Red Ginseng Ameliorates Place Learning Deficits in Aged Rats and in Young Rats with Selective Hippocampal Lesions

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ABSTRACT

Ameliorating mechanisms of red ginseng on learning deficits were investigated in the following 3 experiments; its effects on 1) place learning deficits in aged rats and in young rats with selective hippocampal lesions (behavioral study), 2) long-term potentiation in the hippocampal formation (neurophysiological study), and 3) ChAT (choline acetyl transferase) activity in various brain regions of aged rats (pharmacological study). In the behavioral study, first, performance in the place learning tasks were compared among 3 groups of young and aged rats; control young intact rats (10-12 week old) treated with water, aged rats (28-32 month old) treated with water, and aged rats (28-32 month old) treated with red ginseng (100 mg/kg/day) suspended in water. Second, performance in the place learning tasks was compared among 3 groups of young rats; control intact rats treated with water, rats with bilateral hippocampal lesions treated with water, and rats with bilateral hippocampal lesions treated with red ginseng (100 mg/kg/day). Each rat in these 2 behavioral experiments was tested with the 3 types of the place learning tasks in a circular open field using intracranial self-stimulation (ICSS) as reward. The ICSS reward was delivered if the rat (1) moved distance of 100-160 cm (DMT); (2) entered an experiment-determined reward place within the open field, and this place was randomly varied in sequential trials (RRPST); or (3) entered 2 specific places, and did a shuttle behavior between the 2 places (PLT). Performance of the aged rats in the ginseng group was not significantly different from that of control young rats in ICSS (current intensity, bar press rates), DMT and RRPST. However, treatment with red ginseng significantly ameliorated place-navigation learning deficits in aged rats in the PLT. Similarly, red ginseng ameliorated learning and memory deficits in young rats with hippocampal lesions in the same tasks. In the neurophysiological study using young rats, perfusion of hippocampal slices with non-saponin fraction of red ginseng significantly enhanced magnitudes of the long-term potentiation (LTP) in the CA3 subfield. In the pharmacological study, treatment with red ginseng did not affect ChAT activity in aged rat brain including the hippocampal formation. These results strongly suggest that red ginseng ameliorates learning and memory deficits in aged rats through actions on the CA3 subfield of the hippocampal formation, which were independent of the presynaptic components of the cholinergic system.

Introduction

The medial temporal lobe including the hippocampal formation is most susceptible to the senile dementia including Alzheimer disease. Neuropsychological studies in both monkeys and humans reported that lesions in these regions were responsible for human amnesia (deficits in declarative memory) (Scoville and Milner, 1957; Zola-Morgan *et al.,* 1986; Squire, 1987). Furthermore, it has been reported that hippocampal neurons in both rats and monkeys responded during various spatial and non-spatial learning tasks (Ono *et al.,* 1993; Eifuku *et al.,* 1995; Kobayashi *et al.,* 1997; Nishijo *et al.,* 1993, 1997). Therefore, prophylatic and therapeutic drugs for amnesia due to the senile dementia are expected to act on the medial temporal lobe including the hippocampal formation. On the other hand, Oriental medicines are suitable for aged people since these medicines have less side effects and consequently are safer.

Previously, our neurophysiological studies demonstrated that 1) red ginseng increased amplitude of population spikes in the CA1 pyramidal cell layer in response to single electrical stimulation of Schaffer/commissural fibers *in vitro,* and 2) this augmentative effects of red ginseng were attributed to its non-saponin fraction. Furthermore, our previous behavioral study indicated that oral administration of red ginseng significantly ameliorated place learning deficits of young rats with hippocampal lesions in Morris water maze. In the present study, to elucidate mechanisms of action of red ginseng on learning and memory, we investigated 1) effects of red ginseng on learning deficits in aged rats and in young rats with hippocampal CA1 lesions due to transient forebrain ischemia and in a place-navigation task using intracranial self-stimulation (ICSS) reward (behavioral study), 2) effects of non-saponin fraction of red ginseng on the long-term potentiation (LTP) in the CA3 subfield of the hippocampal formation of young rats (neurophysiological study), and 3) effects of red ginseng on ChAT (choline acetyl transferase) activity in various brain regions of aged rats (pharmacological study).

