Arch Pharm Res Vol 23, No 5, 488-494, 2000

Subacute Nicotine Exposure in Cultured Cerebellar Cells Increased the Release and Uptake of Glutamate

Dong Koo Lim*, Sun Hee Park, and Woo Jeoung Choi

College of Pharmacy and Institute for Drug Development, Chonnam National University, Kwangju 500-757, Korea

(Received May 20, 2000)

Cerebellar granule and glial cells prepared from 7 day-old rat pups were used to investigate the effects of sub-acute nicotine exposure on the glutamatergic nervous system. These cells were exposed to nicotine in various concentrations for 2 to 10 days *in situ*. Nicotine-exposure did not result in any changes in cerebellar granule and glial cell viability at concentrations of up to 500 μM. In cerebellar granule cells, the basal extracellular levels of glutamate, aspartate and glycine were enhanced in the nicotine-exposed granule cells. In addition, the responses of N-methyl-D-aspartate (NMDA)-induced glutamate release were enhanced at low NMDA concentrations in the nicotine-exposed granule cells. However, this decreased at higher NMDA concentrations. The glutaminase activity was increased after nicotine exposure. In cerebellar glial cells, glutamate uptake in the nicotine-exposed glial cells were either increased at low nicotine exposure levels or decreased at higher levels. The inhibition of glutamate uptake by L-trans-pyrollidine-2,4-dicarboxylic acid (PDC) was lower in glial cells exposed to 50 μM nicotine. Glutamine synthetase activity was lower in glial cells exposed to 100 or 500 μM of nicotine. These results indicate that the properties of cerebellar granule and glial cells may alter after subacute nicotine exposure. Furthermore, they suggest that nicotine exposure during development may modulate glutamatergic nervous activity.

Key words: Nicotine, Glutamate, Glutaminase, Glutamate transporters, Glutamine synthetase

INTRODUCTION

Nicotine is well known as a psychostimulant drug with both a reinforcing and dependence-producing action in animals as well as in humans. Nicotine enhances the ion flux and release of neurotransmitters and elicits a variety of physiological and behavioral effects, such as locomotor activity, cerebrovasodilatation, convulsions and antinociception (Martin, 1986). Although nicotine has many effects on CNS functions, its action is primarily focussed on the catecholamine and acetylcholine producing neurons (Fung *et al.*, 1995; Li *et al.*, 1995; Zhang *et al.*, 1994). It has been reported that nicotine elicits the release of glutamate from a synaptosomal preparation (Perez De La Mora *et al.*, 1991) and that the nicotine-induced release of dopamine may be the result of a nicotine-induced release of glutamate (Garcia-Munoz *et al.*, 1996). Recently, it has been reported that nicotine selectively enhances NMDA receptor-mediated synaptic transmission (Aramakis and Metherate, 1998). However, apoptosis of the hippocampal progenitor cells has been reported to be induced by nicotine (Berger *et al.*, 1998). Furthermore, nicotine can affect cognitive abilities and protect against the toxicity of glutamate-induced cellular damage (Akaike *et al.*, 1994; Borlongan *et al.*, 1995; Gattu *et al.*, 1997; Birtwistle and Hall, 1997). Although the evidence supports the fact that nicotine may affect the glutamatergic nervous system, there is a paucity of reports showing nicotine-induced changes in the characteriza-tion of glutamate neurons.

It is generally known that glutamate is the major rapid excitatory neurotransmitter of the mammalian brain, and has various important roles in neurotoxicity and neuronal plasticity (Collingridge and Lester, 1989; Meldrum and Garthwaite, 1990). Moreover, the released glutamate level in the synapse is maintained through the uptake of glutamate in the astrocytes, especially the glial cells, which contain the glutamate/glutamine cycle (Nicholls and Attwell, 1990). Therefore the changes in the glutamate uptake properties may affect the glutamatergic nervous activity. However, detailed studies on the nicotine-induced changes in astrocytes have not been reported. To explore the

Correspondence to: Dong Koo Lim, Ph.D., College of Pharmacy Chonnam National University 300 Yongbong-dong, Bukgu, Kwangju 500-757 Korea
E-mail: dklim@chonnam.chonnam.ac.kr

mechanism of nicotine action, it is necessary to determine the changes in both glutamate release and uptake in addition to the related enzyme activity in the neurons and glial cells after nicotine exposure.

