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Estrogen Replacement Effect of Korean Ginseng Saponin on Learning and Memory of Ovariectomized Mice

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Abstract : Estrogen can influence on the expression of behaviors not associated directly with reproduction, including learning and memory. Recently estrogen has received considerable attention for its effects on neuroprotection and neural circuits in brain areas associated with cognition. Although estrogen replacement therapy may be helpful to postmenopausal women, it also results in a number of harmful side effects. Ginseng also has steroidal qualities and contains several ginsenoside components which have similar backbone structure to estrogen. The objectives of this experiment were 1) to examine the effects of estrogen and 2) to investigate the effects of ginsenosides as estrogenic agent on learning and memory using the Morris water maze, a traditional experimental task for spatial memory. In the experiments designed here, ovariectomized mice were implanted subcutaneously with Silastic capsules containing 17β-estradiol (100~250 μg/m*l*), panaxadiol (PD) and panaxatriol (PT) saponins (15~100 μg/m*l*) diluted with sesame oil. In the first set of experiment, the effects of estradiol on learning and memory during the Morris water maze was examined. When estradiol was delivered via Silastic capsules following training improved spatial memory performance in ovariectomized female mice. In the second set of experiment, three different PD and PT saponin concentrations were delivered via Silastic implants to ovariectomized female mice and their effects were compared with estrogenic effects. Results of three separate experiments demonstrated that estradiol, PD and PT administrated by Silastic implants for 2 weeks prior to water maze training significantly improved spatial memory performance compared to ovariectomized (OVX) mice, as indicated by lower escape latency over trial. The positive effect of estradiol suggests that estrogen can affect performance on learning and memory. In addition, the positive effect of PD and PT saponins suggest that ginsenosides have an estrogen-like effects in mediating learning and memory related behavior action.

Key words : Estrogen, ginsenoside, ovariectomy, learning, memory.

INTRODUCTION

One of the clinical symptoms reported by menopausal and post-menopausal women is a deficit in memory and cognitive function.[1-3] And it has been reported that the Alzheimer's disease, which is characterized at onset by memory impairment, occurs in women during the post-menopausal years exclusively[4] and in young healthy women, changes in performance of memory tasks correlate with changes in ovarian steroid secretion during menstrual cycle,[5-7] and in elderly men with low endogenous levels of sex steroids, testosterone administration improves performance on a measure of spatial ability.[8] Therefore it was suggested that exogenous estrogen administration to postmenopausal women could be associated with a decreased risk of Alzheimer's disease.[9] And during past several decades, effects of sex steroid on performance of learning and memory tasks have been examined. Many accumulated evidences indicate that estrogen can affect performance on various measures of learning and memory in mammals.[10-18]

Recent evidence suggests that postmenopausal estrogen replacement reduces the risk and severity of Alzheimer's disease in women[19-20] but data regarding the therapeutic regimens and contributing mechanisms associated with this important phenomenon currently are still limited.

On the other hand, Korean ginseng contains several steroid compounds such as panaxadiols and panaxtriols. The steroid components contained in ginseng are remarkably similar to anabolic steroids found naturally in the human body.

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Although these ginsenosides are usually mild and subtle in their efficacy compared with other medicines, many evidences[21,23,31] show that ginsenosides are involved in various biological systems such as cardiovascular systems, central or peripheral nervous systems, immune systems, and so on. And these ginsenosides also have been demonstrated to have a powerful function as an adaptogen, invaluable in helping the body adapt to and recover from the effects of stress, disease, and fatigue.

Modern pharmacological studies[21] suggested that ginseng has bi-directional regulation effects in CNS, anti-fatigue, anti-hypertensive and decreased peripheral blood resistance effect. Furthermore, recent study[22,23] suggested that ginseng improved the drug-induced deficit after three-day consecutive administration on passive avoidance performance in rats.

Results of some experiments on the behavior of rats and mice in order to explore the possible pharmacological actions of *Panax ginseng* upon the central nervous system, appear to suggest a stimulating effect on the central nervous system when ginseng saponin is administered in small doses, but that larger doses might result in an inhibitory effect.[21] While some pharmacological studies showed that the Korean ginseng roots improved spatial swimming performance of mice, improved retention of passive avoidance with step-down method and of active avoidance with shuttle-box method.[22,23] These experiments were designed to further investigate estrogenic effects on learning and memory using female mice. Also these experiments show in some aspects that ginsenosides do act like estrogenic effects on the Morris water maze.

MATERIALS AND METHODS

1. Materials

(1) Animals

Female ICR mice were obtained from Dae Han Laboratory Animal Research Center. Animals weight (25~35g) and age (8~12 weeks) were matched at the time of testing. All mice were maintained on a 12/12 hr light/dark schedule at 22±1°C temperature. Mice were group housed in a colony (n=10) with chow and water available *ad libitum*. Testing occurred the light portion (06:00-18:00) of the light/dark cycle. Mice were allowed to adapt to the new environment for 1 weeks before behavioral testing.

(2) Reagents

17-Estradiol (E2) (Sigma Chemical Co.) was dissolved in ethanol as a concentrated stock (10 mg/m*l*) and then serially diluted to its final concentration. Panaxadiol (PD) and panaxatriol (PT) were provided from Korea Ginseng and Tobacco Research Institute. PD and PT were dissolved in 20% ethanol (10 mg/m*l*). Mice were bilaterally ovariectomized (OVX) for 3 weeks and then randomly divided three groups. Each group of mice implanted of 17β-estradiol (E2) (100 μg, 150 μg, 250 μg in 1 m*l* of sesame oil), PD and PT (15 μg, 50 μg, 100 μg in 1 m*l* of sesame oil).

