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Peony and Its Major Constituent, Paeoniflorin, Improve Radial Maze Performance Impaired by Scopolamine in Rats

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OHTA, H., J.-W. NI, K. MATSUMOTO, H. WATANABE AND M. SHIMIZU. *Peony and its major constituent, paeoniflorin, improve radial maze performance impaired by scopolamine in rats.* PHARMACOL BIOCHEM BEHAV **45**(3) 719–723, 1993.—A traditional Chinese medicine, Shimotsu-to has been shown to improve spatial working memory in rats. Shimotsu-to consists of four herbs, Japanese angelica root, cnidium rhizome, peony root, and rehmannia root. In the present study, the effects of aqueous extracts of each component herb on scopolamine (0.3 mg/kg)-induced spatial working memory disruption were examined using an eight-arm radial maze task in rats. Among the four component herbs, peony root extract (0.25 and 1 g dried herb/kg, PO) exhibited the most potent antagonizing effect on the scopolamine disruption of the choice accuracy. Japanese angelica root extract (1 g dried herb/kg, PO) also significantly attenuated the scopolamine disruption, whereas neither cnidium rhizome nor rehmannia root affected it. Paeoniflorin (0.01–1 mg/kg, PO), a major constituent of peony root, dose-dependently attenuated the scopolamine-induced impairment in the choice accuracy. Scopolamine (0.3 mg/kg, IP) significantly decreased the acetylcholine contents in the hippocampus, cortex, and striatum. Although paeoniflorin alone did not affect the acetylcholine contents, pretreatment with paeoniflorin significantly prevented the scopolamine-induced decrease in the acetylcholine content in the striatum, but not in the hippocampus or cortex. These data suggest that peony root mainly contributes to the cognitive enhancing effect of Shimotsu-to and that paeoniflorin may be one of the active constituents of peony root.

Peony root Paeoniflorin Scopolamine Working memory Radial maze Rats

SHIMOTSU-TO is a traditional Chinese medicine, which was first described in the twelfth century classic, the Fue-Ji-Ju-Fang. It has been clinically used to treat menstrual problems and autonomic imbalance in China and in Japan. Recently, Shimotsu-to has been experimentally demonstrated to have various psychotropic effects (12). It markedly decreases the aggressive behavior induced by long-term individual housing and reverses the pentobarbital-induced sleep duration shortened by a stressful manipulation, to the unstressed level in mice (7). In addition, our previous report (13) demonstrated that Shimotsu-to improves working memory performance impaired by scopolamine in an eight-arm radial maze and in a T-maze delayed alternation tasks in rats. Working memory is analogous to recent memory in humans. This type of memory is more severely impaired than remote memory in human dementia.

Shimotsu-to consists of four herbs; Japanese angelica root, cnidium rhizome, peony root, and rehmannia root. In the present study, to clarify which component herbs mainly contribute to the memory-enhancing effect of Shimotsu-to, the effects of aqueous extracts of the individual component herbs on the scopolamine-induced deficit in the radial maze performance were examined. Thereafter, to investigate the action mechanisms, the effects of the active constituent on the acetylcholine contents in brain tissues were measured.

METHOD

Animals

Male Wistar rats obtained from SLC (Shizuoka, Japan) weighing 290–390 g were used. Four or five rats were housed in a cage with free access to water in an air-conditioned room

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and subjected to a 12L : 12D cycle (lights on: 0730–1930 h). The animals were maintained on a restricted feeding schedule designed to keep their body weight at about 85% of the free-feeding level. Prior to maze training, each animal was handled for 5–10 min daily for 2 days and was also given 3 days to adapt to the maze.

Apparatus

Each arm (50 × 12 cm) of eight-arm radial maze extended from an octagonally shaped central hub (30 cm across). The platform was elevated 40 cm above the floor. Small black plastic cups (3 cm in diameter and 1 cm deep), mounted at the end of each arm, served as receptacles for reinforcers (45 mg food pellet; Bio-Serv, USA). Guillotine doors surrounded the hub.