Materials and Methods

Behavioral study

The methods were substantially the same as those described in our previous papers (Fukuda *et al.,* 1992; Kobayashi *et al.,* 1997). In the 1st behavioral study, 19 young (8-10 week old) and aged (28-30 month old) male Fisher 344 rats were used. These rats were divided into 3 groups; young rats treated with water (n=6), aged rats treated with water (n=6), and aged rats treated with ginseng (n=7). In the 2nd behavioral experiment, 16 young rats were divided into 3 groups; control intact rats treated with water (5 ml/kg/day) (n=8), rats with hippocampal lesions treated with water (n=4), and rats with hippocampal lesions treated with red ginseng (100 mg/kg/day) (n=4). Young rats for bilat-

eral hippocampal lesions were subjected to transeint forebrain ischemia with modified 4 vessel occlusion technique by Pulsinelli *et al.* (1982) to produce selective lesions in the CA1 subfield of the hippocampal formation. Suspension of red ginseng (Ginseng Radix rubura, Seikansho, Korea Tobacco & Ginseng Co., Ltd.) was administered daily (100 mg/kg/day, p.o.) 2hr before start of each session from the 4th day of ICSS training (see below in detail). Red ginseng was suspended in water (100 mg in 5 ml of water). Control rats received same amount of water (5 ml/kg, p.o.).

Each rat was anesthetized and implanted bilaterally with monopolar stimulating electrodes aimed at the medial forebrain bundle at the level of the lateral hypothalamic area. After 1 week of recovery, the rats were screened to self-stimulate in an operant chamber equipped with a lever on one wall. Each lever press triggered the delivery of a 0.5 sec train of 0.3 msec negative square wave pulses at 100 Hz. The current intensity for ICSS was determined to produce 40-70 lever presses/min in the operant chamber. The rats were trained for ICSS for 8 days (30 min/day).

After ICSS training, the rats were tested in the 3 kinds of place tasks in a 150 cm diameter circular open field with a 45 cm high wall, painted black on the inside (Fig. 1A). The open field was enclosed by a black curtain (180 cm in diameter and 200 cm high). The ceiling of the enclosure contained 4 small speakers mounted near the circumference, spaced 90° apart, 4 light bulbs individually mounted near the inner edge of each speaker, and a video camera at the center (Fig. 1A). A small light bulb was mounted on the head of the rat. The video camera tracked the 2-dimensional (horizontal) motion of the small bulb. A laboratory microcomputer received the X and Y coordinates of the position of the head through an RS-232C serial port. A program delimited circular areas (reward places) in the open field, and triggered the delivery of current for ICSS when the rat entered the reward place.

In each of the following 3 place tasks, the small electric bulb on the head of the rat was turned on at the start of a trial, and an train of ICSS current was delivered to activate the rat. Each trial was terminated after 50 rewards had been delivered or 10 min had elapsed, whichever occurred first. Each session consisted of 3 trials, and the rats were given training of 1 session/day.

1) Distance movement task (DMT; Fig. 1Ba): Current for ICSS was delivered when the cumulative distance traveled by the rat reached a given distance. The initial distance was 100 cm, and this was increased progressively to 160 cm by 20 cm if the rat acquired 50 rewards in 10 min. The rats were trained until they could acquire 50 rewards within 10 min in a condition of 160 cm moving distance.

2) Random reward place search task (RRPST; Fig. 1Bb): In this protocol a reward place (90 cm diameter) was delimited; its center was chosen at random within a square circumscribed around the open field. The rat was rewarded with ICSS when it entered the reward place, which was then made inactive. After a 5 sec interval, the reward place was moved to a different location and reactivated. The rats were trained until 3 sessions, which contained more than 2 trials with a total of moving distance more than 25 m/trial, were successively observed.