Therefore the present study was designed to determine whether sub-acute exposure of nicotine affects the activities of the glutamatergic nervous system in cultured cerebellar granule and glial cells. Since cerebellar granule cells in the rat brain express nicotinic acetylcholine receptors and contain glutamate as their primary neurotransmitter (Didier *et al.*, 1995), this model is expected to be appropriate to investigate the interactions of nicotine with the glutamatergic nervous system.

MATERIALS AND METHODS

Animals and materials

Male and female Spraque-Dawley rats weighing 200-250 g were housed at room temperature (22-24°C) and a 12-h light / 12-h dark cycle with free access to food and water. N-methyl-D-aspartate (NMDA) and L-trans-pyrollidine-2,4-dicarboxylic acid (PDC) were purchased from Research Biochemical Inc. (Natick, MA, USA) and the [^{14}C]L-glutamate (specific activity, 261.6 mCi/mmol) was purchased from New England Nuclear (Boston, MA, USA). The ion exchange chromatography supports, AG1-X8 (acetate form) were purchased from BIO-RAD (Hercules, CA, USA). Both the fetal bovine serum (FBS) and bovine calf serum (BCS) were purchased from Hyclone (Logan, Utah, U.S.A). All other chemicals were obtained from the Sigma Chemical Co. (St. Louis, MO, USA).

Cerbellar granule and glial cell cultures

The cells were prepared by a minor modification of the method reported by McCaslin and Morgan (1987). Seven day-old rat pups were decapitated and the head partially sterilized by dipping them in 100 % ethanol. The cerebella were excised and placed in a culture medium lacking serum and bicarbonate. The cells were then mechanically dissociated. The growth medium used (5 ml/60 mm dish) was Dulbecco's modified Eagle's medium (DMEM) supplemented with 40 mM $NaHCO_3$, 0.15 mM $CaCl_2$, 66 μM $MgSO_4$, 0.44 mM KCl, 6% FBS and 6% BCS. After 2 days of stabilization, the growth medium was aspirated from the cultures with new growth medium being added. In preparing the cerebellar granule cells, new growth medium containing 35 mM $NaHCO_3$, 0.36 mM $CaCl_2$, 0.17 mM $MgSO_4$, 25 mM KCl, 6% FBS and 6% BCS was added with 5 μM cytosine arabinoside in order to prevent the proliferation of non-neuronal cells. For cerebellar glial cell preparation, the previous growth medium was used again.

Determination of cellular viability

The viable cells were quantified by the method reported by Mosmann (1983). Mitochondrial enzymes in normal cells have the capacity to transform 3-(4,5-dimethyl-thioazol-2-yl)-2,5-diphenyl tetrazolium (MTT) salt into water-insoluble MTT formazan. At 10 DIV, the growth medium was separated by filtration and washed with 0.1 M phosphate buffered saline (pH 7.6). The MTT salt was dissolved in serum-free DMEM at a concentration of 0.5 mg/ml and incubated with the cells at 37°C for 4 hr. The MTT formazan produced was dissolved in 0.04N HCl in isopropanol and scanned at 570 nm with an ELISA reader.

