Modulation in NMDA and $GABA_A$ Receptor Expression after Cerebroventricular Infusion of Ginsenosides

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Abstract

In the present study, we have investigated the effects of centrally administered ginsenoside Rc or Rg1 on the modulation of NMDA receptor and $GABA_A$ receptor binding in rat brain. The NMDA receptor binding was analyzed by quantitative autoradiography using [^{3}H]MK-801 binding, and $GABA_A$ receptor bindings were analyzed by using [^{3}H]muscimol and [^{3}H]flunitrazepam in rat brain slices. Rats were infused with ginsenoside Rc or Rg1 (10 μg/10 μl/hr, i.c.v.) for 7 days, through pre-implanted cannula by osmotic minipumps (Alzet, model 2ML). The levels of [^{3}H]MK-801 binding were highly decreased in part of cortex and cingulated by ginsenoside Rc and Rg1. The levels of [^{3}H]muscimol binding were strongly elevated in almost all regions of frontal cortex by the treatment of ginseoside Rc but decreased by ginsenoside Rg1. However, the [^{3}H]flunitrazepam binding was not modulated by ginsenoside Rc or ginsenoside Rg1 infusion. These results suggest that prolonged infusion of ginsenoside could differentially modulate [^{3}H]MK-801 and [^{3}H]muscimol binding in a region-specific manner. Also, we investigated the influence of centrally administered ginsenoside on the regulation of mRNA levels of the family of NMDA receptor subtypes (NR1, NR2A, NR2B, NR2C) by in situ hybridization histochemistry in the rat brain. The level of NR1 mRNA is significantly increased in temporal cortex, caudate putamen, hippocampus, and granule layer of cerebellum in Rg1-infused rats as compared to control group. The level of NR2A mRNA is elevated in the frontal cortex. In contrast, it was decreased in CA1 area of hippocampus in Rg1-infused rats. However, there was no significant change of NR1 and NR2A mRNA levels in Rc-infused rats. The level of NR2B mRNA is elevated in cortex, caudate putamen, and thalamus in both Rc- and Rg-infused rats. In contrast, NR2B level is decreased in CA3 in Rg1-infused rats. The level of NR2C mRNA is increased in the granule layer of cerebellum in only Rg1 but not Rc infused rats. These results show that

structure difference of ginsenoside may diversely affect the modulation of expression of NMDA receptor subunit mRNA after infusion into cerebroventricle in rats.

Introduction

Ginseng is well known as an herbal medicine and has been used in therapy for thousands of years. In general, ginseng was considered to have tonic, stimulant and sedative properties. Giseng saponin, known as the major effective compound in ginseng, is called ginsenoside. Ginsenosides isolated and purified from ginseng saponin fraction were steroid-derivatives containing carbohydrates. Based on its chemical structure, ginseng saponin is classified into two groups: protopanaxadiol and protopanaxatriol, which quantitatively account for at least 50% and 20% of all ginseng saponin, respectively. It was reported that ginsenoside Rc exhibited a sedative effect but Rg showed stimulative actions on central nervous system (1). Ginsenoside Rg1 inhibits the rat brain cAMP phosphodiesterase activity (2). Several studies have indicated that excitatory amino acids are involved in neuronal survival, synaptogenesis, neuronal plasticity, and learning and memory process (3,4,5). This consideration prompted us to investigate whether the different structure of ginsenoside shows different effect on glutamate-activated receptor function and expression or not.

Glutamate is a major excitatory transmitter in the mammalian central nervous system. This amino acid produces its effect by acting on at least 3 receptor subtypes designated as NMDA, AMPA/kainate and metabotropic receptors. The excitatory amino acids may under certain conditions not only act as neurotransmitters, but also can be strong cytotoxic agents. It is well known that the functional properties of the NMDA receptor vary throughout the central nervous system. Abnormalities in glutamate neurotransmitter systems were believed to be involved in neurological disorders such as epilepsy (6,7), and certain neurodegenerative diseases such as Alzheimer disease (8), and brain and spinal cord damage following ischemia and spinal cord trauma (9,10). The NMDA receptor is a ligand-gated ion channel that is permeable to Ca^{2+}. The activation of NMDA receptor is essential for synaptic plasticity such as long-term potentiation and long-term depression, which are thought to underlie memory acquisition and learning. Ginsenoside Rc increased the glutamate release and elevated intracellular calcium level in cultured cerebellar granule neurons in a dose-dependent manner (11).