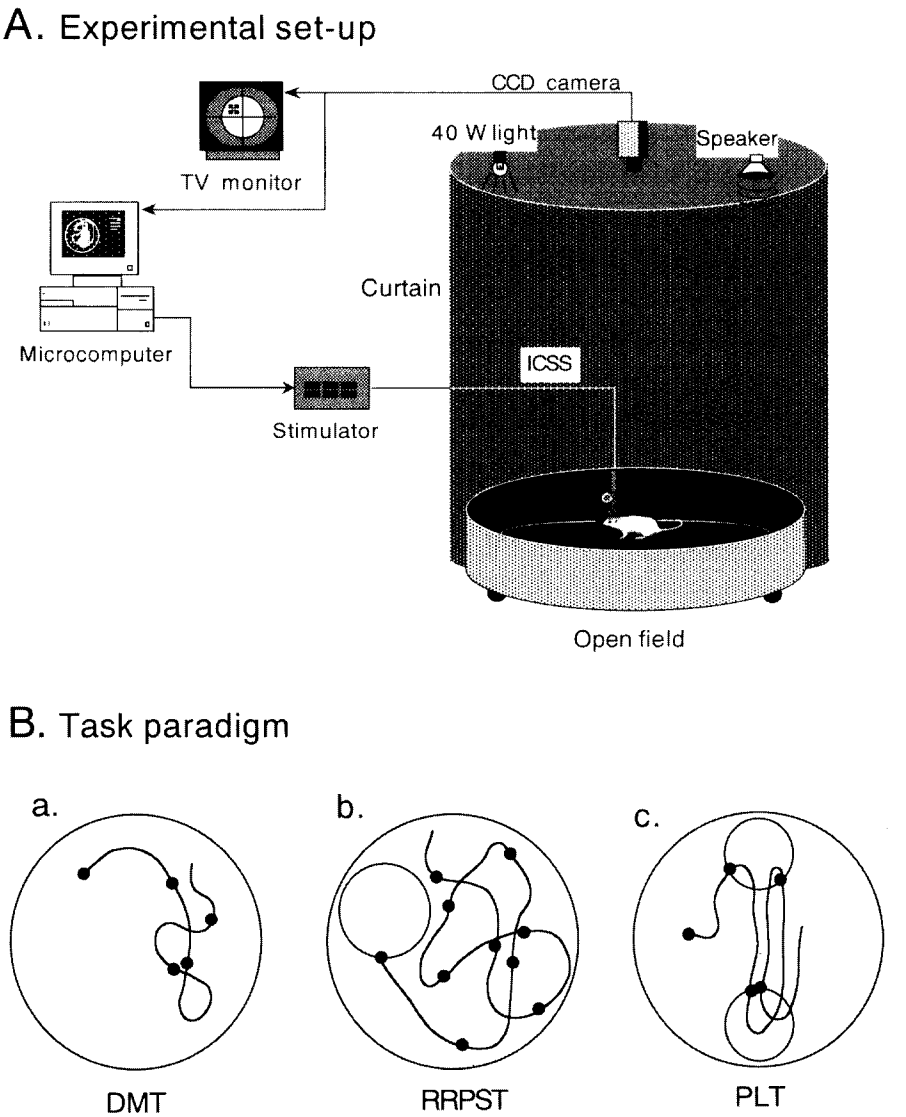

Fig. 1. Experimental set up (A) and task paradigms (B) in the behavioral study.

A: An open field (150-cm-diameter) containing a rat was viewed from top center by a video camera that signaled the rat's position. The open field was enclosed by a black curtain. The video signal was sent to a conventional TV monitor and a digital interface, which sent the X and Y coordinates of a miniature light bulb attached to the head of the rat through a RS-232C serial port to a microcomputer. The microcomputer plotted the trail of the rat, compared the rat's behavior with preset criteria, and triggered intracranial self-stimulation (ICSS) delivery when the criteria were met.