Determinations of glutamate and other amino acids release in granule cells

Cerebellar granule cells grown for 10 days after plating were used. At the end of growing period, the growth medium was aspirated and the cells were placed in a physiological saline hepes (PSH) buffer containing following chemicals; 5 mM hepes, 135 mM NaCl, 3.6 mM KCl, 2.5 mM $CaCl_2$, 10 mM glucose and 44 mM $NaHCO_3$ (pH 7.4). After washing with the PSH buffer for 1 hr, the cells were incubated with/without various concentrations of NMDA at 37°C for 1 hr after which, the buffer was subsequently collected and analyzed. The levels of glutamate and other amino acids were determined by the method used by Schmid *et al.* (1980). The buffer collected from the cultures was treated with an o-phtaldialdehyde derivatizing agent according to the method reported by Shoup *et al.* (1984). Fifty microliters were injected into a high performance liquid chromatography-electrochemical detector (HPLC-ECD). Separation was achieved using a C18 reverse type column (Rainin instrument 15 cm in length) and a 0.1 M sodium phosphate buffer (pH 5.4) containing 37% methanol, with a flow rate 1 ml/min, was used as the mobile phase. The glutamate and other amino acid concentrations were determined by a direct comparison of sample peak heights to those of an external standard containing.

Determinations of glutamate uptake in glial cells

Cerebellar glial cells grown for either 10 or 15 days after plating were used. At the end of growing period, the growth medium was removed. After washing with PSH buffer for 1 hr, the cells were incubated in the presence of 20 μM of glutamate and/or various concentrations of PDC at 37°C for 30 min. The buffer was then collected and the amount of glutamate remaining in the buffer was analyzed by HPLC-ECD as described previously.

Determinations of the activity of glutaminase in granule cells

The glutaminase activity was determined by a minor modification of the method reported by Miulli *et al.* (1993).

Cerebellar granule cells grown for 10 days after plating were mechanically scraped and suspended in deionized water. 0.25 ml of the enzyme samples was incubated with 0.25 ml of 40 mM L-glutamate at 37°C for 20 min. The reaction was quenched by the addition of 0.5 ml 15% trichloroacetic acid and centrifuged at 12,000 rpm for 5 min. The supernatant was diluted with deionized water and 0.2 ml of Nessler's reagent was then added. The glutaminase activity was determined at 400 nm using an UV spectrophotometer. An ammonium acetate solution was used as a standard.

Determination of glutamine synthetase (GS) activity in glial cells

The activity of GS was determined by a minor modification of the method reported by Caldani *et al.* (1982). Cerebellar glial cells, grown for 10 day after plating, were mechanically scraped with a 10 mM imidazole-HCl buffer (pH 6.8) including EDTA, which were then sonnicated. The enzyme samples were incubated with assay buffer at 37°C for 20 min. The buffer was composed of 10 mM [^{14}C]L-glutamate (0.8 mCi/mmol), 15 mM $MgCl_2$, 4 mM NH_4Cl, 1 mM 2-mercaptoethanol, 50 mM imidazole-HCl, 1 mM ouabain and 10 mM ATP. The reaction was quenched by the addition of 1 ml of ice-cold deionized water and then loaded immediately onto a column (Dowex AG1-X8, acetate form). The column was washed with 5 ml of ice-cold deionized water and the eluate was mixed with scintillation solution. The radioactivity was determined by a liquid scintillation spectrophotometer.

Determination of protein concentration

The protein concentration of the cultured cells was determined by the method reported by Lowry *et al.* (1951) using bovine serum albumin as a standard.

Statistics

The Student's t-tests was used to test for statistical significance where two groups were being compared. Analyses of the variance were used to the test statistical significance in four groups. When significant effects were observed, the Newman-Keuls' multiple range test was applied for the degree of significance.

RESULTS

No changes in cell viability were observed when cultured cerebellar granule cells were exposed to nicotine concentrations of 500 μM from 2 to 10 days (data not shown).

Table I shows the levels of extracellular glutamate and other amino acids from sub-acute nicotine-exposed cerebellar granule cells. The basal release of glutamate was increased in nicotine-exposed granule cells, which was observed in granule cells that were exposed to 100 μM nicotine. Furthermore, the aspartate and glycine concentrations were also higher. However, the high basal releases observed in this case were induced upon exposure to 500 μM of nicotine. Changes in the NMDA-induced glutamate release in sub-acute nicotine-exposed cerebellar granule cells are shown in Fig. 1. NMDA increased gluta-

