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Effects of Ginseng Total Saponin on The Altered Glutamatergic Nervous Systems by AF64A in Brain of Rats

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Abstract – To investigate effects of ginseng total saponin (GTS) on the ethylcholine aziridnium ion (AF64A) - induced glutamatergic nervous system, rats were pretreated with the infusion of AF64A (3 nmole) into lateral ventricle and were posttreated with 50 mg/kg of GTS, i.p., for 1 week. Twenty four hours after the last administration, rats were sacrificed and the levels of glutamate and taurine, [^{3}H]dizocilpine ([^{3}H]MK801) binding sites and glutamine synthetase activity were assessed in striatum, hippocampus and frontal cortex. The levels of striatal glutamate after GTS treatment in rats were decreased. And the levels of glutamate were decreased in striatum and frontal cortex and increased in hippocampus by the infusion of AF64A. However, the AF64A-induced changes of glutamate were returned to the control level by the administration of GTS in striatum, frontal cortex and hippocampus. After the infusion of AF64A, the level of taurine was decreased in striatum and increased in hippocampus. GTS administrations in the AF64A-treated rats restored to the control level of taurine in the decreased striatal level of taurine, but not in the elevated level of hippocampal taurine. The specific [^{3}H]MK801 binding sites in hippocampus was significantly decreased but not in striatum and frontal cortex after the administration of AF64A. Although GTS itself did not affect the specific [^{3}H]MK801 binding sites, GTS administrations in the AF64A-treated rats did decrease the binding sites of [^{3}H]MK801 in all examined regions. The activities of striatal glutamine synthetase were decreased after GTS treatment. The activities of striatal glutamine synthetase (GS) were decreased in AF64A-treated groups. However, the decreased striatal GS activities by AF64A were returned to the control level by GTS treatment. Furthermore, GTS administrations in the AF64A-treated rats increased the hippocampal GS activities. The results indicate that GTS may adjust the levels of glutamate and taurine constantly and may induce increase in AF64A-induced decrease of GS activity. Thus, it suggests that GTS may antagonize changes in central glutamatergic nervous system induced by AF64A. Also it suggests that the actions of GTS may differently affect in the disease state.

Keywords □ ginseng total saponin, AF64A, glutamate, taurine, [^{3}H]MK801 binding sites, glutamine synthetase.

The memory disturbance is the main symptom of dementia. Also, disturbances of the picture are dominant in the early symptoms. It has been reported that the number of cholinergic cell body in dementia was decreased (Bartus *et al.*, 1982; Mash *et al.*, 1985). In addition, it has been recently reported that impairments in glutamatergic nervous system might be involved in learning disturbance (Greenamyre and Maragos, 1993).

Ethylcholine aziridinium ion (AF64A) is a nitrogen mustard analog of choline which is selective, irreversible neurotoxin. The AF64A-treated animals have been reported to lose the memory (Chrobak *et al.*, 1988; Lim *et al.*, 1995; Nakahara *et al.*, 1988). Recently, Lim *et al.* (1995) reported that AF64A impaired the motivation as well as the latent memory in Morris water maze. Thus, this toxin has been proposed as a useful tool in the development of animal models of Alzheimer's disease and senile dementia of the Alzheimer type (Hanin *et al.*, 1982). In addition, it has been reported that the administration of AF64A altered the central glutamatergic nervous activities (Ma and Lim, 1996).

It is known that ginseng has various properties, such as tonic, stimulant and sedative properties. It has been reported that extracts of ginseng could increase growth of synapse induced by nerve growth factors and defend degeneration of neuron in frontal cortex (Himi *et al.*, 1989; Lee *et al.*, 1980). The administration of water extracts of ginseng had been reported to progress the spatial per-

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formance interference and learning impairment induced by scopolamine (Nitta *et al.*, 1995). Also, it has been known that chronic administration of extracts of ginseng could directly or indirectly affect various central nervous systems because it had both sided effects, that is, increase or decrease in learning, memory, behavioral mass and sound discrimination ability (Itoh *et al.*, 1989; Saito *et al.*, 1979). To examine the usefulness of ginseng components in protecting the memory impairment, it is necessary to invetigate their actions against the changed neuronal activities in the animal model.