NMDA receptor cloning studies have shown that this receptor is assembled from two distantly

sequence-related subunit, at least one of the NR1 subunit and at least one of four NR2 subunits (NR2A-NR2D) (12,13,14). Recent studies suggest that the ligand binding affinities of recombinant NMDA receptors depend on subunit combination (15). NR1 subunit is essential to expression of NMDA receptor and is widely distributed in brain, whereas the NR2 subunits display more restricted, region-specific patterns of distribution (13,14,15,16,17). It has also been shown that regional and developmental differences in subunit composition of NMDA receptors may underlie their functional and pharmacological heterogeneity (15). The pharmacological characteristics of these heteromeric receptors depend on the NR2 subunit expression (16,17,18). The type of NR2 subunit strongly influences the electrophysiological properties and the drug binding profile of recombinant NMDA receptor (19,20,21). It has been suggested that NR1-NR2A, NR1-NR2B, and NR1-NR2C subunit combinations may account for the antagonist-preferring, agonist- preferring, and cerebellar subtypes of native NMDA receptors, respectively (15). Because, NR1-NR2A and NR1-NR2B receptors show the highest binding affinities for NMDA antagonists and agonists, and NR1-NR2C receptors are expressed only in the cerebellum and have low affinities for NMDA antagonsits and agonists.

GABA is a major inhibitory transmitter in the central nervous system. The $GABA_A$ receptor gates a Cl^- -selective channel in response to the binding of transmitter and contains multiple sites for pharmacologically distinct classes of allosteric modulators. $GABA_A$ receptor-mediated Cl^- conductance is positively modulated at a GABA recognition site, but also at allosteric sites that bind benzodiazepine, barbiturates, and steroids (22,23,24). Biochemical studies have provided evidence that there are at least two different conformations of $GABA_A$ receptor. The high affinity $GABA_A$ sites can be labeled with [^{3}H]muscimol and are associated with the GABA recognition sites and benzodiazepine site can be labeled with [^{3}H]flunitrazepam (25). Ginsenoside (Rb, Rc, Rg1) inhibited the specific [^{3}H]muscimol binding but did not affect [^{3}H]flunitrazepam binding in rat synaptosome (26).

The purpose of the present study was 1) to investigate the role of ginsenoside Rc and Rg1 in the modulation of NMDA receptor and $GABA_A$ receptor expression by using autoradiographic techniques with the receptor binding ligand [^{3}H]MK-801, [^{3}H]muscimol, and [^{3}H]flunitrazepam, and 2) to investigate the changes of NR1 and NR2 (NR2A-2C) subunit mRNA levels after treatment of ginsenoside Rc and Rg1. Ginsenosides are infused into cerebroventricle by using osmotic mini-pump to minimize the indirect effect of ginsenoside in the peripheral system. after prolonged infusion into i.c.v.

Materials and Methods

Chemicals

[^{3}H]MK-801 (20.3 Ci/mmol), [^{3}H]Muscimol (10.1 Ci/mmol), and [^{3}H]flunitrazepam (85.8 Ci/mmol) were purchased from New England Nuclear (Boston, MA, USA). MK-801, Muscimol, and flunitrazepam were obtained from Research Biochemical International (Natick, MA, USA) and all other reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ginsenoside Rc and ginsenoside Rg1 were kindly obtained from Korea Ginseng and Tobacco Research Institute.

Animals and treatment

Male Harlan Sprague-Dawley rats (Daehan Laboratory Animal, Eumsung, Korea) weighing 220-240 g were acclimatized for 1 week with free access to rat chow and tap water. The temperature (24 ± 3°C) and light (12-hr dark) of the housing environment were maintained constantly. All procedures involving rats were performed using protocols approved by the Animal Care and Use Committee of my institution. Rats were implanted with guide cannulae for the drug infusion. Rats were anesthetized by intramuscular injection of ketamine (50 mg/kg) and xylazine (1 mg/kg), before standard stereotaxic surgery performed on a Kopf stereotaxic frame. A 21-gauge stainless steel cannula was implanted in the right lateral ventricle (L: 1.3 mm; A-P: –0.5 mm; and D-V: –4.0 mm) of the rat brain with the bregma chosen as the stereotaxic reference point (27). The cannula was held in place with rapid-setting dental acrylic (Lang Dental Mfg. Co., Chicago, IL, USA) anchored to the skull by an aluminum protective cap and steel screws. Rats were allowed 1 week for recovery before implantation of osmotic minipumps. The minipump was implanted s.c. as described (28) with minor modification. Briefly, under ether anesthesia, a small cut was made behind the ears of the rat and the subcutaneous space was expanded with a hemostatic forceps. Saline vehicle or ginsenoside Rc was filtered through a 0.2 μm sterile syringe filter and was then used to fill an osmotic minipump (Alzet 2ML 1, Alza, Palo Alto, CA, USA). The minipump, which contains ginsenoside Rc, was implanted and connected directly to the cannula via 6-cm long PE-60 polyethylene tubing. The infusion rate was 10 μg/10 μl/hr for 7 days. The incision on the back was closed with cyanoacrylate glue, and dental acrylic was layered on top of the polyethylene tube.