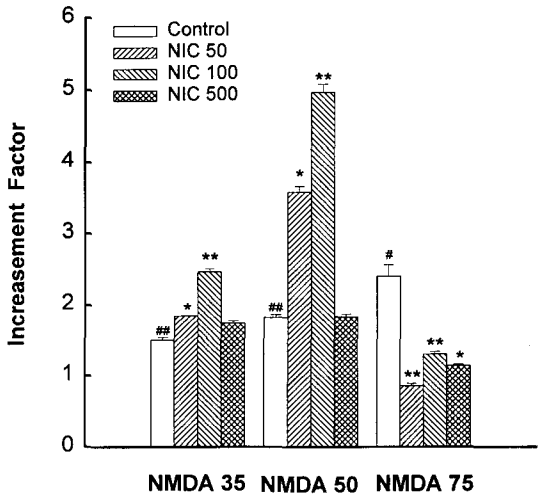

Fig. 1. Effects of subacute nicotine exposure on NMDA-induced glutamate release in cerebellar granule cells. The cultured cells were exposed to various concentrations of nicotine from 2 to 10 days. The designated concentrations of NMDA were exposed for 1 hr at 10 days in culture. Values are mean ± S.E.M. of 4 or 5 different determinations. $^{\#}p<0.05$, $^{\#\#}p<0.01$ significantly different when compared to respective basal group. $^{*}p<0.05$, $^{**}p<0.01$ significantly different when compared to respective control group.

Table I. Effects of subacute nicotine exposure on the extracellular levels of glutamate and other amino acids in cerebellar granule cells

Culture conditions	Glu	Asp	Gly
Control	0.153 ± 0.018	0.284 ± 0.020	0.517 ± 0.026
NIC 50 μM	0.399 ± 0.042**	0.307 ± 0.015	1.302 ± 0.070**
NIC 100 μM	0.463 ± 0.045**	0.363 ± 0.015*	1.278 ± 0.159*
NIC 500 μM	0.314 ± 0.030**	0.508 ± 0.054*	1.405 ± 0.083**

The designated concentrations of nicotine were exposed from 2 to 10 days in culture. Values are mean ± S.E.M. of 4 or 5 different determinations. Units are in μM. *p<0.05 , **p<0.01 significantly different when compared to control group.

Table II. Effects of subacute nicotine exposure on the glutaminase activity in cerebellar granule cells

Culture conditions	Control	Treated
Nic 50 μM	4.34 ± 0.38	5.78 ± 0.19*
Nic 100 μM	3.44 ± 0.26	4.85 ± 0.22**
Nic 500 μM	3.94 ± 0.12	5.48 ± 0.56*

The cultured cells were exposed to designated concentrations of nicotine from 2 to 10 days. Values are mean ± S.E.M. of 4 or 6 different determinations. Units are in mole/min/mg protein. *p<0.05, **p<0.01 sifnificantly different when compared to respective control group.

mate release with increasing NMDA dosage. The NMDA-induced glutamate release was significantly higher in cerebellar granule cells that were exposed to 50 or 100 μM of nicotine than that observed in the control. However, the granule cells exposed to 500 μM of nicotine did not respond to NMDA and further glutamate release was reduced by stimulation with 75 μM of NMDA in all nicotine-treated granule cells. The changes observed in the glutaminase activity of cerebellar granule cells after sub-acute nicotine exposure are shown in Table II. There was a 33-41% increase in granule cell glutaminase activity after sub-acute exposure to various nicotine concentrations.

No significant changes in cell viability were observed when cultured cerebellar glial cells were exposed to nicotine (500 μM) for 2 to 10 days (data not shown). The glutamate uptake in cerebellar glial cells after sub-acute nicotine exposure is shown in Fig. 2. When the cultured cells were exposed to nicotine either from 2 to 10 days or from 10 to 15 days, the glutamate uptake was significantly higher in the glial cells that were sub-acutely treated with 50 μM of nicotine. In addition, there was a marginal increase observed after treatment with 100 μM nicotine. However, glutamate uptake was significantly lower in glial cells sub-acutely treated with 500 μM nicotine. The changes observed in PDC-induced inhibition of glutamate uptake in cerebellar glial cells after sub-acute nicotine exposure are shown in Fig. 3. Glutamate uptake with 50 μM PDC addition were significantly less inhibited in the glial cells sub-acutely exposed to 50 μM of nicotine, but were unchanged after sub-acute exposure to higher nicotine concentrations. Changes in the GS activity of cerebellar glial cells after sub-acute nicotine exposure are shown in Fig. 4. The GS activities were significantly lower in glial cells sub-acutely exposed to 100 or 500 μM of nicotine.