Therefore, the present study is designed to investigate whether ginseng total saponin might have any effects against AF64A-induced changes in glutamatergic nervous activities in rats.

MATERIALS AND METHODS

Animals and materials

Male Sprague-Dawley rats weighing 250~300 g were housed at 21±1 ℃, 50~60 relative humunity on a 12 hour light/12 hour dark schedule. Animals were freely accessible on food and water. Ginseng total saponin (GTS) was supplied from Ginseng Institutes. Acetylcholine mustard-HCl was purchased from Research Biochemical Inc. (Wayland, MA). Ethylcholine aziridinium (AF64A) is synthesized according to the method of Mantione et al. (1983). [^{3}H]Dizocilpine ([^{3}H]MK801 (20.30 Ci/mmol)) was purchased from New England Nuclear (Boston, MA, U.S.A.). All other chemicals were obtained from Sigma Chemical Co. (St Louis, MO, U.S.A.).

Animal treatment

For the administraion of AF64A, rats were anethetized with Equitensin and mounted in a David Kopf stereotaxic apparatus. The skull was exposed and a guide cannula was implanted according to Paxino and Watson (1986) through the dural surface into the lateral ventricle with respect to bregma at the following coordinates: A-0. 8, L±1.4, V-4.4. Skull screws and dental cement were used for fixation of guide cannula. A stainless steel obturator was inserted into the guide cannula. Penicilline 30,000 I.U. was administered to protect from infection after surgery. Before the infusion of AF64A, the rats were allowed to recover from surgery for 4 days, housed singly in their cages. AF64A was infused in both ventricle with the rate of 0.5 μl/min (3 nmol/each side) at 4 days after the surgery. The control groups were infused with the artifitial cerebrospinal fluid (CSF).

Three days after the infusion of AF64A, GTS, 50 mg/kg, was administrated once a day for 1 week intraperitoneal (i.p.). The control groups were treated with saline. Twenty four hours after the last administration of GTS, rats were decapitated and the brains were rapidly removed. Various brain regions such as striatum, hippocampus and frontal cortex were dissected out according to the method of Glowinski *et al.* (1966). Since the glutamatergic nervous system in rat brain is rich in hippocampus, frontal cortex and striatum, those areas are chosen to examine.

Determination of the concentrations of glutamate and taurine

The concentraions of glutamate and taurine in each brain regions were determined according to Ellison *et al.* (1987). The dissected tissues were homogenized in cold methanol/water (50:50, v:v) using a polytron homogenizer in ice bath. The homogenates were centrifuged at 20,000×g for 20 min at 4 ℃ and the supernatant was collected, diluted with cold methanol/water (50:50, v:v) and then treated with derivatizing agent. Derivatizing agent was prepared according to Shoup *et al.* (1984). Fifty microliter was injected into HPLC-ECD system (Waters system). Separations of various amino acids were achieved using a C18 reverse type column (Rainin instrument 15 cm in length) and 0.1 M sodium phosphate buffer containing 37% methanol was used with mobile phase. The concentrations of glutamate and other amino acids were determined by direct comparison of sample peak heights to those of an external standard containing amino acids.

Determination of [^{3}H]MK801 binding sites

Membranes for NMDA receptor binding assays were prepared according to Foster and Wong (1987). The dissected tissues were homogenized in 15 volumes of ice-cold 0.32 M sucrose at low speed. The homogenates were centrifuged at 1,000×g for 10 min and the supernatant was centrifuged at 20,000×g for 20 min and then crude synaptozomal pellet was obtained. This crude synaptozomal pellet was resuspended in deionized water and dispersed with the polytron homogenizer for 30 sec. The suspension was centrifuged at 8,000×g for 20 min. The supernatants, including the buffy layer, were collected, resuspended in deionized water and centrifuged at 48,000×g for 20 min and then the pellet was suspended in 50 mM Tris-HCl buffer (pH 7.4) and stored at -70 ℃

until used. The frozen membrane preparation was thawed, suspended in 75 volumes of 5 mM Tris-HCl buffer (pH 7.4) and centrifuged 3 times at 48,000×g for 20 min at 4 ℃ and the final pellet was suspended in 5 mM Tris-HCl buffer (pH 7.4) containing 1 μM glycine and 30 μM glutamate.

