures. Immunostaining was performed by the methods described previously (17), using the primary antibody (anti-MAP2 antibody (diluted 1:500)) for 24 h. At least 200 neurons were counted in 10 to 20 randomly selected fields at 100 (total magnification) in control cultures to determine the total number of neurons.

Preparation of Cell Extracts—Semi confluent cultures were exposed to each treatment and incubated at 37 °C for various time intervals. Subsequently, cells were lysed in a buffer consisting of 20 mM Tris/HCl, pH 7.0, 2 mM EGTA, 25 mM 2-glycerophosphate, 1% Triton X-100, 2 mM dithiothreitol, 1 mM vanadate, 1 mM phenylmethylsulfonyl fluoride, and 1% aprotinin and centrifuged at 15,000 × g for 30 min at 4 °C. The supernatants were used as the cell extracts.

Immunoblotting and Immunoprecipitation—Protein samples in sodium dodecyl sulfate (SDS) buffer were loaded onto SDS-polyacrylamide gels. After electrophoresis, proteins were electrotransferred to a polyvinylidene difluoride membrane (Immobilon, Millipore). Membranes were incubated with horseradish peroxidase-conjugated anti-rabbit antibody. Immunoreactive bands were detected on the membrane by chemiluminescence with luminol and a laser image analyzer (LumiGLO, New England Biolabs, Inc., Beverly, MA). Protein samples were immunoprecipitated with antibodies (described above) and then incubated with protein G-Sepharose as described previously (18).

Statistics—Data are expressed as the percentage of surviving neurons relative to the number of neurons in control cultures (vehicle only) and represent the mean ± S.E. Statistical significance was determined using one-way analysis of variance followed by Scheffe’s multiple comparisons test.

RESULTS

Pretreatment of Neurons with Aβ Enhances Glutamate Cytotoxicity—Cortical neurons were incubated with Aβ1–40 (1 nm) or Aβ1–42 (100 pm) for 7 days, which did not induce cell death. These are the concentrations of Aβ in the cerebrospinal fluid of AD patients (19). Treatment with a low dose of glutamate (20 μM) alone did not significantly induce cell death (Fig. 1a). Simultaneous exposure to the same dose of Aβ1–40 and 1–42 followed by glutamate caused a significant reduction in the number of neuronal cells (Fig. 1c). These findings suggested that Aβ itself is not neurotoxic at physiological concentrations but makes neurons vulnerable to excitatory amino acids.

We investigated whether the effect on glutamate cytotoxicity depends upon the Aβ structure. Scrambled Aβ, Aβ40–1, or 42–1 did not enhance glutamate cytotoxicity, even when incubated simultaneously with other peptides including 1–40 or 1–42 (Fig. 2a). None of the peptides caused neuronal death when administered alone.

In addition, other fragments of Aβ, such as Aβ1–16 (10 nm), 12–28 (10 nm), and the amyloid P component (27–38) (10 nm), did not significantly reduce the number of viable neurons after exposure to glutamate (Fig. 2b). None of the peptides caused neuronal death when administered alone (data not shown). Although Aβ1–16 enhanced glutamate cytotoxicity, this was not statistically significant.

Nicotine Protects Neurons from Aβ-enhanced Glutamate Cytotoxicity—We used the 1–40 and 1–42 fragments of Aβ, because these fragments are found in the brains of AD patients and are the most potent combination for the enhancement of glutamate cytotoxicity. Cortical neurons were incubated with both Aβ1–40 (1 nm) and Aβ1–42 (100 pm) for 7 days, which enhanced glutamate cytotoxicity. This effect was inhibited by MK801 when incubated with glutamate, indicating that the toxicity was mediated via N-methyl-D-aspartate receptors (Fig. 3). Pretreatment of the cultures with nicotine (0.5 μM, 7 days) and Aβ significantly reduced Aβ-enhanced glutamate cytotoxicity (Fig. 3). Previously, we reported that nicotine protects neurons from glutamate-induced cytotoxicity (8–10). Therefore, we hypothesized that the protective effect of nicotine against Aβ-enhanced glutamate cytotoxicity depends upon its effect on glutamate toxicity.


**PI3K Contributes to the Protective Effect of Nicotine against Glutamate-induced Cytotoxicity**—To investigate the mechanism of the protective effect of nicotine, we focused on the PI3K cascade because PI3K has been reported to protect cells from apoptosis through Akt activation (13). The glutamate toxicity model was adopted because a low concentration of Aβ alone was not toxic or only enhanced glutamate toxicity. Furthermore, we showed that nicotine did not directly affect the Aβ conformation as described previously (20).