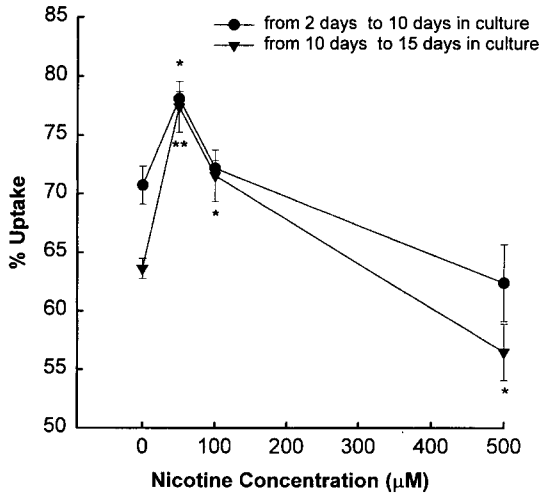

Fig. 2. Effects of subacute nicotine exposure on glutamate uptake in cerebellar glial cells. The cultured cells were exposed to various concentrations of nicotine from 2 to 10 days or from 10 to 15 days. The 20 μM of glutamate was exposed for 30 min at either 10 or 15 days in culture. Values are mean ± S.E.M. of 4 or 5 different determinations. *p<0.05, **p< 0.01 significantly different when compared to respective control group.

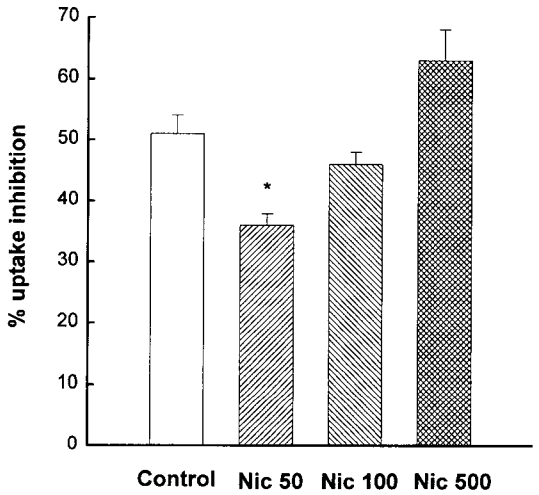

Fig. 3. Effects of subacute nicotine exposure on PDC-induced inhibition of glutamate uptake in cerebellar glial cells. The cultured cells were exposed to designated concentrations of nicotine from 2 to 10 days. The 20 μM of glutamate and 50 M of PDC was exposed for 30 min at 10 days in culture. Values are mean ± S.E.M. of 4 or 5 different determinations. *p<0.05 significantly different when compared to control group.

DISCUSSION

The present results demonstrate that the activity of the central glutamatergic nervous system is changed after sub-acute nicotine exposure to the developing cells.

Different effects that nicotine has on the cell viability are reported. While nicotine reduces cell viability in the matured cortical and hippocampal progenitor cells, it does not affect the viability of the differentiated hippocampal cells (Akaike *et al.*, 1994; Berger *et al.*, 1998). The present results indicate that nicotine exposure during the development of the cerebellar cells does not affect their viability. It has been reported that the cytotoxic