Tissue preparation

Rats infused with ginsenoside Rc were decapitated 7 hrs after the disconnection of osmotic minipumps. After the decapitation, rat brains were removed immediately and were frozen in liquid nitrogen for 20 sec. Horizontal sections, 14 μm-thickness, were cut on a cryostat microtome at −18°C, thaw-mounted on gelatin-coated microscope slides and stored at −80°C until used.

Autoradiographic procedures

Receptor autoradiography of [^{3}H]MK-801 was performed according to the method of Sakurai et al. (29) with modifications. In brief, tissue sections were thawed and dried at room temperature, pre-washed in 50 mM Tris-HCl buffer (pH 7.4) for 30 min at 4°C and blown-dry under a stream of room-temperature air before the [^{3}H]MK-801 binding. Tissue sections were incubated in 50 mM Tri-HCl buffer containing 10 nM [^{3}H]MK-801 and 30 μM glutamate/10 μM for 120 min at room temperature, rinsed with cold 50 mM Tris-HCl buffer two times for 30 min each, dipped once in ice-cold distilled water, and immediately dried in a stream of cool air. Non-specific binding was determined in the presence of non-radioactive 50 μM MK-801. Receptor autoradiography of [^{3}H]muscimol was performed according to the method of Titulaer et al. (30), and receptor autoradiography of [^{3}H]flunitrazepam was performed according to the method of Carlson et al. (31). Dried tissue sections were placed in X-ray cassettes with a set of tritium standards ([^{3}H]Micro-scale RPA 510, Amersham) for [^{3}H]MK-801, [^{3}H]muscimol and [^{3}H]flunitrazepam binding, and juxtaposed to Hyperfilm or β-max film (Amersham). Following a certain exposure period (4-week for [^{3}H]MK-801, 6-week for [^{3}H]muscimol and 2-week for [^{3}H]flunitrazepam binding at 4°C), the film was developed in Kodak D19 at room temperature for 3 min and fixed for 5 min. Autoradiograms were analyzed by a digital scanning densitometer (Personal Densitometer, Molecular Dynamics, Sunnyvale, CA, USA), operating on the image acquisition and analysis program ImageQuant 3.3 (Molecular Dynamics). Plastic standards exposed simultaneously with the brain sections were used as reference with the resulting binding values given as radioactivity levels estimated for gray matter areas (nCi/mg tissue). Non-specific binding was less than 5% of the total binding and was negligible for analyzing the autoradiograms.

Oligonucleotide Probe and Labeling

The purified oligodeoxynucleotide probes complementary to rat NMDA receptor subunit cDNA of NR1 (5'-CGT GCT AAG GAA ACT CAG GTG GAT ACT CTT GTC AGA GTA GAT

GGA-3' residues 375-420), NR2A (5'-AGA AGG CCC GTG GGA GCT TTC CCT TTG GCT AAG TTT C-3' residues 567-579), NR2B (5'-GGG CCT CCT GGC TCT CTG CCA TCG GCT AGG CAC CTG TTG TAA CCC-3' residues 557-572) and NR2C (5'-TGG TCC ACC TTT CTT GCC CTT GGT GAG GTT CTG GTT GTA GCT-3' residues 562-576) were obtained from New England Nuclear (Boston, MA) (16). The probe was labeled at its 3' end using terminal deoxynucleotidyl transferase and [α-^{35}S]dATP (New England Nuclear, Boston, MA). Ten picomoles of probe in the tailing buffer (0.1 M potassium cacodylate, 25 mM Tris base, 1.0 mM cobalt chloride, and 0.2 mM dithiothreitol, pH 7.0) and 50 pmol [α-^{35}S]dATP were incubated with 36 units of terminal deoxynucleotidyl transferase at 37°C for 30 min. The reaction was terminated by adding 400 μl of Nensorb-20 solution on ice. The labeled probe was eluted in 50% ethanol using the Nensorb-20 column chromatography.