Prolonged exposure to glutamate (50 μM, 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 either LY294002 (10 μM) or wortmannin (100 nM), both PI3K inhibitors, with nicotine reduced the protective effect of nicotine against glutamate cytotoxicity (13). Conversely, the α7-selective nicotinic receptor agonist, α7-Bungarotoxin (αBTX, 1 nM), an α7-selective nicotinic receptor antagonist, also blocked the protection, indicating that the α7 subtype of nicotinic receptors is involved in this effect (Figs. 4a and 5).

**3-(2,4)-Dimethoxybenzylidene anabaseine (DMXB, 10 μM), an α7-selective nicotinic receptor agonist (21), also exerted a protective effect against glutamate-induced cytotoxicity. This effect was inhibited by 1 nM αBTX, indicating that the effect of DMXB is mediated via α7 receptors. Furthermore, the protection was also reduced by 10 μM LY294002 (Fig. 4b). From these findings, we concluded that α7 nicotinic receptor stimulation exerts a neuroprotective effect against glutamate cytotoxicity and that the PI3K cascade is involved in this effect. We previously showed that α4β2 subunit nicotinic receptor stimulation also exerted a protective effect against Aβ- and glutamate-induced cytotoxicity (10, 12). This effect, however, was not inhibited by LY294002 (data not shown).

Furthermore, a non-receptor tyrosine kinase inhibitor, PP2 (10 μM), also reduced the protective effect. This implies that Src is involved in the mechanism of protection. Cycloheximide (1 mg/ml) inhibited the protection, implying that some protein synthesis is necessary. In contrast, PD98059 (50 μM), a mitogen-activated protein kinase (MAPK) kinase (MAPKKK), also known as MEK1) inhibitor, did not reduce the protective effect of nicotine (Figs. 4a and 5).

Nicotine Activates Akt through PI3K Activation and Up-regulates Bcl-2—Akt is a serine/threonine protein kinase and a putative effector of PI3K. When PI3K is activated, it phosphorylates Akt. To investigate the activation of Akt by nicotine through PI3K, we examined the level of phosphorylated Akt detected by an antibody specific for phospho-Akt using Western blotting. In preliminary experiments, phosphorylation of Akt was detected just after the application of nicotine. The levels of phosphorylated Akt increased and reached a plateau after around 60-min stimulation and were maintained for 24 h (data not shown). Therefore, 60-min stimulation was adopted for the following experiments.

Nicotine-induced Akt phosphorylation was blocked by the simultaneous application of LY294002, showing the involvement of PI3K (Fig. 6a). PD98059 did not alter the phosphorylation effect of nicotine. Akt phosphorylation was blocked by αBTX, indicating that the phosphorylating effect of nicotine is mediated by α7 nicotinic receptors (Fig. 6, b and c). Conversely, dihydro-β-erythroidine (100 nM), a selective α4β2 nicotinic receptor antagonist, did not block nicotine-induced Akt phosphorylation (Fig. 6c). PP2 blocked Akt phosphorylation, indicating the involvement of tyrosine kinase. 10 μM MK801 did not block Akt phosphorylation, showing that the secondary release of glutamate has no effect (Fig. 6b).

Bcl-2 proteins are anti-apoptotic proteins, which can prevent cell death induced by a variety of toxic insults (22). It has been reported that Akt activation leads to the overexpression of Bcl-2 (23). Because nicotine could activate Akt via PI3K, we examined the protein levels of Bcl-2. Treatment with nicotine
for 24 h induced the augmented level of Bcl-2, and the nicotine-induced up-regulation of Bcl-2 was reduced by LY294002, indicating the involvement of PI3K signal transduction (Fig. 6a).

\[ \text{DISCUSSION} \]

There is still a controversy about the role of Aβ and glutamate in the pathogenesis of AD. However, amyloid accumulation is one of the earliest changes in AD pathology, and this peptide may cause neuronal death in the central nervous system (2). The precise mechanism of Aβ-induced cytotoxicity remains unknown, although various hypotheses have been suggested. Oxidative stress, or free radical generation, is one of the candidates for the cause of Aβ-induced cytotoxicity. Previous reports have shown that Aβ stimulates NO production through Ca\(^{2+}\) entry triggered by activated N-methyl-D-aspartate-gated channels (24). Other reports have suggested that Aβ inhibits glutamate uptake and causes extracellular glutamate increase (7). There are also some reports that have proposed that Aβ enhances the toxicity induced by excitotoxins (25, 26). These reports indicated that Aβ-induced cytotoxicity might be, at least in part, mediated via glutamate toxicity. The present study also indicated that Aβ enhances glutamate neurotoxicity.