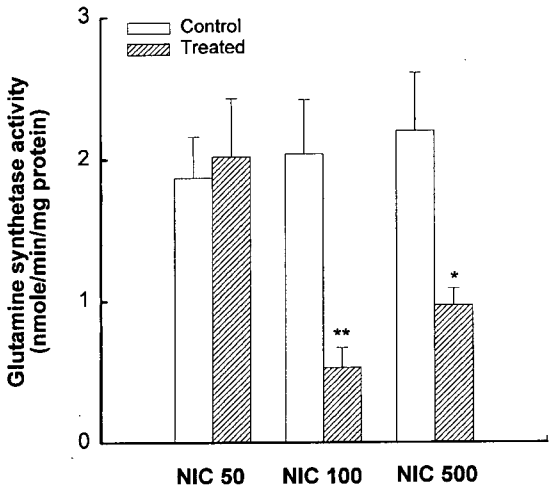

Fig. 4. Effects of subacute nicotine exposure on GS activity in cerebellar glial cells. The cultured cells were exposed to designated concentrations of nicotine from 2 to 10 days. Values are mean ± S.E.M. of 4 or 6 different determinations. *$p<0.05$, **$p<0.01$ significantly different when compared to respective control group.

effect of nicotine on cells depends upon the extracelluar calcium levels and the buffering ability of cells (Berger *et al.*, 1998). Thus, it implies that the buffering ability of cerebellar cells to calcium may increase during growth and cells are as a result resistant to nicotine cytotoxicity.

It has been reported that nicotine increases the extracellular levels of amino acids in certain rat brain regions (Perez De La Mora *et al.*, 1991; Toth *et al.*, 1993; Vidal, 1997). Consistent with these reports, the present results suggest that the extracellular glutamate, aspartate and glycine levels are higher after sub-acute nicotine exposure. Since nicotine induces the influx of sodium and calcium through the nicotinic acetylcholine receptors, the increases in glutamate release may be due to higher intracellular calcium levels during nicotine exposure. It has been reported that depending on its concentration, nicotine facilitates or suppresses the NMDA-induced peak current (Akaike *et al.*, 1991). The present results indicate that the responses to NMDA in the cerebellar granule cells are different depending on the nicotine concentrations. In addition, NMDA-induced glutamate release in nicotine-exposed granule cells is enhanced at low concentrations, but reduced at high concentrations. Thus the present results suggest that nicotine exposure during the growth of cells may induce changes in neuron characteristics. It has been reported that nicotine increases the intracelluar free calcium levels (Kim and Pae, 1996). It is well known that changes in the free calcium levels in cells affect various biochemical processes. Although the changes in the granule cells may be due to calcium influx during nicotine exposure, the exact mechanism is unknown. It has been reported that the presence of NMDA causes a strong down-regulation of calcium uptake through the NMDA receptor channel (Oster and Schramm, 1993; Resink *et al.*, 1996). Thus, the decreases in NMDA response may be attributable to receptor desensitization by sustained stimulation with high NMDA concentrations and released glutamate during nicotine exposure. It has been reported that chronic NMDA or KCl stimulation of the cerebellar granule cells induces glutaminase activation (Alavez *et al.*, 1997). Thus the increase in glutaminase activity in nicotine-treated cerebellar granule cells suggests that nicotine may stimulate the NMDA receptor resulting in the activation of enzymes involved in glutamate synthesis.

It has been well known that glutamate transporters in the astrocytes keep the extracellular glutamate concentration at low levels (Fairman *et al*, 1995; Mennerick and Zorumski, 1994). This is important because excessive stimulation of the NMDA receptor can lead to neuronal injury. It has been reported that the glutamate transporter exists in two neurotransmitter-bound states (Grunewald and Kanner, 1995). In addition, the two types of glutamate transporter exist in the glial cells with a different affinity to PDC (Kondo *et al.*, 1995). The present results indicate that the glutamate uptake is higher in the glial cells exposed to low nicotine concentrations. Since glutamate uptake was significantly lower after acute nicotine exposure (data not shown), the higher uptake of glutamate observed may reflect cellular adaptation to sub-acute nicotine exposure. Although there are no explanations for the decrease in glutamate uptake after sub-acute exposure to high nicotine concentrations, it may be due to the destruction of glutamate transporter function in the astrocytes. Furthermore, the changes in PDC-induced inhibition of glutamate uptake in nicotine-exposed cerebellar glial cells suggest the possibility that nicotine can induce either conformational changes in the glutamate transporters or preferential development of the PDC-insensitive glutamate transporters. It has been reported that the activities of both the glutamate transporter and the GS are coupled for neurotransmitter clearance (Derouiche and Rauen, 1995). However, increases in glutamate transporter activity but not in GS activity were observed after exposure to low nicotine concentrations. Since both glutamate uptake and GS activity in the astrocytes are influenced by direct contact with neuronal cells, co-cultures are needed to further investigate the delicate changes in astrocyte glutamatergic activity.