Binding sites using [^{3}H]MK801 was determined according to Ebert *et al.* (1991). Aliquots (200 μl) of the membrane preparations (200-250 μg/tube) were incubated with [^{3}H]MK801 for 4 hours at 25 ℃. Non-specific binding was determined by adding 100 μM unlabled MK801. After incubation, the samples were filtered through Whatman GF/B fiber filters and washed twice using 5 ml ice-cold 5 mM Tris-HCl buffer (pH 7.4) and then transfered to vials containing scintillation solution and counted with liquid scintillation spectrophotometer.

Determination of glutamine synthetase (GS) activity

The activity of GS was determined following the method of Patel *et al.* with a minor modification (1982). The dissected tissues were washed in cold imidazole buffered saline containing 20 mM imidazole-HCl (pH 6.8), 5.4 mM KCl, 137 mM NaCl, 5.5 mM glucose and homogenized in 10 mM imidazole-HCl (pH 6.8) including ethylenediamine tetraacetic acid. Fifty microliter assay buffer composed of 50 mM imidazole-HCl (pH 6.8), 15 mM $MgCl_2$, 10 mM ATP, 10 mM L-[U-^{14}C] glutamate (0.8mCi/mmol), 4 mM NH_4Cl, 1 mM β-mercaptoethanol, 1 mM ouabain and homogenates were incubated for 30 min at 37 ℃. After incubation, the reaction was terminated by adding 1 ml of ice-cold water and then loaded immediately into a column (Dowex AG-1 X8, acetate form and Amberlite IRP-G9). Columns were washed with deionized water and the elutes were transfered to vials containing scintillation solution and counted with liquid scintillation spectrophotometer.

Determination of protein concentration

The protein contents of tissue homogenates were determined by the method of Lowry *et al.* (1951) using bovine serum albumine as a standard.

Statistics

The statistical significance of differences was determined using Student's t-tests.

RESULTS

Effects of the administration of GTS on the levels of glutamate and taurine

Changes in the levels of glutamate in striatum, hippocampus and frontal cortex after the administration of GTS in AF64A-treated group are shown in Fig. 1. After GTS treatment in the control group, the level of glutamate was significantly decreased in striatum (33.5%). Also, the concentration of glutamate after the infusion of AF64A was decreased in striatum and frontal cortex, 35.6% and 48.7%, respectively. But the level of glutamate in hippocampus was significantly increased after AF64A treatment. However, in group exposed to GTS for 7 days in AF64A-treated animals, the level of glutamate in three brain regions was not different with the control group. Especially, the AF64A-induced increases in the concentration of hippocampal glutamate were significantly decreased to normal level by the administration of GTS.

Changes in the levels of taurine in striatum, hippocampus and frontal cortex after the administration of GTS in AF64A-treated group are shown in Fig. 2. The administration of GTS did not affect the level of taurine. The level of taurine after infusion of AF64A was differently affected: significant decrease in striatum, increase in hippocampus and no change in frontal cortex. However, the AF64A-induced decreases in the level of strital taurine were returned to the control level after post-