In Situ Hybridization

In situ hybridization was performed according to the method of Tseng et al. (32). The probe solution (5×10^5 dpm/μl) was diluted with the hybridization buffer [50% formamide, 10% dextran sulfate, 1% Denhardt s solution, 100 mM DTT, 0.025% tRNA from Escherichia coli and 0.05% DNA from salmon testes in 4 x standard sodium citrate buffer (SSC, 1 × SSC: 0.15 M NaCl and 15 mM sodium citrate, pH 7.0)]. Final radioactivity of the hybridization solution was about 1×10^4 dpm/μl. Frozen brain sections were dried at room temperature for 10 min and fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS), pH 7.4, for 10 min. The slides were rinsed three times in PBS for 3 min, rinsed once in 2 × SSC for 3 min, and dipped once in deionized water. Each brain slice was hybridized with 35 hybridization buffer under a coverslip to prevent tissue drying and was incubated overnight at 40°C in a high humidity environment. After hybridization, coverslips were carefully removed in 1 × SSC and the slides were rinsed in 1 × SSC three times for 3 min each to remove excess hybridization buffer. Slides were then washed twice, for 15 min each, in 2 × SSC + 50% formamide at 55!, followed by washing twice for 15 min in 1 × SSC at room temperature. Finally, slides were briefly dipped in deionized water and dried by air. Competition hybridization was carried out in the presence of an excess amount (70-fold) of unlabeled probe which showed negligible non-specific hybridization in the final image.

Quantification and statistics

The density in each region was recorded by marking 4 to 10 areas over bilateral sides of the

brain according to the size and shape of the region. The mean values were determined from 5 rats and expressed as the mean ± SE, in nCi/mg or nCi/g tissue. Data were analyzed using analysis of variance followed by Newman-Keul's test.

Results

Distribution of [^{3}H]MK-801 binding

Autoradiographs of [^{3}H]MK-801 binding in rat brain are shown in Fig. 1. When the autoradiographs of [^{3}H]MK-801 were quantified, binding was highest in the hippocampus and moderately high in the cortex, caudate putamen, septum, thalamus, and granule layer of cerebellum, and low in brain stem and molecular layer of cerebellum. Binding of [^{3}H]MK-801 was significantly decreased (6-47%) in the frontal cortex by the infusion of ginsenoside Rc or ginsenoside Rg1.

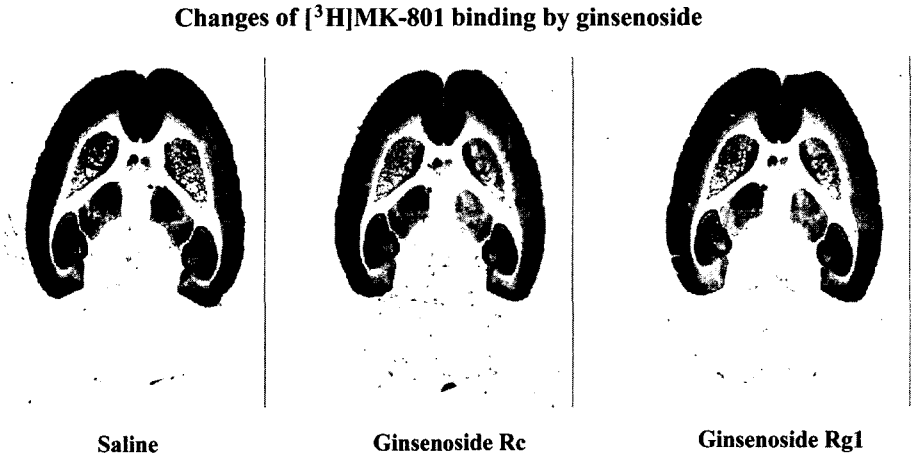

Fig. 1. Representative autoradiograms of [^{3}H]MK-801 in ginsenoside-infused rats. The tissue sections were incubated with 10 nM [^{3}H]MK-801 in the presence of 30 μM glutamate and 10 μM glycine in 50 mM Tris-HCl buffer for 2hr at 25°C.

Distribution of [^{3}H]muscimol binding

The distribution of $GABA_A$ receptor binding(e.g. [^{3}H]muscimol and [^{3}H]flunitrazepam binding) shows some discrepancies in some regions of the rat brain. Autoradiographs of [^{3}H]muscimol binding are shown in Fig. 2. When the density of binding was quantitated, it was highest in the granular layer of cerebellum, moderately high in the cortex and thalamus, and low in the hip-