(3) The Morris water maze

The Morris water maze consists of a round tank (pool; 80 cm diameter, 48 cm depth) of water. The pool and platform are white. The platform (10×10 cm square) was located 0.5~1 cm below the surface of the water, and make it invisible for the mouse with non fat powder milk.

The pool was divided into 4 quadrants with the platform in a fixed position in one quadrant. Water temperature was maintained at 26°C.

2. Procedures

(1) Learning training

For each trial, the mouse was placed in the water at a same location. A maximum of 2 min was allowed, during which the mouse had to find the platform and climb onto it. It gets on the platform & usually rears and looks around, where it was allowed to stay for 20 sec. The inter-trial-interval (ITI), within 10 trials, was 4~5 min. After a some training swims, the mouse will go direct to the platform. After 20 sec lift it off the platform and gently dry it. The latency to mount the escape platform was recorded on the trial and used as a measure of learning.

(2) Memory training

At least 24 h elapsed between the last trial of one day and the first trial of the next day. All methods are same with learning session. An one-trial retention test session was conducted 1 day, 5 day, 9 day after completion of the training.

(3) Ovariectomy

In all experiments, female mice were ovariectomized under ketamine anesthesia. Administration of drugs was conducted 3 weeks after surgery.

(4) Administration of drug

3 weeks after ovariectomy surgery, all mice were subcutaneously implanted with silastic tubing (SF medical Co.) filled with 17β-estradiol (E2) and ginsenosides in sesame oil. Estrogen and ginsenoside were packed into a 0.5 cm long silastic tube (id, 1.47 mm: od, 1.96 mm) which was sealed at both ends with a medical adhesive

silicon glue (Dow Corning) and then subcutaneously implanted to the dorsal of the neck under ketamine anesthesia. The incision was closed with surgical clips and animal allowed to recover. Behavior test was conducted 2 weeks after surgery.

(5) Serum estrogen assay

Mice were anesthetized with an intraperitoneally (i.p.) injection of a ketamine in sterile 0.1% PBS (1:1 v/v; 0.1 m*l*/100g body weight) and blood was collected by cardiacpuncture for the determination of serum levels of estradiol. Eppendorf tubes containing whole blood were placed on ice for 20~30 mim and then centrifuged at 2000 ×g for 10 min. Serum was collected into eppendorf tubes and stored at 2~8°C for up to 24 hour, and should be frozen at -20°C for longer periods. Serum levels of estradiol were determined by enzyme-linked immunosorbent assay (ELISA) using commercially available kits (Diagnostic Products Co.). The lower level of detectability was 4.6 pg/m*l*.

(7) Statistic

Results of the escape latency were expressed as means ±S.E.M. Statistical analysis of the escape latency values from intact (Sham control) and OVX, estradiol treated, ginsenosides treated mice was determined using Student's unpaired *t*-test. The value were considered statistically significant with P value<0.05.

RESULTS

1. Learning and memory of OVX

The ovariectomized (OVX) mice were tested to remove any impairing effect of estrogen on performance. OVX mice showed significantly longer escape latencies than intact mice on the acquisition (Fig. 1).

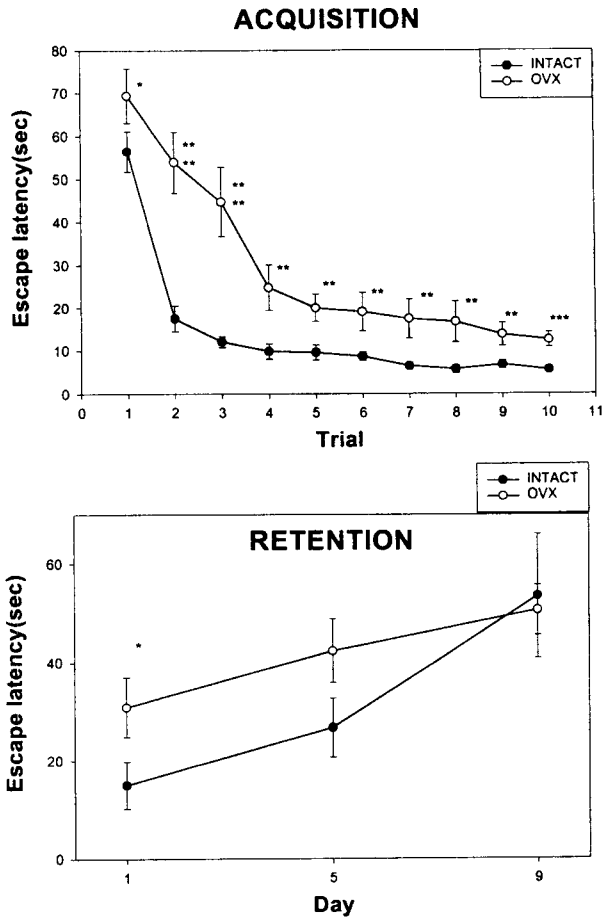

Fig. 1. Acquisition and retention of escape latency in the Morris water maze with intact and ovariectomized (OVX) female mice. The error bars present the standard error of the means. Statistical significance of differences between OVX and intact groups was assessed by Student's unpaired *t*-test (*P<0.05, **P<0.005, ***P<0.00005, ****P<0.0000005). INTACT : Not ovariectomized (n=30), OVX : Ovariectomized (n=30)