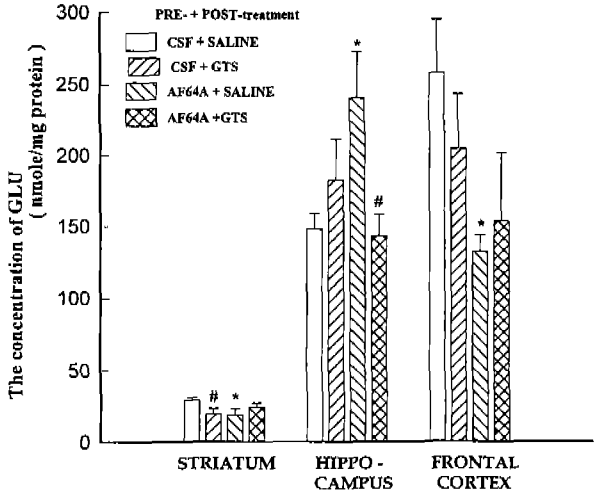

Fig. 1. Effects of the administration of ginseng total saponin on the levels of glutamate in AF64A-treated rats.

Rats were pretreated with AF64A into lateral ventricle and were posttreated with ginseng total saponin, 50 mg/kg, i.p, for 1 week. Twenty four hours after the last administration, rats were sacrificed and each brain regions was dissected out. Each values represents mean ± S.E.M. for 4 animals.
*significantly different when compared with the control value $P<0.05$.

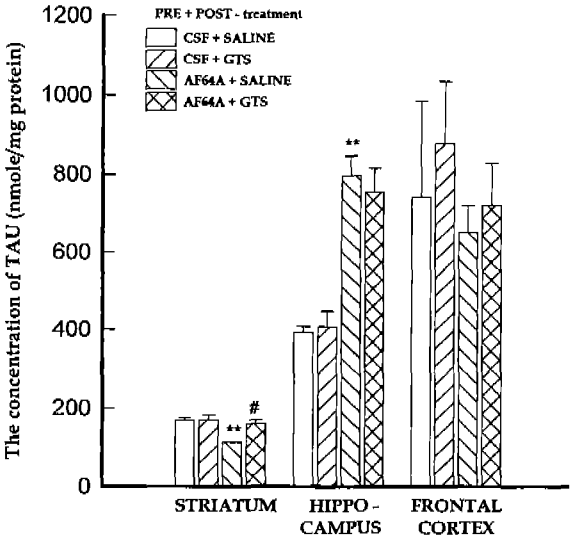

Fig. 2. Effects of the administration of ginseng total saponin on the levels of taurine in AF64A-treated rats.

Legends are the same as Fig. 1. **significantly different when compared with the control value $P < 0.01$. #significantly different when compared with the posttreated value $P<0.05$. AF64A: Ethylcholine aziridium ion, CSF: Cerebrospinal fluid, TAU: Taurine.

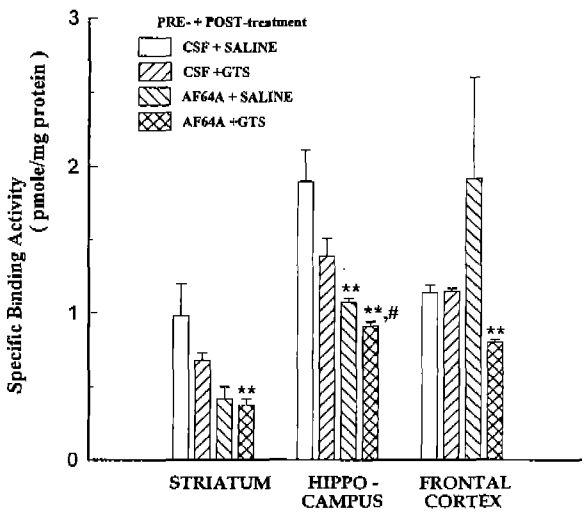

Fig. 3. Effects of the administration of ginseng total saponin on [3H]MK801 binding in AF64A-treated rats.

Legends are the same as Fig. 1. Each values are mean±S.E.M. for 4 separate determination, done in duplicate. **significantly different when compared with the control value $P<0.01$. #significantly different when compared with the posttreated value $P<0.05$. [^{3}H]MK801: [^{3}H]Dizocilpine, AF 64A: Ethylcholine aziridium ion, CSF: Cerebrospinal fluid.

treatment with GTS in AF64A-treated animals. But the increased level of hippocampal taurine by AF64A was not affected by the GTS administration.