The present data show that only a combination of Aβ 1–40 and Aβ 1–42 enhanced glutamate cytotoxicity. The concentra-

**Fig. 5.** Immunostained images showing the protective effect of nicotine against glutamate neurotoxicity. A, control; B, 50 μM glutamate alone; C, nicotine (10 μM) + glutamate; D, nicotine + LY294002 (10 μM) + glutamate; E, nicotine + αBTX (1 μM) + glutamate; F, nicotine + PD98059 (50 μM) + glutamate. Bar = 1 × 10^{-2} m

**FIG. 4.** a, effect of nicotine on glutamate-induced cytotoxicity. Cultures were exposed to glutamate (50 μM) for 24 h followed by incubation in EMEM for 24 h, which induced cell death. Nicotine (1 μM) was preincubated with the cultures for 24 h. If used, LY294002 (LY), wortmannin (Wort), α-bungarotoxin (BTX), PP2, cycloheximide (Cyclo), and PD98059 (PD) were added to the medium containing nicotine. After the preincubation, medium was replaced by glutamate-containing medium for 24 h and finally replaced with EMEM, as described above. LY294002, wortmannin, and PI3K inhibitors all significantly reduced the protective effect of nicotine. Furthermore, PP2, a Src family tyrosine kinase inhibitor, also reduced the protective effect of nicotine. *p < 0.05 compared with glutamate alone; **, p < 0.01 compared with glutamate + nicotine. NS, not significant. b, effect of DMXB on glutamate-induced cytotoxicity. DMXB (10 μM) was added to the medium and incubated for 24 h. LY294002 significantly reduced the protective effect. *p < 0.05 compared with glutamate alone; **, p < 0.05 compared with glutamate + nicotine.

α7 Nicotinic Acetylcholine Receptors Physically Associate with PI3K and Fyn—The present results show that nicotine protects neurons from glutamate cytotoxicity by activating PI3K, which activates Akt and up-regulates Bcl-2. Nicotinic receptors are ionotropic, and there have been no reports of ionotropic receptors activating PI3K directly.

Therefore, we hypothesized that nicotinic receptors may act as metabotropic receptors, directly transmitting signals to PI3K. In other words, nicotinic receptors might associate with PI3K. To demonstrate this, lysates of cortical neurons were immunoprecipitated with a monoclonal anti-α7 nicotinic receptor antibody. Immunoprecipitated (IP) samples were then separated by SDS-polyacrylamide gel electrophoresis and stained with a polyclonal anti-PI3K p85 subunit antibody using the Western blot technique. The PI3K p85 subunit was detected in this IP sample, indicating that α7 nicotinic receptors bind to the PI3K p85 subunit (Fig. 7a). Conversely, lysate immunoprecipitated with the polyclonal anti PI3K p85 subunit antibody contained protein detected by the monoclonal anti-α7 nicotinic receptor antibody. Non-receptor type tyrosine kinase, Fyn, was also co-immunoprecipitated with the α7 nicotinic receptors (Fig. 7b). The α 4 subunit of the nicotinic receptor was also investigated and was not detected in lysates immunoprecipitated with the PI3K p85 subunit, Fyn, or the α7 nicotinic receptor antibody (Fig. 7c).
Fig. 6. Representative data of phosphorylation of Akt/PKB by nicotine detected by Western blot using antibody specific for phosphorylated Akt. a, nicotine (Nic, 10 μM) increased the levels of the phosphorylated Akt compared with the total Akt levels. This phosphorylation was significantly inhibited by LY294002 (10 μM), PD98059 (50 μM), a MEK1 inhibitor, did not interfere with Akt phosphorylation. Each inhibitor was added simultaneously with glutamate. PD, PD98059; LY, LY294002. n = 6. b, Akt phosphorylation was inhibited by αBTX (1 mM) and PP2 (10 μM), a Src inhibitor. MK801 (10 μM) did not reduce the phosphorylation of Akt induced by nicotine. BTX, αBTX, MK, MK801. n = 6. c, Akt phosphorylation was not inhibited by dihydro-β-erythroidine (100 nM) in contrast to αBTX. BTX, αBTX, n = 6. d, Bcl-2 was up-regulated after nicotine treatment. LY294002 (10 μM) inhibited the up-regulation of Bcl-2, indicating that nicotine-induced up-regulation of Bcl-2 is mediated via the PI3K cascade. n = 6.

Fig. 7. Association of the α7 nicotinic receptor with PI3K and Fyn. a, the PI3K was detected in IP produced using the anti-α7 nicotinic receptor antibody (left). Conversely, the α7 nicotinic receptor was detected in IP produced using the anti-p85 subunit PI3K antibody (right). n = 6. nACHR, nicotinic acetylcholine receptor. b, Fyn was detected in IP produced using the anti-α7 nicotinic receptor antibody. Conversely, α7 nicotinic receptors were detected in IP produced using the anti-Fyn antibody (right). n = 6. c, the α4 subunit of the nicotinic receptor was not detected in any of the IP samples produced using the anti-PI3K p85 subunit, Fyn, or the α7 nicotinic receptor antibody.