2. Learning and memory of E2

The effects of pretraining implant of 17β-estradiol (100 μg/m*l*, 150 μg/m*l*, 250 μg/m*l*) on acquisition and retention is shown in Fig. 2. In the acquisition test trials, a significant effects on group 1 (17β-estradiol 100 μg/m*l* implant) trial 2 (**P<0.005) and trial 3 (*P<0.05); group 2 (17β-estradiol 150 μg/m*l* implant) trial 2 (**P<0.005) and trial 3 (*P<0.05), trial 5 (*P<0.05), trial 10 (*P<0.05); group 3 (17β-estradiol 250 μg/m*l* implant) trial 2 (**P<0.005) and trial 3 (*P<0.05) were observed. In retention test trials, a significant effects on group 1 (17β-estradiol 100 μg/m*l* implant) day 1 (*P<0.05) and day 5 (*P<0.05), day 9 (*P<0.005); group 2 (17β-estradiol 150 μg/m*l* implant) day 1 (*P<0.05), day 5 (*P<0.05) and day 9 (*P<0.005); group 3 (17β-estradiol 250 μg/m*l* implant) day 1 (*P<0.05), day 5 (*P<0.05) and day 9 (*P<0.005) were observed. Similar tests revealed that the escape latencies of mice given each of the three doses of 17β-estradiol were significantly lower than those of ovariectomized (OVX) mice on acquisition test trial 2 (P<0.05, all groups), retention test day 9 (P<0.05, all groups). Furthermore, E2 (17β-estradiol)-implant mice showed a marked acceleration in their rate of learning between trial 1 and 2. The implant of 17β-estradiol is indicating a time-dependent effect of estradiol on memory storage processes.

Plasma E2 was obtained from behaviorally tested animals. The mean serum levels of estradiol detected in each

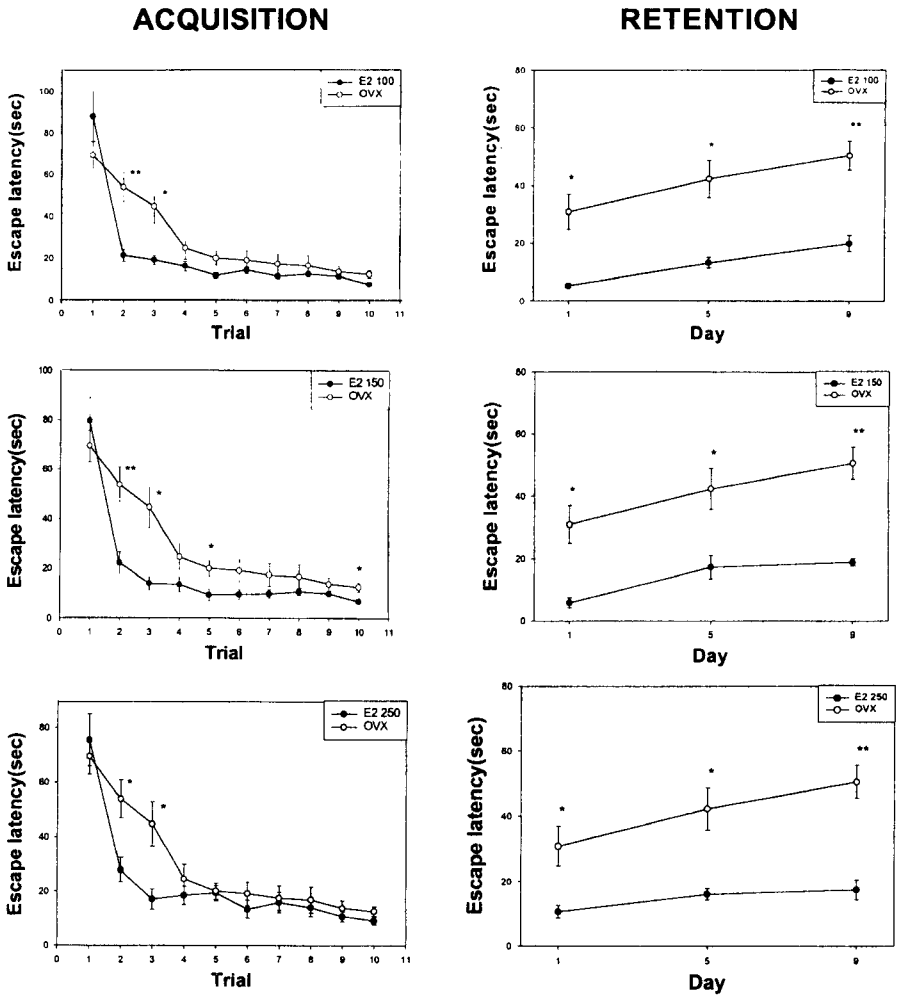

Fig. 2. Effects of pretraining implant of 17β-estradiol (100 μg/m*l*, 150 μg/m*l*, 250 μg/m*l*) on acquisition and retention task. Statistical significance of differences between E2-implant and OVX groups was assessed by Student's unpaired *t*-test (*P<0.05, **P<0.005). E2 100 : 17β-estradiol 100 μg/m*l* implant (n=10), E2 150 : 17β-estradiol 150 μg/m*l* implant (n=10), E2 250 : 17β-estradiol 250 μg/m*l* implant (n=10), OVX : Ovariectomized (n=30).

Table 1. Serum levels of estradiol detected after implant 100, 150, 250 μg/m*l* E2 (17β-estradiol) for two weeks

Dose (μg/m*l*)	2 Weeks
OVX	10.7±3.0
INTACT	62.4±2.3
E2 100	67.0±3.4
E2 150	74.0±1.4
E2 250	74.5±1.2

Values are mean serum levels of estradiol (pg/m*l*)±S.E.M

group are summarized in Table 1.

Serum E2 concentrations were 62.4±2.3 pg/m*l* for the INTACT and ovariectomy reduced serum E2 concentrations to below 10.7±3.0 pg/m*l* for the OVX. As expected, implants of 100, 150, 250 μg/m*l* estradiol administered two weeks resulted in serum levels of estradiol in the high physiological (67.0~74.5 pg/m*l*) range. Four mice were sampled.