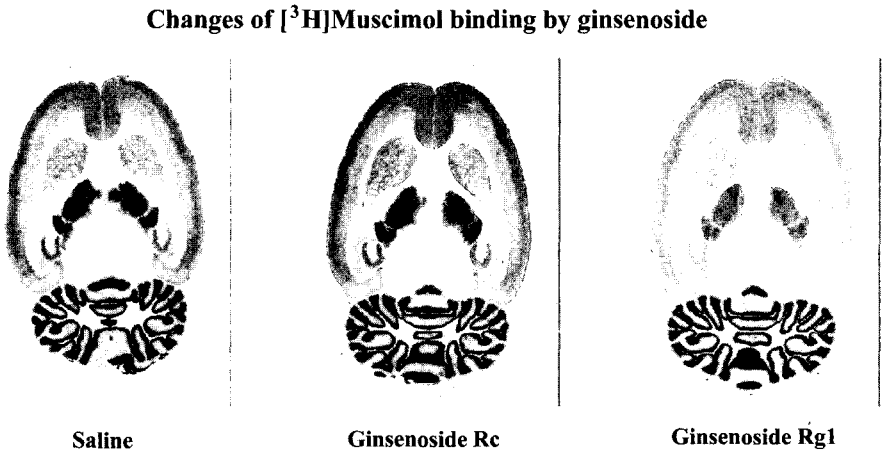

Fig. 2. Representative autoradiograms of [^{3}H]muscimol in ginsenoside-infused rats. The tissue sections were incubated with 5 nM [^{3}H]muscimol in the presence of 150 mM NaCl for 30 min at 4°C.

pocampus and caudate putamen (Fig. 2). Binding of [^{3}H]muscimol was highly increased in the cortex (44-86%) and caudate putamen (71%) by the infusion of ginsenoside Rc. However, the binding was strongly decreased in the cortex (34-50%) and cerebellar molecule layer (35%) but by the infusion of ginsenoside Rg1. But the binding was significantly elevated in the cerebellar granule layer (14%) by the treatment of ginsenoside Rg1.

Distribution of [^{3}H]flunitrazepam binding

When the autoradiographs of [^{3}H]flunitrazepam were quantitated, binding was found to be

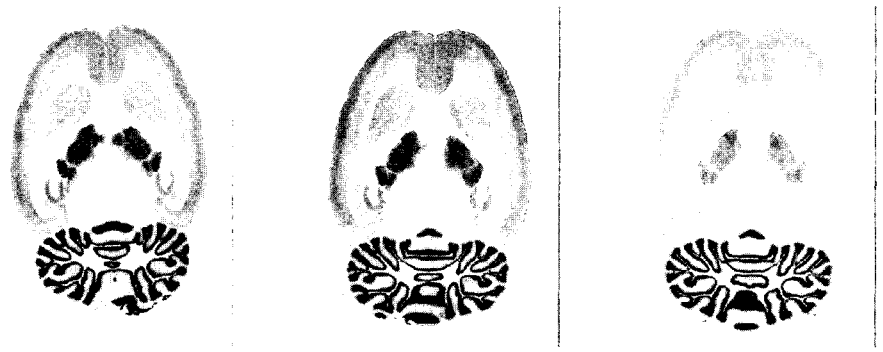

Fig. 3. Representative autoradiograms of [^{3}H]flunitrazepam in ginsenoside-infused rats. The tissue sections were incubated with 1 nM [^{3}H]flunitrazepam in the presence of 150 mM NaCl for 90 min at 4°C.

highest in the cortex and hippocampus, moderately high in the caudate putamen, brainstem and molecular layer of the cerebellum, and low in the thalamus and granular layer of the cerebellum (Fig. 3). However, the binding of [^{3}H]flunitrazepam was not changed by the treatment of ginsenoside Rc or ginsenoside Rg1 although it was significantly increased (9%) in part of the frontal cortex (layer IV) by ginsenoside Rc.

Distribution of NR1 and NR2A-2C Subunit mRNA

In this study, the distribution of NR1 and NR2 subunit mRNA is in agreement with those of previous reports (13,16,17). The levels of NR1 and NR2A mRNA are the highest in the hippocampus and the granule layer of cerebellum, and moderately high in the cortex (Fig. 4). The level of NR2B mRNA is high in the hippocampus and moderate levels are found in the cortical area, thalamus, and caudate putamen, whereas NR2B mRNA can barely be detected in the cerebellum (Fig. 4). The level of NR2C subunit mRNA is exclusively localized to the granule layer of cerebellum (Fig. 4).

Changes of NR1 and NR2A-2C Subunit mRNA. The level of NR1 mRNA is significantly increased in the temporal cortex (19%), caudate putamen (20%), hippocampus (15-27%), and granule layer of the cerebellum (17%) in Rg1-infused rats as compared to control group. However, there is no change in NR1 mRNA level in Rc-infused rats although it is decreased in the septum (10%). The level of NR2A mRNA is elevated in frontal cortex (13%), in contrast, it is decreased in CA1 area of the hippocampus (10%) in Rg1-infused rats. However, there is no significant change in Rc-infused rats. The level of NR2B mRNA is elevated in the cortex (10-20%), caudate putamen (12-14%), and thalamus (12-19%) in both Rc- and Rg-infused rats. In contrast, the level of NR2B is decreased in CA3 (18%) in Rg1-infused rats. The level of NR2C subunit mRNA is increased in the granule layer (8%) of the cerebellum in Rg1-infused rats. However, there is no change in the NR2C level in Rc-infused rats (Fig. 4).