B: In the distance moving task (DMT) (a), a computer program computed moving distance from the trail. The rat acquired ICSS rewards if it moved in the fixed distance (i.e., 100, 120, 140, 160 cm). In the random reward place search task (RRPST) (b), a computer program delimited a circular reward place (thick line circle) at some randomly selected coordinate. The rat was rewarded with ICSS when it entered the reward place, which was then made inactive (changed to thin line circle). After a 5 sec interval, the reward place was moved to a different location and reactivated. In the place learning task (PLT) (c), the rat received rewards in two target areas (thick line circles) when it returned to one reward place after a visit to the other reward place.

3) Place learning task (PLT; Fig. 1Bc): Two 40 cm diameter reward places were located diametrically opposite to one another in the open field. The rat was rewarded in both reward places, when it returned to one of them after a visit to the other one. The rats were trained for 21 day with this protocol.

Neurophysiological study

Experiments were performed on young male Fisher 344 rats (150-200 g, n=20). The rats were rapidly decapitated, and the brains were dissected out and rapidly cooled (4℃) in oxygenated in artificial cerebrospinal fluid (ACSF). Transverse slices (400 ㎛ in thickness) were cut from the isolated hippocampal formation using a mciro-slicer (Dosaka, E.M., Osaka, Japan). The slices were preincubated for more than 1 hr at 32℃ in ACSF containing (in mM) NaCl 124, KCl 5, KH_2PO_4 1.24, $MgSO_4$ 1.3, $CaCl_2$ 2.6, $NaHCO_3$ 26 and glucose 10, which was continuously bubbled with a mixture of 95% O_2 and 5% CO_2. A slice was transferred to a recording chamber and perfused at a rate of 2.0 ml/min with ACSF. A glass electrode filled with 0.9% NaCl (resistance, 3-8 MΩ) and a pair of stimulating electrodes were placed on the CA3 subfield and mossy fibers, respectively. A rectangular test pulse (duration, 0.1/msec; frequency, 0.2-0.5 Hz) was delivered to the stimulating electrodes at a current that elicited 40-60 % of maximum spike amplitude (usually, 0.1-0.5 mA). Conventional electrophysiological techniques for extracellular recordings were employed to record population spikes from the CA3 subfield of the hippocampal formation. Population spikes were averaged for every 8 records, and spike amplitudes were measured every 10 min. The amplitude of population spikes was measured as the difference between the initial negative deflection and next positive one.

After recording baseline responses to test pulses for at least 20 min, perfusion of non-saponin fraction of red ginseng (0, 0.4, 4, and 12 ㎍/ml of ACSF) was applied for 10 min. Twenty min after the end of perfusion of non-saponin fraction, tetanizing stimulation (duration, 0.1 m/sec; intensity, 0.2-0.4 mA; frequency, 100 Hz; No. of train, 100) was applied to the mossy fibers.

Pharmacological study

Aged rats (n=10) were decapitated, and the brains were dissected out and rapidly cooled. Then, the olfactory bulb, septum, hippocampal formation, striatum, amygdala, dorsolateral frontal cortex, parietal cortex, cerebellum were dissected out on a chilled glass. These tissues were frozen immediately in liquid nitrogen and stored at -80℃. The tissues were sonicated in 0.05 M Tris-Triton buffer, pH 7.4 (diluted 1:20, wet w/v), and centrifuged. The supernatant was transferred to another tube, and soluble protein levels were determined by Bradford method (Bio-Rad protein assay, Nippon Bio-Rad Laboratories KK, Tokyo). Duplicate samples from each region of each animal were diluted 1:5 with Tris-phosphate buffer up to a final volume of 50 ㎕. Reaction cocktail (50 ㎕; containing NaCl, eserine, choline chloride, albumin, and C^{14}-labeled acetyl CoA in Na phosphate buffer) was added to the

tissue samples, mixed, and incubated at 37℃ for 20 min. The amount of acetylcholine synthesized was calculated and expressed as nanomoles per milligram of protein per min.