Procedures

One daily training trial was conducted with each rat. At the beginning of trial, two food pellets were placed in each receptacle. A rat was placed on the center hub with all guillotine doors lowered. Then, all the doors were simultaneously opened to allow the rat to choose arms freely. When the rat entered one of the arms, the doors to the remaining seven arms were closed. The open door was closed after the rat returned to the center hub. All the doors were then raised again simultaneously. The trial was judged complete when the rat had visited all eight arms or had spent 10 min on the maze. Entry into an arm that the rat had not previously visited was recorded as a correct response and reentry was counted as an error. The number of correct responses before committing the first error (the number of initial correct responses) was calculated as the index of radial maze performance. A trial in which an animal made no errors, or only one error at the eighth choice, was defined as a "successful" trial. The percentage of successful rats (percent successful rats) was also used as an index. This index is thought to be highly sensitive to drug-induced behavioral changes in the radial arm maze (13). The total running time was divided by the total number of choices to calculate the running time. Only the rats which "succeeded" in trials for 5 consecutive days were used for experiments.

Drugs

Japanese angelica root (*Angelica acutiloba* KITAGAWA), cnidium rhizome (*Cnidium officinale* MAKINO), peony root (*Paeonia lactiflora* PALLAS), and rehmannia root (*Rehmannia glutinosa* LIBOSCHITZ) were purchased from Tochimoto (Osaka, Japan). To prepare the aqueous extract of each, 25 g of the dried herb was boiled with 250 ml distilled water at 100°C for 1 h. After filtration, the filtrate was freeze dried. The yield of Japanese angelica root, cnidium rhizome, peony root, and rehmannia root extracts were 28, 19, 44, and 36%, respectively. Paeoniflorin was extracted from peony root and purified as described by Shibata et al. (9) with a modification. The purity of the paeoniflorin was about 99%. Each herbal extract or paeoniflorin was orally (PO) administered 90 min before testing. Doses of the extracts were expressed as dried herb weight per kg body weight. Scopolamine hydrobromide (Nakalai Tesque, Kyoto, Japan) dissolved in physiological saline was intraperitoneally (IP) injected 30 min before testing. Drugs were tested in a counterbalanced order to exclude the possible involvement of order effects. Animals treated with scopolamine alone were always present in any pharmacological trial.

Determination of Acetylcholine

Rats were killed by microwave irradiation (2450 MHz, 9 kW, 1.0 s). The cerebral cortex, hippocampus, and striatum were dissected out rapidly and homogenized in 1 ml perchloric acid (0.2 N) containing 2 nmol ethylhomocholine as an internal standard. The homogenates were centrifuged at 10,000 × *g* at 4°C for 20 min. The supernatants were extracted with diethylether. The ACh levels were determined using HPLC equipped with an electrochemical detector. In this study, scopolamine (0.3 mg/kg) was administered IP, 45 min prior to microwave irradiation. Paeoniflorin (0.1 or 1 mg/kg, PO) was administered 60 min before the scopolamine injection.

Statistical Analysis

The effects of the drugs on the number of initial correct responses and the running time were analyzed by the Kruskal-Wallis analysis of variance followed by Mann-Whitney's *U*-test for multiple comparisons. The percentage of successful rats was analyzed using Fisher's exact probability test. Thus the distribution of success or failure was compared among several pairs of rats. The effects of the drugs on the acetylcho-