3. Learning and memory of PD

The effects of pretraining implant of panaxadiol (PD) saponins (15 μg/m*l*, 50 μg/m*l*, 100 μg/m*l*) on acquisition and retention is shown in Fig. 3. In the acquisition test, a significant effects on group 1 (PD 15 μg/m*l* implant) trial 1 (***P<0.0005) and trial 2 (**P<0.005) and trial 3 (*P<0.05); group 2 (PD 50 μg/m*l*) trial 1 (*P<0.05), trial 2 (**P<0.005), trial 3 (*P<0.05) and trial 5 (*P<0.05); group 3 (PD 100 μg/m*l*) trial 1 (**P<0.005), trial 2 (**P<0.005), trial 3 (*P<0.05) and trial 5 (*P<0.05) were observed. In the retention test, a significant effects on

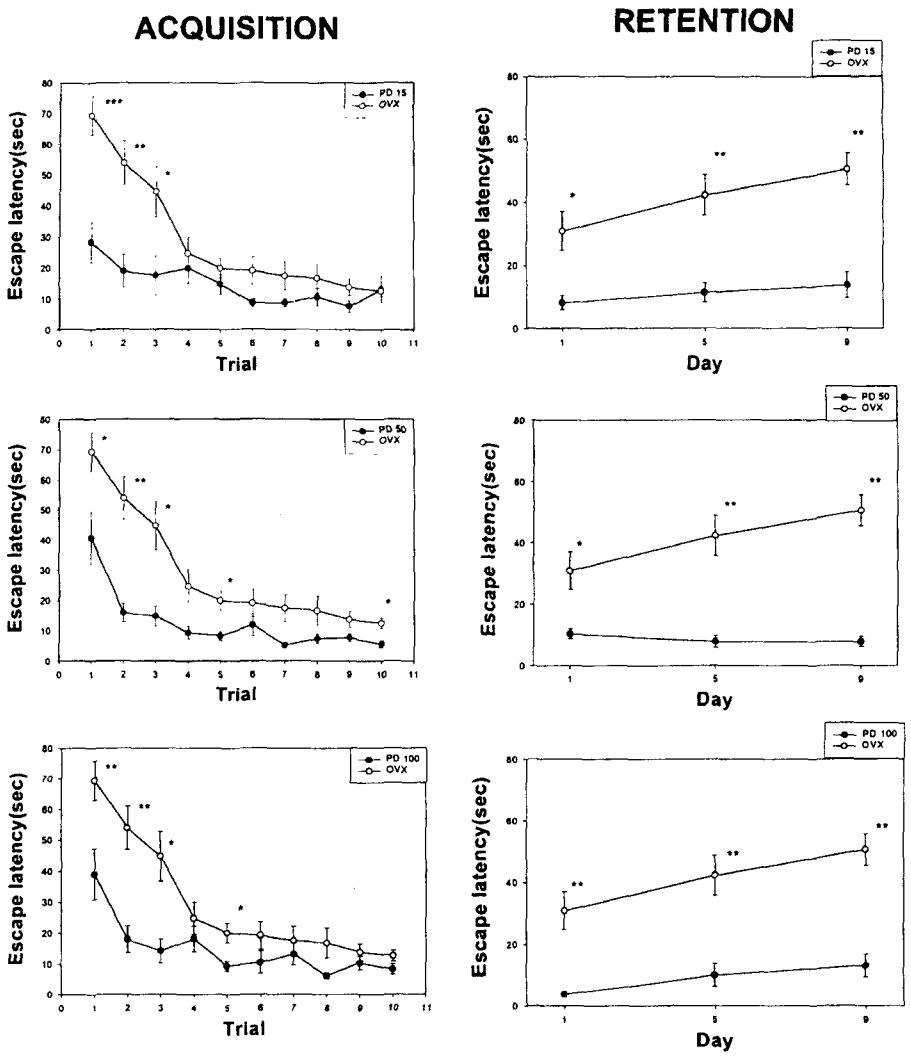

Fig. 3. Effects of pretraining implant of panaxadiol (PD) saponins (15 μg/m*l*, 50 μg/m*l*, 100 μg/m*l*) on acquisition and retention task. Statistical significance of differences between PD-implant and OVX groups was assessed by Student's unpaired *t*-test (*P<0.05, **P<0.005). PD 15 : PD saponins 15 μg/m*l* implant (n=10), PD 50 : PD saponins 50 μg/m*l* implant (n=10), PD 100 : PD saponins 100 μg/m*l* implant (n=10), OVX : Ovariectomized (n=30).

group 1 (PD 15 μg/m*l* implant) day 1 (*P<0.05), day 5 (*P<0.05) and day 9 (***P<0.0005); group 2 (PD 50 μg/m*l*) day 1 (*P<0.05), day 5 (**P<0.005) and day 9 (***P<0.0005); group 3 (PD 100 μg/m*l*) day 1 (*P<0.05), day 5 (*P<0.05) and day 9 (***P<0.0005) were observed. Similar tests revealed that the escape latencies of mice given each of the three doses of PD saponins were significantly lower than those of ovariectomized mice on acquisition test trial 2 (P<0.005, all groups), retention test day 9 (P<0.0005, all groups). Furthermore, PD-implant mice showed a marked acceleration in their rate of learning between trial 1 and 2. Results are indicating a time-dependent effect of PD saponins more on memory storage processes than learning task. In addition, the effects of pretraining implant of panaxadiol (PD) saponins (15 μg/m*l*, 50 μg/m*l*, 100 μg/m*l*) on acquisition and retention is shown in Fig. 4. However, there was no significant differences between PD-implant and INTACT groups both in the acquisition and retention tests.