Discussion

The present study investigated the effect of ginsenoside Rc and ginsenoside Rg1 on the changes of NMDA and $GABA_A$ receptors in rat brain slices after prolonged infusion into ventricle. The biding of [^{3}H]MK-801 was decreased in part of frontal cortex by the infusion of ginsenoside Rc and Rg1. However, the binding of [^{3}H]muscimol was increased in frontal cortex and

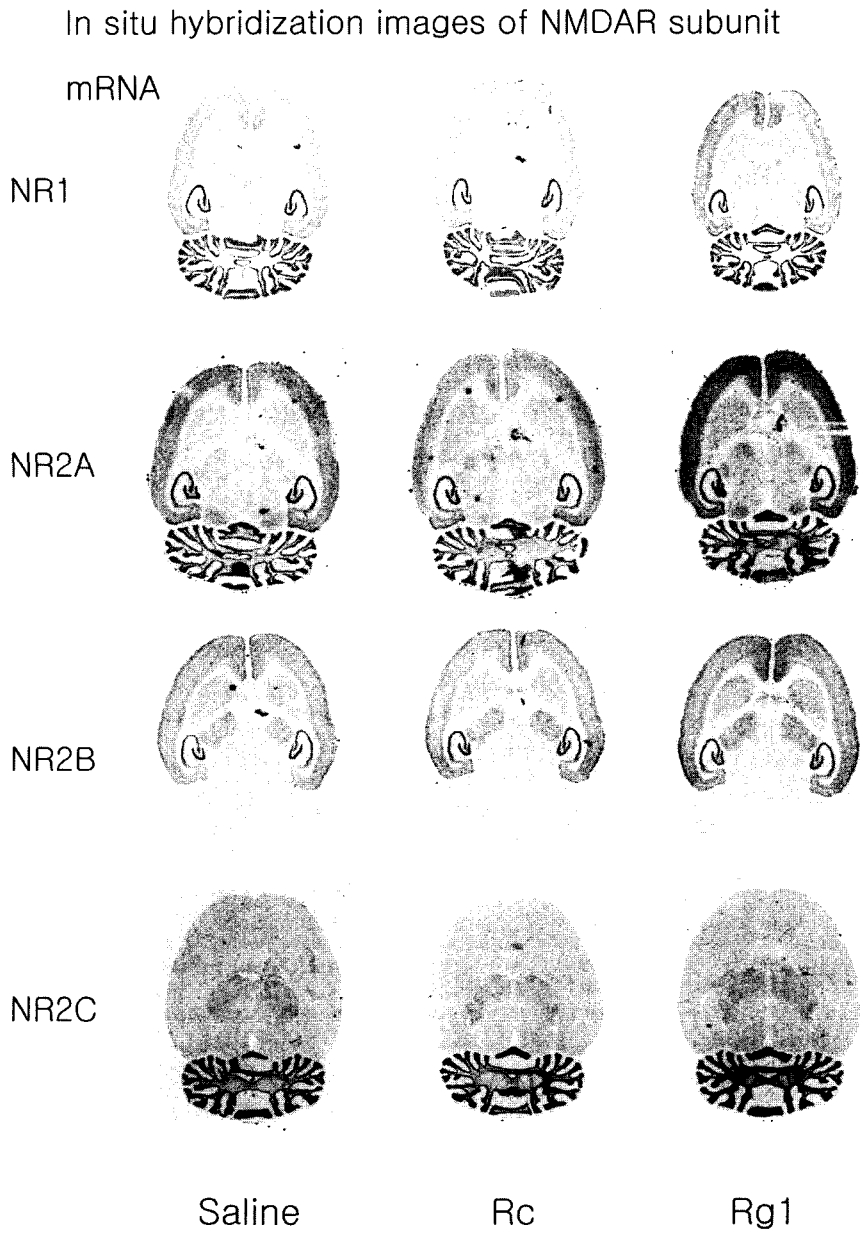

Fig. 4. Representative autoradiograms of in situ hybridization of a 3' end ^{35}S-dATP-labeled synthetic oligonucleotide probe, complementary to rat cDNA coding for NMDA receptor NR1 and NR2 subunit mRNA in rats.

striatum by the infusion of ginsenoside Rc but the binding was decreased in the cortex by the infusion of ginsenoside Rg1. The binding of [^{3}H]flunitrazepam was not changed by the infusion

of ginsenoside Rc or ginsenoside Rg1 in our experiment.