Results and Discussion

Behavioral study

In the 1st behavioral study, there were no differences in training period to reach the criterion in the DMT task (moving distance = 160 cm) among the 3 groups; young rats with water, aged rats treated with water, and aged rats treated with ginseng [1-way ANOVA: $F(2, 16)=0.668$, $p>0.05$]. In the RRPST task, mean moving distance per trial for these 3 groups after reaching the criterion for the RRPST task were 3354 ± 144 (mean$\pm$SEM), 2944 ± 488, and 4267 ± 358 cm, respectively. The post-hoc test indicated that mean moving distance of aged rats treated with red ginseng was significantly greater than that of aged rats treated with water [Newman-Keuls test after 1-way ANOVA, $p<0.05$]. Figure 2 shows results in the PLT task. Number of reward acquired per trial gradually increased in the 3 groups (A). However, there were some differences in learning curves among the 3 groups. The number of rewards suddenly increased after day 4 and reached the maximum (i.e., 50 rewards per trial) on day 10 in young rats treated with water, while it did not reach maximum in both groups of aged rats even on the last day of the experiment. There were significant differences in mean number of rewards for each successive 3 days from day 4 to 18 among the 3 groups [Newman-Keuls test after 2-way ANOVA, $p<0.05$]; mean number of rewards was larger in young rats treated with water than aged rats treated with ginseng; and larger in aged rats treated with ginseng than aged rats treated with water. However, there were no significant differences for initial 3 days [2-way ANOVA: $F(2, 57)=2.262$, $p>0.05$]. From day 19 to 21, mean number of reward was significantly larger in young rats treated with water than aged rats treated with ginseng and water [Newman-Keuls test after 2-way ANOVA, $p>0.05$]. When moving distance for each 3 successive days was compared, the same results as those for number of reward were obtained from day 4 to 21 [Newman-Keuls test after 2-way ANOVA, $p<0.05$] (B).

The 2nd behavioral experiment with the same protocol was performed using 3 groups of young rats with and without hippocampal lesions. Essentially similar results as those in aged rats were obtained, i.e., 1) there were no significant differences in current intensity in ICSS bar-press behavior, and in performance in DMT and RRPST tasks among 3 groups, and 2) oral administration of red ginseng significantly ameliorated place learning deficits in young rats with hippocampal lesions in the PLT task (data not shown).

It has been reported that red ginseng has several peripheral effects (Choi *et al.*, 1998; Wang *et al.*, 1998). Therefore, the present results in aged rats might be ascribed to peripheral effects such as improvement in motor performance other than those on the central nervous system. However, this is