In summary, nicotine exposure during development affects the properties of both neurons and glial cells. Moreover, the alterations observed in the glutamatergic nervous system after nicotine exposure may either have beneficial or detrimental effects in both cell behaviors and viability depending on the degree of the exogenous stimuli.

ACKNOWLEDGEMENTS

This work was supported by the Brain Korea 21 Project.

REFERENCES

Akaike, N., Harata, N. and Tateishi, N., Modulatory action of cholinergic drugs on NMDA response in dissociated rat nucleus basalis of Meynert neurons. *Neurosci. Lett.*, 130, 243-247 (1991).

Akaike, A., Tamura, Y., Yokota, T., Shimohama, S. and Kimura, J., Nicotine-induced protection of cultured cortical neurons against N-methyl-D-aspartate receptor-mediated glutamate cytotoxicity. *Brain Res.*, 644, 181-187 (1994).

Alavez, S., Gutierrez, L. and Moran J., Characterization of the activation of glutaminase induced by N-methyl-D-aspartate and potassium in cerebellar granule cells. *J. Neurosci. Res.*, 45, 637-646 (1997).

Aramakis, V. B. and Metherate, R., Nicotine selectively enhances NMDA receptor-mediated synaptic transmission during postnatal development in sensory neocortex. *J. Neurosci.*, 18, 8485-8494 (1998).

Berger, F., Gage, F. and Vijayaraghavan, S., Nicotinic receptor-induced apoptotic cell death of hippocampal progentor cells. *J. Neurosci.*, 18, 6871-6881 (1998).

Birtwistle, J. and Hall, K., Does nicotine have beneficial effects in the treatment of certain diseases? *Br. J. Nurs.*, 5, 1195-1202 (1997).

Borlongan, C. V., Shytle, R. D., Ross, S. D., Shimizu, T., Freeman, T. B., Cahill, D. W. and Sanberg, P. R., (-)-Nicotine protects against systemic kainic acid-induced excitotoxic effects. *Exp. Neurology*, 136, 261-265 (1995).

Caldani, M., Rolland, B., Fages, C. and Tardy, M., Glutamine synthetase activity during mouse brain development. *Experientia*, 38, 1199-1202 (1982).

Collingridge, G. L. and Lester, R. A. J., Excitatory amino acid receptors in the vertebrate central nervous system. *Pharmacol. Rev.*, 41, 143-210 (1989).

Derouiche, A. and Rauen, T., Coincidence of L-glutamate/L-aspartate transporter (GLAST) and glutamine synthetase (GS) immunoreactions in retinal glia: evidence for coupling of GLAST and GS in transmitter clearence. *J. Neurosci. Res.*, 42, 131-143 (1995).

Didier, M., Berman S. A., Lindstrom, J. and Bursztajn, S., Characterization of nicotinic acetylcholine receptors expressed in primary cultures of cerebellar granule cells. *Mol. Brain Res.*, 30, 17-28 (1995).

Fairman, W. A., Vandenverg, R. J., Arriza, J. L., Kavanaugh, M. P. and Amara, S. G., An excitatory amino-acid transporter with properties of a ligand-gated chloride channel. *Nature*, 375, 599-603 (1995).

Fung, Y. K., Schmid, M. J., Anderson, T. M. and Lau, Y., Effects of nicotine withdrawal on central dopaminergic systems. *Pharmacol. Biochem. Behav.*, 53, 635-640 (1996).

Garcia-Munoz, M., Patino, P., Young, S. J. and Groves, P. M., Effects of nicotine on dopaminergic nigrostriatal axons requires stimulation of presynaptic glutamatergic receptors. *J. Pharmacol. Exp. Ther.*, 277, 1685-1693 (1996).