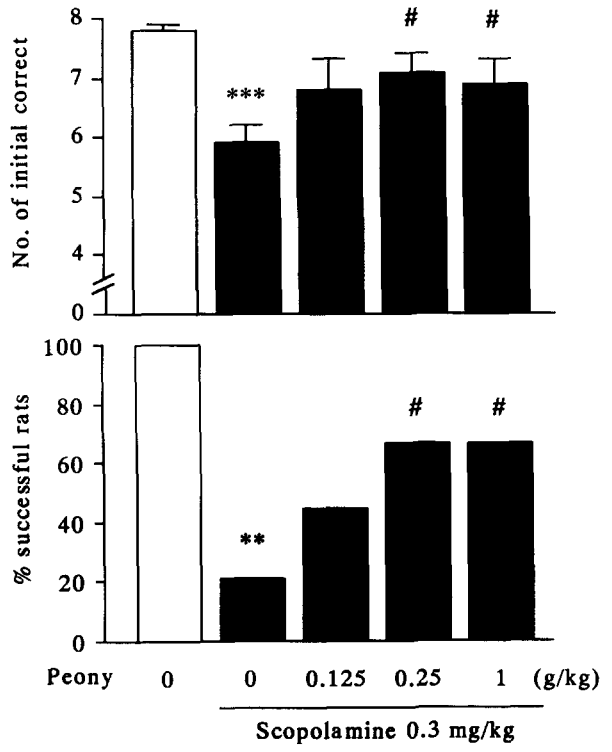

FIG. 1. Effect of peony root extract on scopolamine-induced disruption of radial maze performance. The top panel represents the mean (± SE) number of correct responses before committing the first error. The bottom panel represents the percentage of rats that completed the trial with no error, or only one error at the eighth choice. Saline (open column, $n = 10$) or scopolamine hydrobromide was administered IP 30 min before testing. Peony root extract 0 g/kg ($n = 24$), 0.125 g/kg ($n = 9$), 0.25 g/kg ($n = 12$), or 1 g/kg ($n = 12$) was administered PO 60 min before the scopolamine injection. Doses of herbal extract are expressed as dried herb weight per kg body weight. $^{**}p < 0.01$, $^{***}p < 0.001$ compared with the saline group. $\#p < 0.05$ compared to scopolamine alone (Mann-Whitney's *U*-test, top panel; Fisher's exact probability test, bottom panel).

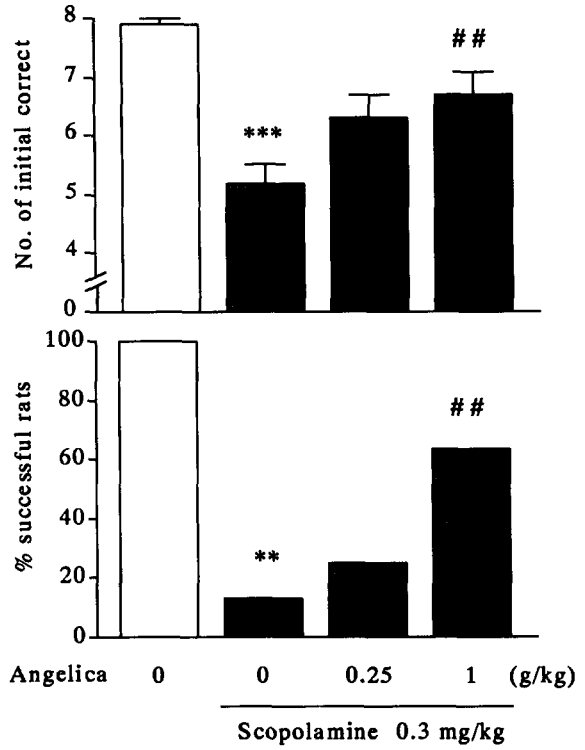

FIG. 2. Effect of Japanese angelica root extract on scopolamine-induced disruption of radial maze performance. Explanations are as described in the legend to Fig. 1. Saline (open column, $n = 10$) or scopolamine was administered IP 30 min before testing. Japanese angelica root extract 0 g/kg ($n = 23$), 0.25 g/kg ($n = 12$), or 1 g/kg ($n = 11$) was administered PO 60 min before the scopolamine injection. $^{**}p < 0.01$, $^{***}p < 0.001$ compared with the saline group. $^{\#\#}p < 0.01$ compared with scopolamine alone (Mann-Whitney's U-test, top panel; Fisher's exact probability test, bottom panel).

line contents were analyzed using ANOVA followed by Duncan's test for multiple comparison. Differences with a $p < 0.05$ were considered statistically significant.