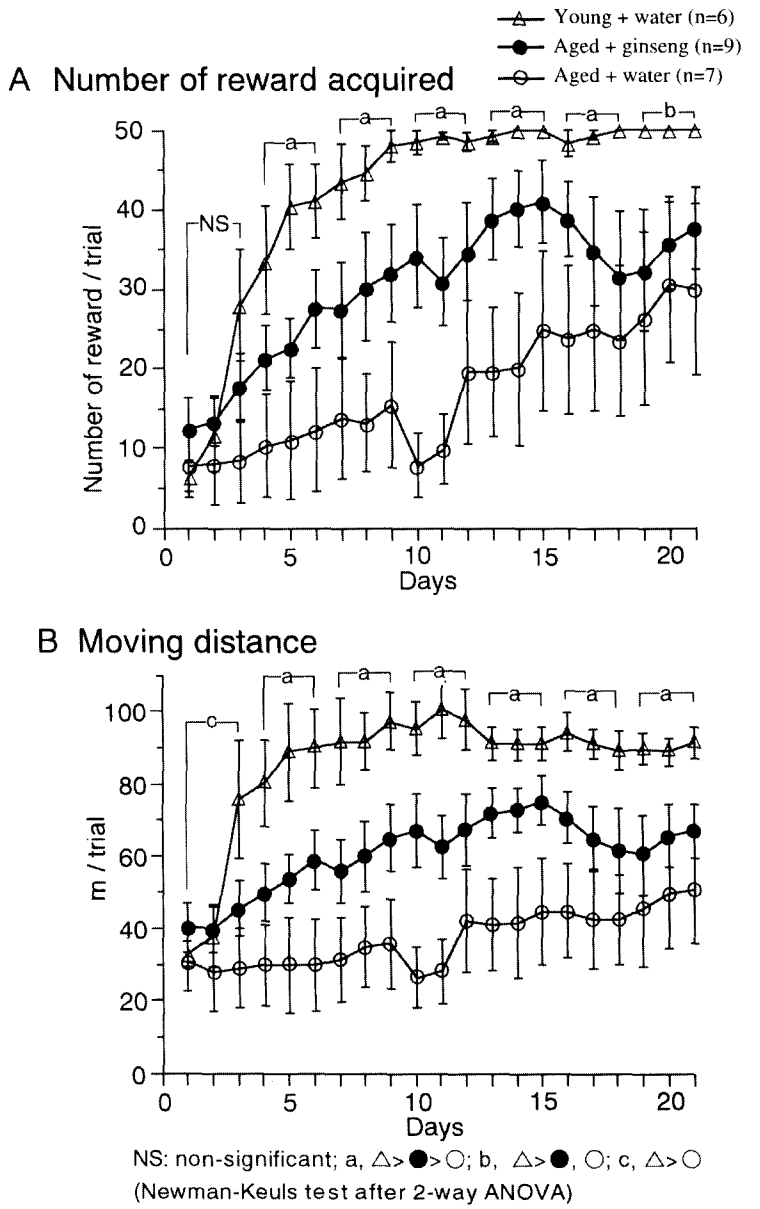

Fig.2. Comparison of performance in the PLT among 3 groups of rats.

A: Mean number of rewards acquired; B: Mean moving distance. Mean values of the 3 trials in each day in each 3 groups are shown. Note that the performance of the both aged rats was significantly lower than that of the young rats, and that the aged rats treated with red ginseng showed significant improvement in the PLT. Open triangles, young intact rats; closed circles, aged rats treated with ginseng, open circles, aged rats treated with water. NS, non-significant difference among 3 groups; >, significant difference by Newman-Keuls test after 2 way-ANOVA ($p<0.05$).