Changes in the NMDA receptor by the administration of GTS

Change in the specific binding sites of [3H]MK801 after AF64A pretreatment and GTS posttreatment is shown in Fig. 3. GTS administration did not affect the binding sites of [^{3}H]MK801 in all examined regions. The [^{3}H]MK801 binding sites after the infusion of AF64A were significantly decreased in hippocampus. However, those in striatum and frontal cortex were not affected. When GTS was administered in AF64A-pretreated animals, the specific binding sites of [^{3}H]MK801 were significantly decreased in all examined brain areas compared with the control level. Also the specific binding sites of [^{3}H]MK801 in hippocampus were further significantly decreased compared with the level of the AF64A-treated animals.

Effects of the administration of GTS on glutamine synthetase activities

Change in the activities of glutamine synthetase after AF64A pretreatment and GTS posttreatment is shown in Table I. The activity of glutamine synthetase by GTS administration was significantly decreased in striatum. Also the glutamine synthetase activity was significantly decreased in striatum by the infusion of AF64A. However, the AF64A-induced decreases in the activity of glutamine synthetase were returned to the control level by the administration of GTS. Also the activity of glutamine synthetase in hippocampus was significantly increased by the GTS administration in AF64A-pretreated animals. But the activity of glutamine synthetase in frontal cortex did not alter at any treatment.

Table 1. Effects of ginseng total saponin on glutamine synthetase activities in AF64A-treated rats.

Regions / Treatments	Striatum	Hippocampus (nmole/min/mg protein)	Frontal cortex
Artificial CSF			
+ Saline	2.64±0.22	1.01±0.15	2.98±0.24
+ GTS	1.08±0.10##	0.68±0.09	3.12±0.34
AF64A			
+ Saline	1.38±0.18**	0.98±0.08	2.36±0.28
+ GTS	2.09±0.28	1.45±0.09##,**	2.78±0.16

Rats were pretreated with AF64A and were posttreated with ginseng total saponin, 50 mg/kg, i.p, for 1 week. Twenty four hours after the last administration, the activities of glutamine synthetase were measured in each brain regions. Each value represents mean±S.E.M. for 5 or 11 animals.

**means significance at $P<0.01$ compared with artificial CSF and AF64A and ##does at $P<0.01$ compared with saline and total saponin. CSF: Cerebrospinal fluid. AF64A : Ethylcholine aziridium ion, GTS: Ginseng total saponin.

DISCUSSION

The present results demonstrate that AF64A-induced changes, such as the levels of glutamate and taurine and the activity of glutamine synthetase, are returned to the respective control by the subacute administration of GTS. However, the specific binding site of [^{3}H]MK801 is further decreased by the GTS treatment.

It is known that glutamate is the major rapid excitatory neurotransmitter of mammalian brain and plays a critical role in a variety of pathophysiological disorder such as epilepsy, brain ischemia and learning (Meldrum and Garthwaite, 1990). The present results in the basal level of glutamate are somewhat higher than the reports by other scientists (Abe *et al.*, 1992; Hotnagl *et al.*, 1991; Ma and Lim, 1996). It has been reported that exposure to stress increased the excitatory glutamatergic nervous activities and induced the different changes in the various brain regions (Gilad *et al.*, 1990; Nowak *et al.*, 1995). Thus, the discrepancy might be due to the stress, such as the daily injection.