RESULTS

Effects of Component Herbs on Scopolamine Disruption of Maze Performance

In all experiments, scopolamine (0.3 mg/kg, IP) significantly decreased both the number of initial correct responses and the percentage of successful rats (Figs. 1-4). The running time of scopolamine-treated rats (16.4 ± 0.9 s) was significantly longer than that of control rats (8.9 ± 1.0 s) ($p < 0.001$).

Pretreatment with peony root extract attenuated the scopolamine disruption of the choice accuracy of radial maze performance in a dose-dependent manner (Fig. 1). Peony root extract at doses of 0.25 and 1 g/kg significantly increased the scopolamine-induced decrease in the initial correct responses and the percentage of successful rats. However, neither dose affected the running time prolonged by scopolamine (data not shown).

Extract of Japanese angelica root also dose-dependently antagonized the disruptive effect of scopolamine on the accuracy of radial maze performance (Fig. 2). At a dose of 1 g/kg, this extract exhibited a significant increase in the initial correct responses and the percentage of successful rats impaired by scopolamine. Japanese angelica root extract did not shorten the running time prolonged by scopolamine (data not shown).

Neither cnidium rhizome (Fig. 3A) nor rehmannia root (Fig. 3B) extract affected the radial maze performance disrupted by scopolamine.

Effect of Paeoniflorin on Scopolamine Disruption of Maze Performance

Pretreatment with paeoniflorin (0.01-1 mg/kg) dose-dependently attenuated the scopolamine disruption of the choice accuracy (Fig. 4). Paeoniflorin at a dose of 1 mg/kg significantly increased the initial correct responses and the percentage of successful rats impaired by scopolamine. The

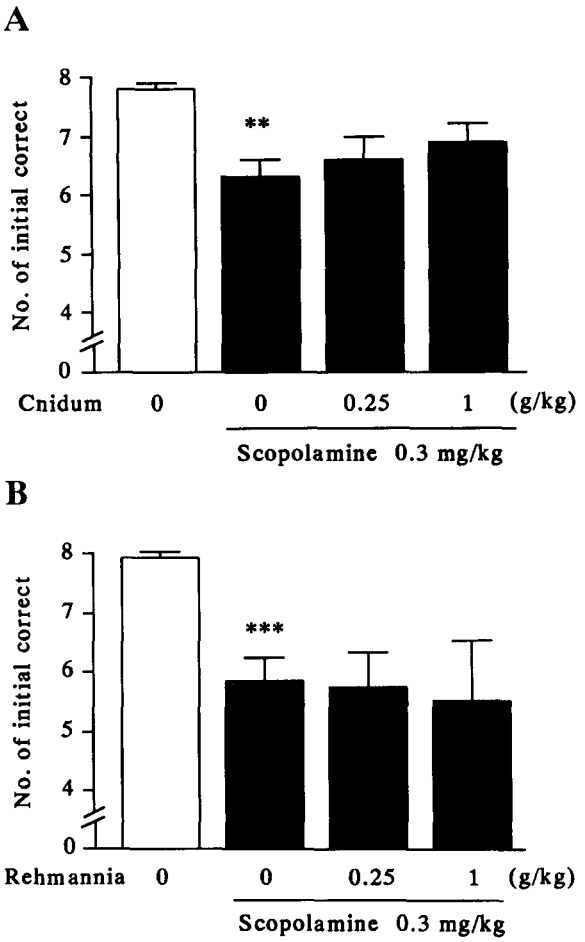

FIG. 3. Effects of cnidium rhizome extract or rehmannia root extract on scopolamine-induced disruption of radial maze performance. (A) Saline (open column, $n = 10$) or scopolamine was administered IP 30 min before testing. Cnidium rhizome extract 0 g/kg ($n = 22$), 0.25 g/kg ($n = 12$), or 1 g/kg ($n = 10$) was administered PO 60 min prior to scopolamine injection. (B) Saline (open column, $n = 10$) or scopolamine was administered IP 30 min before testing. Rehmannia root extract 0 g/kg ($n = 12$), 0.25 g/kg ($n = 6$), or 1 g/kg ($n = 6$) was administered PO 60 min prior to scopolamine injection. $^{**}p < 0.01$, $^{***}p < 0.001$ compared with the saline group (Mann-Whitney's U-test).