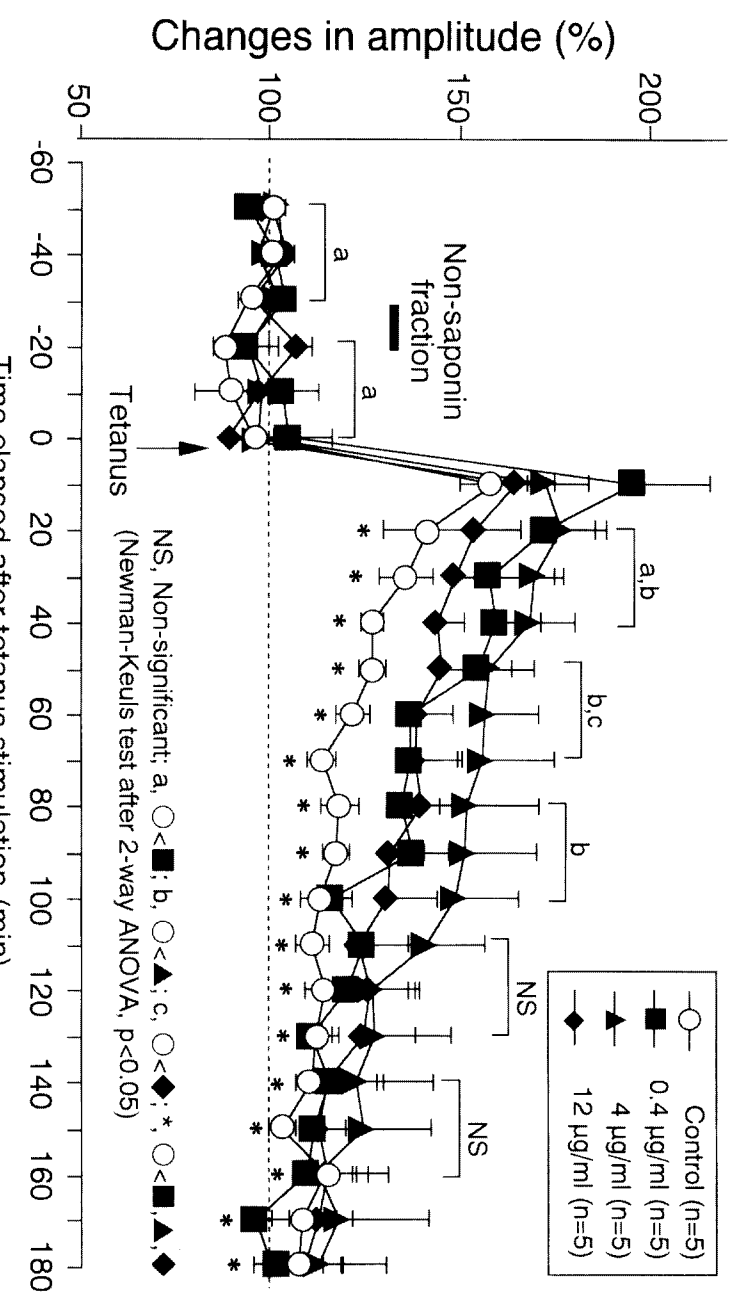

Fig.3. Effects of non-saponin fraction of red ginseng on long-term potentiation (LTP) in the hippocampal formation.

Non-saponin fraction was applied for 10 min, 20 min before tetanus stimulation. Effects of tetanus stimulation were significantly greater in the slices treated with non-saponin fraction than in the control slices. Ordinate, percent changes in spike amplitude from pre-application of non-saponin (baseline); abscissa, time after tetanus stimulation. Other descriptions as for Fig.2.

unlikely. First, the trails of aged rats treated with red ginseng gradually changed during the PLT task similarly to those of young intact rats; the aged rats treated with red ginseng gradually navigated along a line directly connecting the 2 reward areas. Second, in the 2nd experiment using young rats with hippocampal lesions, there were no differences in performance in the DMT and RRPST tasks among the 3 groups of young rats. In the DMT and RRPST tasks, the rats did not necessarily recognize spatial cues (i.e., lamp and speaker) in contrast to the PLT task in which the rats had to recognize the reward areas in reference to the spatial cues. However, the rats must navigate in the same open field to acquire same ICSS rewards in all 3 tasks. These results strongly suggest that cognitive demand in the PLT task is larger than that in the DMT and PLT task although the motivational level and motor performance required to perform the 3 tasks are same. In the present study, learning deficits of the young rats with hippocampal lesions were observed only in the PLT task. Red ginseng ameliorated these learning deficits in the PLT task. The present results strongly suggest that red ginseng ameliorated learning deficits in aged rats rather than ameliorated motor deficits.

Neurophysiological study

Figure 3 shows effects of non-saponin fraction on LTP in the CA3 subfield. There were no significant differences in spike amplitude among 4 groups treated with different concentration of non-saponin fraction before [2-way ANOVA: $F(3, 48)=0.465$, $p>0.05$] and after [2-way ANOVA:$F(3, 48)=1.334$, $p>0.05$] application of non-saponin fraction. However, after application of tetanus stimulation, spike amplitude of the non-saponin group was significantly larger than that of the control group [Newman-Keuls test after 2-way ANOVA, $p<0.05$]. The results indicated that non-saponin fraction of red ginseng significantly enhanced LTP in the CA3 subfield of the hippocampal formation.

Pharmacological study

There were no significant differences in ChAT activity expressed as ACh production in each region between aged rats treated with red ginseng and water (data not shown). Resent pharmacological studies also reported that specific lesions of cholinergic neurons in the septal nuclei of young and aged rats had no effects on place navigation learning in Morris water maze (Baxter *et al.*, 1995; Baxter and Gallagher, 1995). Taken together, the results in our behavioral, neurophysiological and pharmacological studies suggest that red ginseng ameliorated learning deficits of aged rats at least through the non-cholinergic action on the CA3 subfield of the hippocampal formation.

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