4. Learning and memory of PT

The effects of pretraining implant of panaxatriol (PT) saponins (15 μg/m*l*, 50 μg/m*l*, 100 μg/m*l*) on acquisition and retention is shown in Fig. 5. In the acquisition test, group 1 (PT 15 μg/m*l* implant) trial 9 (**P<0.005) and trial 10 (*P<0.05); group 2 (PT 50 μg/m*l*) trial 2

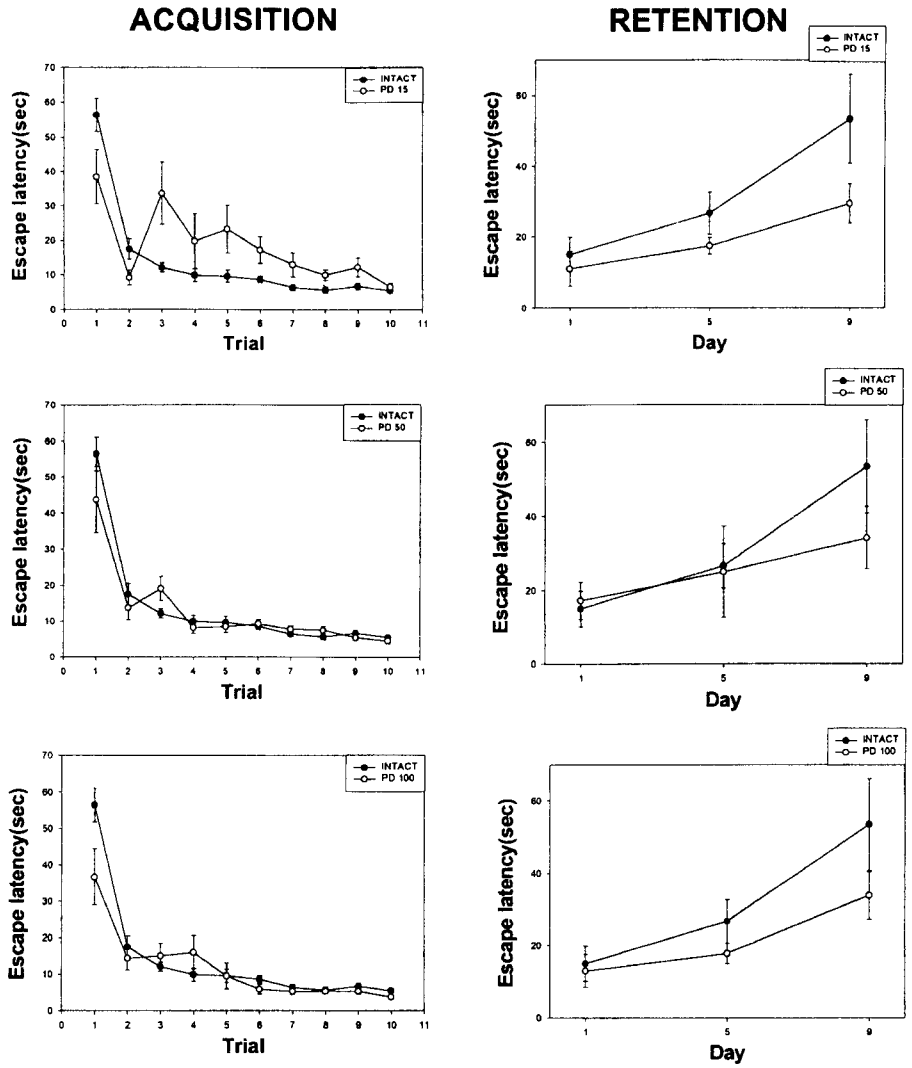

Fig. 4. Effects of pretraining implant of panaxadiol (PD) saponins (15 μg/m*l*, 50 μg/m*l*, 100 μg/m*l*) on acquisition and retention test in INTACT animals. PD 15 : PD saponins 15 μg/m*l* implant (n=10), PD 50 : PD 50 μg/m*l* (n=10), PD 100 : PD 100 μg/m*l* (n=10), OVX : Ovariectomized (n=30).

(**P<0.005) and trial 3 (*P<0.05); group 3 (PT 100 μg/m*l*) trial 1 (*P<0.05) and trial 3 (*P<0.05) show significant effects. In the retention test, a significant effects on group 1 (PT 15 μg/m*l* implant) day 5 (*P<0.05) and day 9 (***P<0.0005); group 2 (PT 50 μg/m*l*) day 5 (**P<0.005) and day 9 (***P<0.0005); group 3 (PT 100 μg/m*l*) day 1 (*P<0.05) and day 9 (***P<0.0005) were observed. Similar tests revealed that the escape latencies of mice given each of the three doses of PT saponins were significantly lower than those of ovariectomized mice on acquisition test trial 2 (P<0.005, only PT50 groups), retention test day 9 (P<0.0005, all groups). Furthermore, only PT50-implant mice showed a marked acceleration in their rate of learning between trial 1 and 2. The implant of PT saponins is indicating more time-dependent effect on memory storage processes than learning task. The effects of pretraining implant of panaxatriol (PT) saponins (15 μg/m*l*, 50 μg/m*l*, 100 μg/m*l*) on acquisition and retention is also shown in Fig. 6. Acquisition and retention tests show no significant differences between PT-implant and INTACT groups.