It has been reported that AF64A, irreversible neurotoxin, impaired the choline uptake in the cholinergic nervous terminals (Chrobak *et al.*, 1988; Nakahara *et al.*, 1988). It has also been reported that the intracerebroventricular administration of AF64A induced a decrease in the level of glutamate (Abe *et al.*, 1992; Hotnagl *et al.*, 1991; Ma and Lim, 1996). The present results showed the similarity in striatum and frontal cortex and the dissimilarity in hippocampus with those reports. Although the exact reason of the increase in hippocampus is unknown, it is possible that the interaction between AF64A-induced toxic effects and daily injection might exaggerate the glutamatergic nervous activities. At any rate, the AF64A-induced changes, such as decrease in the level of total glutamate in striatum and frontal cortex and increase in that of hippocampus were returned to the control level by the administration of GTS. It has been reported that ginsenoside (Rg1) had a life-prolonging effects on neurons (Himi *et al.*, 1989) and ginseng root saponins had the protective role to the hypobaric hypoxia (Liu and Xiao, 1992). It is known that hypoxic state in brain may involve the glutamate-induced neuronal degeneration (Meldrum and Garthwaite, 1990). Thus, the protective effects of GTS on AF64A-induced changes in the level of glutamate as the present results suggest that GTS might antagonize changes in the glutamatergic nervous systems. It has been reported that taurine has a primary role in volume regulation of neurons and glias (Dutton *et al.*, 1991) and the concentrations of taurine affect the excitatory amino acid-induced neurotoxicities (Wu *et al.*, 1995). The present results reveal that GTS administration restores the decreased striatal levels of taurine by AF64A administration to the control level of taurine, but not in the elevated level of hippocampal taurine. It has been reported that the effluxes of taurine from cells are affected by many ions, such as K^+, Na^+ and Ca^{+2} (Dutton *et al.*, 1991). Although the further studies are needed, it suggests that GTS may affect the concentration of such ions. Furthermore, it also suggests that GTS has a regional specific effects. However, other amino acids such as glycine, glutamine and aspartic acid were not altered by the administration of GTS (data not shown).

The various changes in the subtypes of glutamate receptors have been reported in Alzheimer's disease; either reduced (Greenmyre *et al.*, 1987), unaltered (Cowburn *et al.*, 1988), or increased (Ulas *et al.*, 1994). It has been reported that NMDA and non-NMDA receptor agents affect memory and learning function (Pettit *et al.*, 1994; Staubli *et al.*, 1994). The present results indicate that NMDA receptors in hippocampus are significantly decreased but not in striatum and frontal cortex. The present results are different with the previous results (Ma and Lim, 1996). Although the exact reason are not known, the discrepancy might be due to the exposure duration and the daily injection. It is well known that the chronic administration of either agonists or antagonists induces the down- or up-regulation of its receptors. Therefore, the decreased NMDA receptor densities in hippocampus may be due to the elevated glutamate in hippocampus to maintain neuronal balance. The present results reveal that GTS itself do not affect NMDA binding sites, but do decrease the binding sites of [^{3}H]MK801 in AF64A-treated group. Thus, it suggests that GTS may differently affect the specific binding sites of [^{3}H]MK801 on normo- and patho-physiological states. The present results also indicate that GTS further exaggerates the decreased binding sites of [^{3}H]MK801 by AF64A treatment. Although the changes in the characteristics of NMDA receptors for various doses of GTS treatment are needed to further illustrate, it suggests that the administration of GTS should be carefully considered according to the diseased state.

It has been reported that the released glutamate is replenished via glutamate-glutamine cycle in amino acidergic nervous system (Kugler, 1993). Glutamine synthetase converts glutamate into glutamine in glial cells. And generated glutamine is diffused into extracellular space and enters the presynaptic terminals. The glutamine serves as a precursor for presynaptic cytoplasmic glutamate through the mitochondrial glutaminase (Nicolls and Attwell, 1990). The present results reveal that the striatal activities of glutamine synthetase are decreased by GTS as well as AF64A treatment. Since glutamine synthetase is one of the markers of astocytes, GTS itself may deteriorate the striatal astrocytes. However, the present results indicate that the AF64A-induced changes in the activities of glutamine synthetase are increased by the administration of GTS. Although the effectiveness of GTS in the diseased states is further illustrated, the present result suggests that GTS may increase the activities of glutamine synthetase in AF64A-induced pathological states and has properties to increase the reduced activities of glutamine synthetase.

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REFERENCES

Abe, E., Murai, S., Masuda, Y., Saito, H. and Itoh, T. (1992). Reversal by 3,3',5-triido-L-thyronine of the working memory deficit, and the decrease in acetylcholine, glutamate and γ-aminobutyric acid induced by ethylcholine aziridinium ion in mice. *Naunyn-Schmiedeberg's Arch Pharmacol.* **346**, 238-242.