NMDA receptors mediate rapid neuronal excitation at most synapses, while $GABA_A$ receptors mediate neuronal inhibition in the central nervous system. It has been demonstrated that ginsenoside Rb and Rc exhibit a sedative effect and Rg showed stimulative actions on the CNS (1). It is interesting that ginsenoside Rc decreased the [^{3}H]MK-801 binding and elevated the [^{3}H]muscimol binding in the frontal cortex and caudate putamen in our experiment. Although ginsenoside Rg1 decreased the [^{3}H]MK-801 binding in the cortex as done by Rc, Rg1 decreased the [^{3}H]muscimol binding in the cortex. It might be one of the clues how ginsenoside Rc exhibits sedative actions and ginsenoside Rg1 shows stimulative actions on CNS. However, it is not clear how the changes of receptor expression (ligand binding) of cortex are not parallel with those of hippocampus. Ginsenosides cause some behavioral changes by modulating neurons that seems to be related to $GABA_A$ receptor and changes the effects of barbiturate and convulsant, suggesting that the pharmacological actions of ginsenosides might be induced via the $GABA_A$ receptor complex. In fact, ginsenoside Rc and Rg1 decreases the [^{3}H]muscimol binding with concentration of 100 μM in synaptic membrane of the rat frontal cortex (26). However, neither ginsenoside Rc nor Rg1 (each up to 100 μM) affects NMDA receptor binding in synaptic membrane of the rat cortex by using [^{3}H]MK-801 as a ligand (data not shown). These results raise a question how does ginsenoside Rc and ginsenoside Rg1 affect NMDA receptor and $GABA_A$ receptor expression in different manner? One of the possibilities is that inhibitory action of ginsenoside Rc to muscimol results in the up-regulation of $GABA_A$ receptor (at least muscimol binding site) subunit expression after prolonged infusion. The other possibility is that ginsenoside did not affect directly receptor site but modulate endocrine system or intracellular calcium levels. Recently, it has been known that glucocorticoids induce parallel changes in the mRNA level of NMDA receptor subunit and antagonist ([^{3}H]MK-801) binding sites in the hippocampus (33), and that ginsenoside Rg1 binds to the glucocorticoid receptor in a dose-dependent manner (34). Interestingly, ginsenoside Rc is a steroid-derivative containing furanoside (penta-ring) among the ginsenosides. Similarly one of cardiotonic compounds, ouabain (selective Na^+, K^+-ATPase inhibitor) has a steroid backbone containing sugar and penta-ring and it can increase intracellular calcium level (35). Also, ginsenoside Rc but not ginsenoside Rg1 strongly increases the glutamate release and intracellular calcium level in cultured cerebellar granule neurons (11).

Molecular cloning has revealed a multiplicity of $GABA_A$ receptor subunit which, based on homology, can be divided into subunit classes with multiple members; α1-6, β1-4, γ1-4, and

δ(22). [^{3}H]Muscimol labeling reveals that the GABA recognition site is located on the β-subunit (36) or on both the α- and β-subunit (37), while [^{3}H]flunitrazepam labeling indicates that the benzodiazepine recognition site is located mainly on the α-subunit (38) or on both the α- and γ-subunit (39). In our experimental results, the binding of [^{3}H]muscimol was modulated by the prolonged infusion of ginsenoside Rc or ginsenoside Rg1, but the binding of [^{3}H]flunitrazepam was not changed. Based on these results, is seems possible that rearrangement of $GABA_A$ receptor subunit composition may affect its function during chronic ginsenoside treatment and results in physiological and biochemical alterations. However, it is not clear how the binding of [^{3}H]muscimol was exclusively elevated in cortex and caudate putamen by Rc but decreased by Rg1 in our results. One of the possibilities is that different brain regions have different $GABA_A$ receptor subunit combinations and would be regulated differentially by ginsenoside treatment.

The present study investigated the effects of ginsenosides on the expression of NMDA receptor subunit mRNA after prolonged intracerebroventricular infusion in the rat brain. Ginsenosides Rc and Rg1 are the major protopanaxadiol and protopanaxatriol components of ginseng saponin, respectively. Our data show that NR1 mRNA is significantly increased in the caudate putamen, hippocampus, and granule layer of cerebellum in Rg1-infused rats. However, there is no change in the level of of NR1 mRNA in Rc-infused rats although it is decreased in the septal area. The changes in the level of NR2 subunit are somewhat complex. The NR2A mRNA is significantly increased in frontal cortex, but rather decreased in the CA1 area of the hippocampus in Rg1-infused rats, while NR2A mRNA is not changed in Rc-infused rats. The NR2B mRNA level is increased in the temporal cortex, entorhinal cortex, and thalamus, but decreased in the CA3 of the hippocampus in Rg1-infused rats. Also the level of NR2B mRNA is increased in the temporal cortex and caudate putamen in Rc-infused rats. The NR2C mRNA is detected exclusively in the granule layer of the cerebellum in rats treated by Rg1 but not by Rc. These results suggest that regulation of the expression of NR1 and NR2 mRNA is variable to the structure difference of ginsenoside. Comparatively, Rg1 has stronger effect on the changes in the level of of NR1 and NR2 mRNA than Rc. With same concentration of ginsenoside, Rg1 affects all of the expression of NR1 and NR2 subunit mRNA, but Rc only moderately affects the expression of NR2B subunit mRNA.