DISCUSSION

There are no universally accepted definitions of learning and memory. All studies of learning and memory are

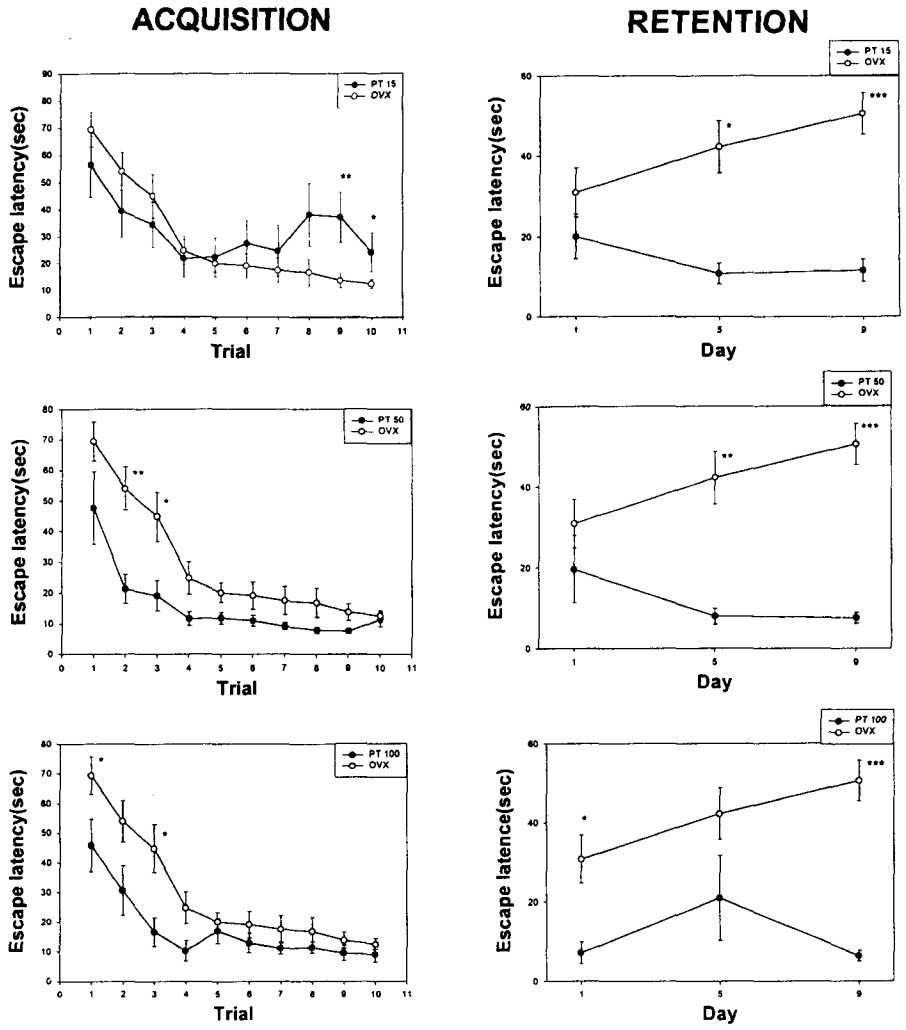

Fig. 5. Effects of pretraining implant of panaxatriol (PT) saponins (15 μg/m*l*, 50 μg/m*l*, 100 μg/m*l*) on acquisition and retention task. Statistical significance of differences between PT-implant and OVX groups was assessed by Student's unpaired *t*-test (*P<0.05, **P<0.005). PT 15 : PT saponins 15 μg/m*l* implant (n=10), PT 50 : PT saponins 50 μg/m*l* implant (n=10), PT 100 : PT saponins 100 μg/m*l* implant (n=10), OVX : Ovariectomized (n=30).

faced with the obvious but troublesome fact that learning and memory cannot be studied directly. It can only make suggestions about learning and memory on the basis of the animal behavior observations. Many types of tasks and training procedure are used in studies of learning and memory. This experiment aim, in the water maze, is to test the capacity of mice to learn a spatially-encoded information and to demonstrate retention of an experiences by performing task. Estradiol delivered by Silastic implants training significantly improved escape latency during acquisition and memory of the Morris water maze in ovariectomozed mice. In addition, panaxadiol saponins (PD) and panaxatriol saponins (PT) implants training significantly improved more retention task than acquisition task in ovariectomized mice. The first retention test trial provides the most sensitive measure of the animal's memory for the previous day of training, and hence the lower escape latencies exhibited by estradiol-treated mice on this trial clearly indicate a memory-enhancing effect of the hormone. A number of recent reports have documented that estrogen can positively influence performance on certain learning and memory tasks.[18,24,25] The results of the present experiment further support to the hypothesis that estrogen can promote learning and memory on certain types of tasks, in this case by improving spatial memory performance during acquisition of the Morris water maze. Although the mechanism by which estrogen affects learning and memory performance have