Bartus, R. T., Dean, R. L., Beer, B. and Lippa, A. S. (1982). The cholinergic hypothesis of geriatric function. *Science* **217**, 408-417.

Chrobak, J. J., Hanin, I., Schmechel, D. E. and Walsh, T. J. (1988). AF64A induced working memory impairment: behavioral, neurochemical and histological correlates. *Brain Res.* **463**, 107-117.

Cowburn, R., Hardy, J., Roberts, P. and Briggs, R. (1988). Presynaptic and postsynaptic glutamatergic function in Alzheimer' disease. *Neurosci. Lett.* **86**, 109-113.

Dutton, G. R., Barry, M., Simmons, M. L. and Philibert, R. A. (1991). Astrocyte taurine. *Ann. N.Y. Acad. Sci. U.S.A.* **633**, 489-500.

Ebert, B., Wong, E. H. F. and Krogsgaard-Larsen, P. (1991). Identification of a novel NMDA receptor in rat cerebellum. *Eur. J. Pharmacol.-Mol. Pharmacol. 208*, 49-52.

Ellison, D. W., Beal, M. F. and Martin, J. B. (1987). Amino acid neurotransmitters in postmortem human brain analyzed by high performance liquid chromatography with electrochemical detection. *J. Neurosci. Methods.* **19**, 305-315.

Foster, A. C. and Wong, E. H. F. (1987). The novel anticonvulsant MK-801 binds to the activated state of the N-methyl-D-aspartate receptor in rat brain. *Br. J. Pharmacol.* **91**, 403-412.

Gilad, G. M., Gilad, V. H., Wyatt, R. J. and Tizabi, Y. (1990). Region-selective stress-induced increase of glutamate uptake and release in rat forebrain. *Brain Res.* **525**, 335-338.

Glowinski, J. and Iversen, L. L. (1966). Regional studies of catecholamine in the rat brain-I. the disposition of ^{3}H-norepinephrine, ^{3}H-dopamine and ^{3}H-DOPA in various regions of the rat. *J. Neurochem.* **13**, 655-669.

Greenamyre, J. T. and Maragos, W. F. (1993). Neurotransmitter receptors in Alzheimer's disease. *Cerebrovasc. Brain Metab. Rev.* **5**, 61-94.

Greenamyre, J. T., Penny, J. B., D'Amato, C. J. and Young, A. B. (1987). Dementia of the Alzheimer' type: changes in hippocampal L-[^{3}H]glutamate binding. *J. Neurochem.* **48**, 543-551.

Hanin, I., Mantione, C. R. and Fisher, A. (1982). AF64A-induced neurotoxicity: a potential animal model in Alzheimers' disease. In Alzheimer's Disease: A report of progress (Aging) (S. Corkin, K. L. Davis, L. H. Growdon, E. Usdin, and R. J. Wurtman, Eds.), pp. 267-270. Raven, New York,

Himi, T., Saito, H. and Nishiyama, N. (1989). Effect of ginseng saponins on the survival of cerebral cortex neurons in cell cultures. *Chem. Pharm. Bull.* **37**, 481-484.

Hortnagl, H., Berger, M. L., Reither, H. and Hornykiewicz, O. (1991). Cholinergic deficit induced by ethylcholine aziridinium (AF64A) in rat hippocampus: Effect on glutamatergic systems. *Naunyn-Schmiedeberg's Arch Pharmacol.* **344**, 213-219.

Itoh, T., Zang, Y. F., Murai, S. and Saito, H. (1989). Effects of panax ginseng root on the vertical and horizontal motor activities and on brain monoamine-related substances in mice. *Plant Medica.* **55**, 429-433.

Kugler, P. (1993). Enzymes involved in glutamatergic and GABAergic neurotransmission. *Int. Rev. Cytol.* **147**, 285-336.