Discussion of the peptides used in this study were almost the same level as that detected in the cerebrospinal fluid of AD patients (19). Other fragments did not enhance the toxicity, even when administered simultaneously with Aβ1–40 or Aβ1–42. Therefore, the enhancing effect appears to be related to the structure of the peptides so that the combination of Aβ1–40 and Aβ1–42 might play a specific role in making neurons vulnerable to glutamate. The full length of both peptides appears to be necessary.

We previously showed that nicotinic receptor stimulation protects neurons from Aβ- and glutamate-induced cell death (8–12). In the present study, we showed that nicotinic receptor stimulation, especially α7 receptor stimulation, inhibits glutamate toxicity and that PI3K-Akt signal transduction contributes to this effect. In addition, the Bcl-2 family is stimulated downstream of the PI3K-Akt cascade and works as an anti-neuronal death factor. It is proposed that PI3K-Akt activation promotes cell survival, and up-regulation of Bcl-2 is one of the major reasons for cell survival (23, 27). Nicotinic receptor stimulation transduces these survival signals besides its role as a transmitter. The β sheet conformation of Aβ might influence its function, such as toxicity or modulation of survival signals. However, in our experiments, nicotine and nicotinic agonists did not influence the β sheet conformation (20). Instead, signal transduction was shown to be important for the protective effect of nicotine.

Our hypothesis for the survival signal transduction is shown in Fig. 8. It is not clear from our experiments whether other Src family members besides Fyn are associated with α7 receptors. However, a relationship between nicotinic receptors and Fyn was implicated because catecholamine release induced by nicotine is dependent upon the presence of Fyn and extracellular Ca2+, and no other Src member was detected (28). In our preliminary data, removal of extracellular Ca2+ suppressed Akt phosphorylation induced by nicotine (data not shown). We showed that an inhibitor of Src tyrosine kinase reduced Akt phosphorylation. In addition, PI3K and Fyn are physically associated with α7 nicotinic receptors. Therefore, nicotinic receptor stimulation might phosphorylate Akt by signal transduction through Fyn to PI3K, and extracellular Ca2+ might contribute to this process.

In the brain, nicotinic receptors include several subtypes with differing properties and functions. The abundant presence of α7 receptors in the hippocampus, neocortex, and basal ganglia (29), in conjunction with the memory-enhancing activity of selective α7 nicotinic agonists such as DMXB (30), suggests a significant role for α7 receptors in learning and memory. In addition, the protective action of nicotine is mediated, at least partially, through α7 receptors. Recently it was reported that Aβ1–42 binds to α7 receptors (31), and this might inhibit α7 nicotinic receptor-dependent learning and memory. The reduction of α7 receptor activation might cause neurons vulnerable
to various toxic insults such as glutamate. In our study, however, the lysate immunoprecipitated with anti-α7 antibody did not contain Aβ 1–42 (data not shown). This might be because the antibody we used was different from that used in the report (31), but we could not prove that enhancement of glutamate toxicity depends upon the reduction of α7 nicotinic receptors. In addition, Aβ 12–28, which suppresses the formation of the α7-Aβ complex (31), did not inhibit the enhanced glutamate toxicity induced by the combined exposure to of Aβ 1–40 and Aβ 1–42 (Fig. 2b). Therefore, it is unlikely that the protective effect of nicotine depends upon the displacement of α7-Aβ binding.

Recently, it was shown that ionotropic receptors have properties similar to metabotropic receptors. AMPA receptors are physically associated with a member of the Src family, Lyn (15). The AMPA receptor activates Lyn, which then activates MAPK. Through the Lyn-MAPK pathway, AMPA receptors generate intracellular signals and transmit them from the cell surface to the nucleus. Nicotinic receptors are known to be ionotropic receptors. The present study indicated that nicotinic receptors also have metabotropic properties, which contribute to neuronal survival. It is likely, however, that many unrecognized receptor functions still remain.

The cholinergic system is affected in dementia-causing diseases, AD among others, and a reduction in the number of nicotinic receptors in these diseases has been reported (32, 33). It is of interest that down-regulation of nicotinic receptors can result in neuronal cell death or neurodegeneration (34). Nicotine might function not only as a cholinergic agonist but also as a neuroprotective agent. Our present study suggests that nicotinic receptor stimulation could protect neurons from Aβ-enhanced glutamate toxicity. Thus, by an early diagnosis of AD and protective therapy with nicotinic receptor stimulation, we could delay the progress of AD.

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