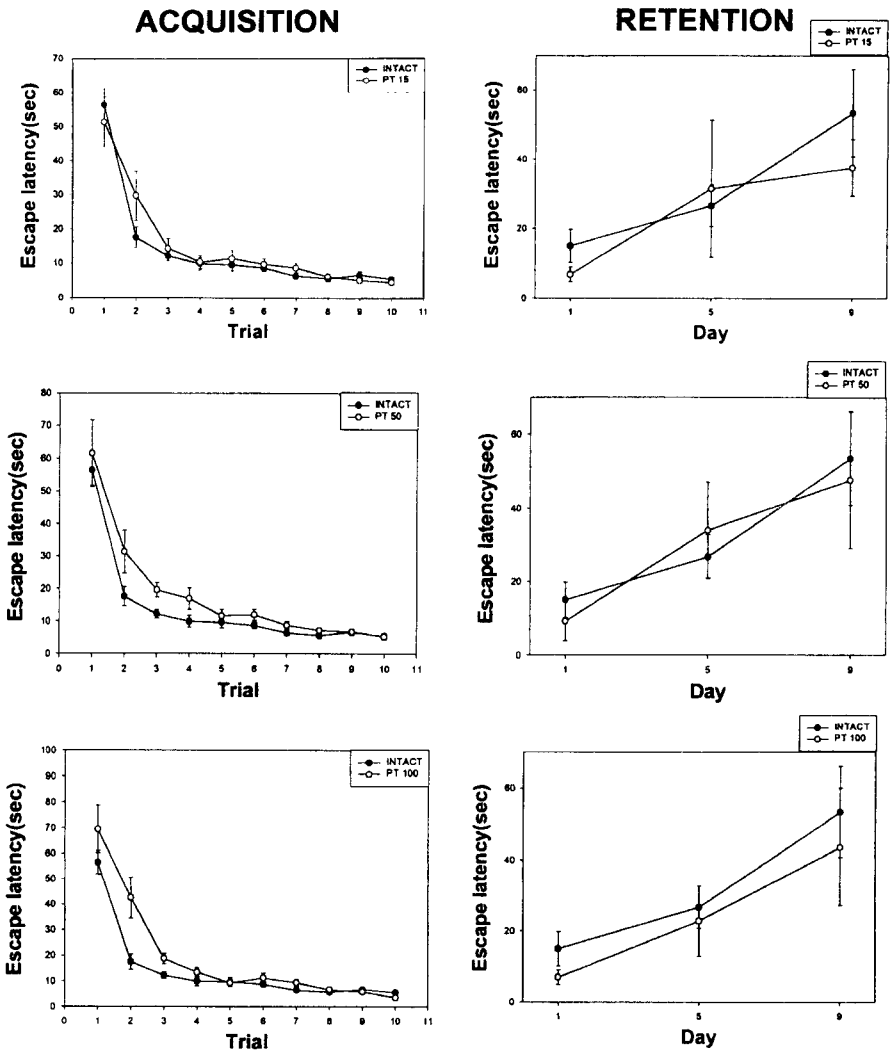

Fig. 6. Effects of pretraining implant of panaxatriol (PT) saponins (15 μg/m*l*, 50 μg/m, 100 μg/m) on acquisition and retention test in INTACT animals. PT 15 : PT saponins 15 μg/m implant in INTACT animals (n=10), PT 50 : PT 50 μg/m (n=10), PT 100 : PT 100 μg/m (n=10), OVX : Ovariectomized (n=30).

not been identified, there are several interesting possibilities such as through the restructuring of dendrites and synapses in the hippocampus,[26] prevention of reduction in hippocampal sprouting induced by gonadectomy.[27] Recent reports indicate that estrogen replacement in postmenopausal women is correlated with a later age of onset and a reduced incidence and severity of Alzheimer's disease, and illness characterized by memory impairment.[9,19-20,28-30] Although the duration and level of estrogen exposure necessary to slow the development and proliferation of Alzheimer's disease are unknown, longer durations of exposure are correlated with greater reductions in risk in some studies. The pharmacological action of *Panax ginseng* compiled from the numerous reports can be summarized as follows: On central nervous systems, the effect of *Panax ginseng* is stimulatory in smaller doses and somewhat depressive in larger doses, seems to increase the mental efficiency of man, protect organism from various physical and chemical stresses and stimulate the growth and basal metabolic rates of experimental animals. Ginseng also prolongs the survival time of animals under adverse influences, increases the physical and mental efficiency, and postpones the onset of fatigue and increases the working capacities. And slight hypotensive effect is also observed. Ginseng tends to stimulate the biosynthesis of nucleic acid and release of histamine and serotonin. Anticancer effects of ginseng seem to be due to indirect action rather than direct action on cancer cell, by improving the host

condition. Recent clinical trials of ginseng have obtained some good results, but still it is necessary to broaden the scope covering many kinds of organ and disease. And analysis of the chemical components and newer standardized concepts and methods appear to be the pre-requisites for further study of the pharmacological effects and mechanisms of *Panax ginseng*.[31] These experiments show that ginsenosides do act like already known estrogen for behavioral effects. Although the Morris water maze remains a valuable preclinical test with better validity and specificity than many other behavioral tests, measures of performance in the Morris water maze should not be considered synonymous with cognitive function. However, there are not yet enough evidences as an estrogenic effects. Further researches are required for the confirmation and the detailed mechanism of ginseng action in the central nervous system.