Lee, Y. M., Saito, H. and Kasaya, Y. (1980). Potentiation of the NGF-mediated neurite outgrowth by the ginsenoside Rg1 in organ cultures of chick embryonic ganglia. *Jap. J. Pharmacol.* **29**, 191-198.

Lim, D. K., Wee, S. M., Ma, Y. and Yi, E. Y. (1995). Effects of ethylcholine aziridinium, scopolamine and morphine on behaviors in morris water maze. *Arch. Pharm. Res.* **18**, 346-350.

Liu, C. X. and Xiao, P. G. (1992). Recent advances on ginseng research in china. *J. Ethnopharmacol.* **36**, 27-38.

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

Ma, Y. and Lim, D. K. (1996). Effects of i.c.v. administration

of ethylcholine aziridinium (AF64A) on the central glutamatergic nervous systems in rats. *Arch. Pharm. Res.* in press.

Mantione, C. R., Zigmond, M. J., Fisher, A. and Hanin, I. (1983). Selective presynaptic cholinergic neurotoxicity following intrahippocampal AF64A injection in rats. *J. Neutochem.* **41**, 251-255.

Mash, D. C., Flynn, D. D. and Potter, L. T. (1985). Loss of M2 muscarinic receptors in the cerebral cortex in Alzheimer's disease and experimental cholinergic denervation. *Science* **228**, 1115-1117.

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

Nakahara, N., Igo, Y., Mizobe, F. and Kawanishi, G. (1988). Effects of intracerebroventricular injection of AF64A on learning behaviors in rats. *Jpn. J. Pharmacol.* **48**, 121-130.

Nitta, H., Matsumoto, K., Shmizu, M., Ni, X. H. and Watanabe, H. (1995). Panax ginseng extract improve the scolpolamine-induced disruption of 8-arm radial maze performance in rats. *Biol. Pharm. Bull.* **18**, 1439-1442.

Nowak, G., Redmond, A., McNamara, M. and Paul, I. A. (1995). Swim stress increases the potency of glycine at the N-methyl-D-aspartate receptor complex. *J. Neurochem.* **64**, 925-927.

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

Patel, A. J., Hunt, A., Gordon, R. D. and Balazs, R. (1982). The activities in different neural cell types of certain enzymes associated with the metabolic compartmentation glutamate. *Developmental Brain Res.* **4**, 3-11.

Paxinos, G. and Watson, C. (1986). The brain in stereotaxic coordinates. Academic Press, New York.

Pettit, H. O., Lutz, D., Gutierrez, C. and Eveleth, D. (1994). I. C.V. infusions of $ACPD_{(1S,3R)}$ attenuate learning in a morris water maze paradigm. *Neurosci. Lett.* **178**, 43-46.

Saito, H., Tsuchiya, M., Naka, S. and Takagi, K. (1979). Effects of panax ginseng root on acquisition of sound discrimination behaviour in rats. *Jap. J. Pharmacol.* **29**, 319-324.

Shoup, R. E., Allison, L. A. and Mayer, G. S. (1984). o-Phthalaldehyde derivatives of amines for high-speed liquid chromatography/electrochemistry. *Analytical Chem.* **56**, 1089-1096.

Staubli, U., Rogers, G. and Lynch, G. (1994). Facilitation of glutamate receptors enhances memory. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 777-781.

Ulas, J., Weihmuller, F. B., Brunner, L. C., Joyce, J. N., Marshall, J. F. and Cotman, C. W. (1994). Selective increase of NMDA-sensitive glutamate binding in the striatum of Parkinson's disease, Alzheimer's disease, and mixed Parkinson's disease/Alzheimer's disease patients: an autoradiographic study. *J. Neurosci.* **14**, 6317-6324.

Wu, I. Y., Tang, X. W., Deupree, D. I., Medina, I., Yang, C. Y. and Chiang, L. Y. (1995). Effect of GABA, taurine and fullerol-1 on excitatory amino acid-induced neurotoxicity. *Society for neurosci. abstracts.* **21**, 786-789.