When the heteromeric receptors are constructed from the NR1 subunit and a subunit of the NR2 subfamily, NR1-NR2A receptors display the highest affinity for competitive antagonists and bind [^{3}H]CGP39653 and [^{3}H]MK-801, whereas NR1-NR2B receptors show the highest

affinity for [^{3}H]glutamate (15). NR1-NR2C receptors exhibit lower affinities for glutamate and NMDA and display lower affinities for antagonist as compared to NR1-NR2A and NR1-NR2B receptors (15). These receptor variants (NR2A, NR2B, and NR2C), therefore, could constitute the rat forebrain "antagonist-preferring", "agonist-preferring", and "cerebellar subtype" NMDA receptors, respectively (15). Therefore, our data can be interpreted as reflecting an increase in the number of the antagonist-preferring receptors in cortex in Rg1-infused rats, as associated with the increase in NR2A mRNA observed. Also, it can be interpreted as an increase in the number of the agonist-preferring receptors in a part of cortex and thalamus in Rg1-infused rats, as associated with the increase in NR2B mRNA. In contradiction, ginsenoside Rg1 induced downregulation of the NR2A and NR2B mRNA levels, especially, in the hippocampal area after treatment. The levels of NR2A and NR2B mRNA are decreased in the hippocampal area by infusion of ginsenoside Rg1. These results show that there are some differences in regional variation between the cortical and hippocampal area in Rg1 treated rats.

NMDA receptors mediate rapid neuronal excitation at most synapses, while $GABA_A$ receptors mediate neuronal inhibition in the central nervous system. It has been demonstrated that ginsenoside Rb and Rc exhibited a sedative effect and Rg showed stimulative actions on the CNS (1). It is interesting that ginsenoside Rg1 but not Rc elevated NR1, and NR2A, NR2B mRNA levels in the cortical area in our experiment. It might be one of the clues how Rg exibits stimulative actions on CNS. Ginsenoside Rc and Rg1 decreased the [^{3}H]muscimol binding with concentration of 100 μM in synaptic membrane of the rat frontal cortex (26), and Rg2 blocked $GABA_A$ receptor functions in bovine chromaffin cells (40). However, neither ginsenoside Rc nor Rg1 (each up to 100 μM) affected NMDA receptor binding in synaptic membrane of the rat cortex by using [^{3}H]MK-801 (data not shown). These results arise a question how does ginsenoside affect NR subunit expression? One of the possibilities which affect modulation of NMDA receptor subunit mRNA is that ginsenosides influence on the modulator of mRNA expression results in the up-regulation of subunit expression. Recently, it has been known that glucocorticoids induce parallel changes in the mRNA level of NMDA receptor subunit and antagoinist ([^{3}H]MK-801) binding sites in the hippocampus. In intact animals, corticosterone increases the mRNA levels of NR2A and NR2B but not NR1 subunit of NMDA receptor in the hippocampus (33). It is an interesting report that ginsenoside Rg1 binds to the glucocorticoid receptor in a dose-dependent manner (34). In fact, our experiments show that the levels of NR2A and NR2B mRNA are significantly decreased but the level of NR1 mRNA is highly increased after ginsenoside Rg1

infusion. These results suggest an intriguing possibility that inhibition of the binding of glucocorticoids by ginsenoside Rg1 results in the down-regulation of NMDA receptor subunits, at least NR2A and NR2B, in the hippocampus. However, it is not clear how the change of subunit expression of cortex is not parallel with that of the hippocampus.

In summary, 1) the current results suggest that the binding of NMDA receptor ([^{3}H]MK-801)is decreased by prolonged infusion of ginsenoside Rc and ginsenoside Rg1. However, the binding of [^{3}H]muscimol is modulated variably by prolonged infusion of ginsenoside Rg1; increased by Rc but decreased by Rg1. In addition, the binding of [^{3}H]MK-801 or [^{3}H]muscimol was changed in a region-specific manner by the treatment of ginsenoside. 2) Our results show that prolonged infusion of ginsenoside into the ventricle alters NMDA subunit mRNA levels in a region-specific manner, and show that structural difference of ginsenoside may diversely affect the modulation of mRNA expression of NMDA receptor subunit in rats.

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