요 약

스테로이드 호르몬의 일종인 에스트로젠은 생식기능에 영향을 미치는 것 외에 학습 및 기억과 관련된 기능에도 영향을 미치는 것으로 알려져 있다. 최근, 에스트로젠은 기억과 관련된 뇌세포 신경망의 발달과 뇌 기능 장애를 방지할 수 있다는 부분에서 상당한 관심의 대상이 되고 있다. 그러나 에스트로젠 대체 치료가 폐경기의 많은 여성들에게 도움을 주기도 하지만 여러 부작용을 유발하는 것으로도 알려져 있다. 인삼 역시도 스테로이드 특성을 보이며 에스트로젠과 유사한 화학구조를 가지는 여러 성분을 가지고 있다. 본 실험의 목적은 첫째로 공간 기억력을 측정하기에 여러 장점을 가지고 있으면서 다른 어떠한 행동학적 실험보다 학습과 기억의 동물 모델로 잘 알려진 방법인 Morris water maze를 이용하여 에스트로젠의 효과를 확인하고, 두 번째는 인삼이 학습과 기억에서 에스트로젠과 같은 효과를 나타낼 수 있는지를 확인하는 것이다. 본 실험은 인위적으로 난소를 제거한 쥐에 17β-estradiol(100~250 μg/m*l*), panaxadiol(PD), panaxatriol(PT) saponins(15~100 μg/m*l*)을 sesame oil에 녹인 capsule을 implant 했다. 첫 번째 실험에서 난소를 제거한 쥐에 에스트로젠을 투입했을 때 학습과 기억의 효과를 확인했다. 두 번째 실험에서는 난소를 제거한 쥐에 3가지 다른 농도에서의 PD, PT를 투입했을 때 학습과 기억에 대한 에스트로젠의 효과와 비교해 보았다. 2주 동안의 implant 후 water maze 실험결과 세 그룹 모두 난소를 제거한 그룹보다 기억력이 향상되었다. 이러한 결과를 토대로 에스트로젠이 학습과 기억에 영향을 준다는 것을 확인할 수 있었고 PD, PT 또한 학습과 기억에 관련된 행동에서 에스트로젠과 같은 효과를 나타낼 수 있다는 것을 확인할 수 있었다. 이러한 동물모델에서의 연구를 통하여 인삼이 에스트로젠 장기결핍치료에서 나타나는 여러 호르몬 부작용을 극복할 수 있는 에스트로젠 대체물질로 개발되어 기억력 저하를 수반하는 Alzheimer's disease 및 여러 퇴행성 중추신경 질환의 치료제로 대체의학의 natural compound 이용에 그 기초 기전을 제공할 수 있으리라 여긴다.

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REFERENCES

1. Brown, M. D. : The management of the menopause and post-menopausal years. *University Park Press.*, Baltimore, p. 109 (1976).
2. Furuhjelm, M., Fedor-Freybergh, P. : The influence of estrogens on the psyche in climacteric and post-menopausal women. (1976).
3. Kopera, H. : *Front Hormone Res* **2**, 118 (1973).
4. Sulkava, R., Wikstrom, J., Aromma, A., Raitasalo, R., Lahtela, K., and Palo, J. : *Neurology* **35**, 1025 (1985).
5. Hampson, E. and Kimura, D. : *Behavioral Neuroscience* **102**, 456 (1988).
6. Hampson, E. : *Brain and Cognition* **14**, 26 (1990).
7. Phillips, S. and Sherwin, B. B. : *Psychoneuroendocrinology* **17**, 485 (1992).
8. Janowsky, J.S., Oviatt, S. K. and Orwoll, E. S. : *Behavioral Neuroscience,* **108**, 325 (1994).
9. Paganini-Hill, A. and Henderson, V. W. : *Am. J. of Epidemiology* **140**, 256 (1994).
10. Burke, A. W. and Broadhurst, P. L. : *Nature* **209**, 223 (1966).
11. I'Kard, W. L., Bennett, W. C., Lundin, R. W. and Trost, R. C. : *Psycho. Rec.* **22**, 249 (1972).
12. Drewett, R. F. : *Animal Behav* **21**, 772 (1973).
13. Gray, P. : *Hormones Behav* **8**, 235-241 (1977).
14. Sifkakis, A., Spyraki, C., Sitaras, N. and Varonos, D. : *Physiol. Behav* **21**, (1978).
15. Williams, C. L., Barnett, A. M. and Meck, W. H. : *Behavioral Neuroscience* **104**, 84 (1990).
16. Alliot J. and Giry N. : *Neuroreport* **2**, 101 (1991).
17. Williams, C. L. and Meck, W. H. : *Psychoneuroendocrinology* **16**, 157 (1991).
18. Luine, V. N. and Rodriguez, M. : *Behavioral and Neural Biology* **62**, 230 (1994).
19. Henderson, V. W., Paganini-Hill, A., Emanuel, C. K., Dunn, M. E. and Buckwalter, J. G. : *Archives of Neurology* **51**, 896 (1994).
20. Tang, M., Jacobs, D., Stern, Y., Marder, K., Schofield, P., Gurland, B. and Andrews, H. : *Lancet* **348**, 429 (1996).
21. Hong, S. A., Park, C. W., Kim, J. H., Hong, S. K., Chang, H. K. and Kim, M. S. : *Korean J. Pharm* **10**, 1 (1974).
22. Hong, S. A., Park, C. W. and Chang, H. K. : *Korean J. Pharm* **12**, 63 (1976).
23. Petkov, Y. D. and Mosharrof, A. H. : *Am. J. Chin. Med.* **15**, 19 (1987).

24. Singh, M., Meyer, E. M., Millard, W. J., and Simpkins, J. W. : *Brain Res* **644**, 305 (1994).
25. Williams, C. L. : *Soc. Neurosci. Abs.* **22**, 1164 (1996).
26. O'Keefe, J. and Nadel, L. : The hippocampus as a Cognitive Map. *Oxford Univ. Press*, London (1978).
27. Morse, J. K., Scheff, S. W. and Dekosky, S. T. : *Exp. Neurol* **94**, 649 (1986).
28. Fillit, H., Weinerb, H., Cholst, I., Luine, V., McEwen, B., Amandor, R. and Zabriskie, J. : *Psychoneuroendocrinology* **11**, 337 (1986).
29. Honjo, H. Ogino, Y., Naitoh, K., Urabe, M., Kitawaki, J., Yasuda, J., Yamamota, T., Ishihara, S., Okada, H., Yonezawa, T., Hayashi, K. and Nambara, T. : *J. of Steroid Biochem* **34**, 521 (1989).
30. O'neal, M. F., Means, L. W., Poole, M. C. and Hamm, R. J. : *Psychoneuroendocrinology* **21**, 51 (1996).
31. Hong, S. A., Lim, J. K., Park, C. W. and Cha, I. J. : *J. Ginseng Res* **3**, 66 (1979).