Gattu, M., Pauly, J. R., Boss, K. L., Summers, J. B. and Buccafusco J. J., Cognitive impairment in spontaneously hypertensive rats: role of central nicotinic receptors. I. *Brain Res.*, 771, 89-103 (1997).

Grunewald, M. and Kanner, B., Conformational changes monitored on the glutamate transporter GLT-1 indicate the existence of two neurotransmitter-bound states. *J. Biol. Chem.*, 270, 17017-17024 (1995).

Kim, W. K. and Pae, Y. S., Cellular mechanism of nicotine-mediated intracelluar calcium homeostasis in primary culture of mouse cerebellar granule cells. *Korean J. Pharmacol.*, 32, 13-21 (1996).

Kondo, K., Hashimoto, H., Kitanaka, J., Sawada, M., Suzumura, A., Marunouchi, T. and Baba, A., Expression of glutamate transporters in cultured glial cells. *Neurosci. Lett.*, 188, 140-142 (1995).

Lawrence, A. D. and Sahakian, B. J., Alzheimer disease, attention and the cholinergic system. *Alzheimer. Dis. Assoc. Disord.*, 9, 43-49 (1995).

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, 193, 265-275 (1951).

McCaslin, P. P. and Morgan, W. W., Cultured cerebellar cells as as in vitro model of excitatory amino acid receptor function. *Brain Res.*, 417, 380-384 (1987).

Meldrum, B. and Garthwaite, J., Excitatory amino acid neurotoxicity and neurodegenerative disease. *Trends in Pharmacol. Sci.*, 11, 379-387 (1990).

Mennerick, S. and Zorumski, C. F., Glial contributions to excitatory neurotransmission in cultured hippocampal cells. *Nature*, 368, 59-62 (1994).

Miulli, D. E., Norwell, D. Y. and Schwartz, F. N., Plasma concentrations of glutamate and its metabolites in patients with Alzheimer's disease. *J. Am. Osteopath. Assoc.*, 93, 670-676 (1993).

Mosmann, T., Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Meth.*, 65, 55-63 (1983).

Oster, Y. and Schramm, M., Down-regulation of NMDA receptor activity by NMDA. *Neurosci. Lett.*, 163, 85-88 (1993).

Nicholis, D. and Attwell, D., The release and uptake of excitatory amino acids. *Trends in Pharmacol. Sci.*, 11, 462-468 (1990).

Perez De La Mora, M., Mendez-Franco, J., Salceda, R., Aguirre, J. A. and Fuxe, K., Neurochemical effects of nicotine on glutamate and GABA mechanisms in the rat brain. *Acta. Physiol. Scand.*, 141, 241-250 (1991).

Resink, A., Villa, M., Benke, D., Hidaka, H., Mohler, H. and Balazs, R., Characterization of agonist-induced down-regulation of NMDA receptors in cerebellar granule cell cultures. *J. Neurochem.*, 66, 369-377 (1996).

Schmid, R., Hong, J. S., Meek, J. and Costa, E., The effects of kainic acid on the hippocampal content of putative neurotransmitter amino acids. *Brain Res.*, 200, 355-

362 (1980).
Shoup, R. E., Allison, L. A. and Mayer, G. S., O-phthalaldehyde derivatives of amines for high-speed liquid chromatography/electrochemistry. *Analytical Chem.*, 56, 1089-1096 (1984).
Toth, E., Vizi, E. S. and Lajtha, A., Effect of nicotine on levels of extracellular amino acids in regions of the rat brain in vivo. *Neuropharmacology*, 32, 827-832 (1993).
Vidal, C., Nicotinic receptors in the brain. Molecular biology, function and therapeutics. *Mol. Chem. Neuropathol.*, 28, 3-11 (1997).
Zhang, X., Gong, Z. and Nordberg A., Effects of chronic treatment with (+)- and (-)-nicotine on nicotinic acetylcholine receptors and N-methyl-D-aspartate receptors in rat brain. *Brain Res.*, 644, 32-39 (1994).