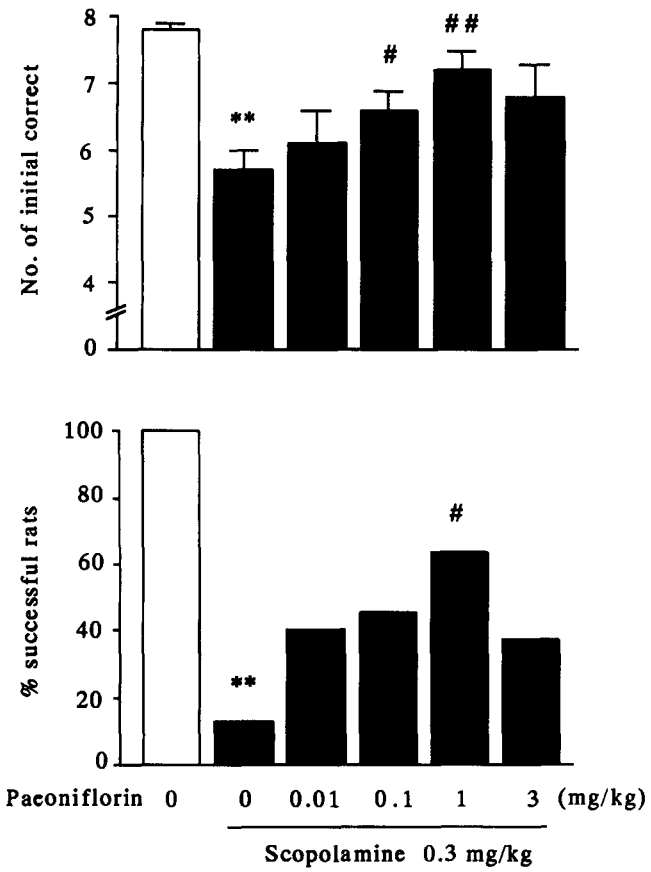

FIG. 4. Effect of paeoniflorin on scopolamine-induced disruption of radial maze performance. Explanations are as described in the legend to Fig. 1. Saline (n = 10) or scopolamine was administered IP 30 min before testing. Paeoniflorin 0 mg/kg (water, n = 15), 0.01 mg/kg (n = 10), 0.1 mg/kg (n = 11), 1 mg/kg (n = 11), or 3 mg/kg (n = 8) was administered PO 60 min before the scopolamine injection. $^{**}p < 0.01$ compared with the saline group. $\#p < 0.05$, $\#\#p < 0.01$ compared with scopolamine alone (Mann-Whitney's U-test, top panel; Fisher's exact probability test, bottom panel).

effect of 3 mg/kg paeoniflorin, however, was less potent than that of 1 mg/kg. Neither dose of paeoniflorin reversed the scopolamine-induced prolongation of the running time (data not shown).

Effect of Paeoniflorin on Brain Acetylcholine Levels

Paeoniflorin (0.1 and 1 mg/kg) alone did not affect the ACh contents in the hippocampus, cerebral cortex, or striatum of the scopolamine-untreated rats (Table 1). Scopolamine (0.3 mg/kg) significantly decreased the ACh levels in these three brain regions (Table 1). Pretreatment with paeoniflorin significantly prevented the scopolamine-induced decrease in the ACh content in the striatum, but not in the hippocampus or the cerebral cortex (Table 1).

DISCUSSION

In the present study, peony root extract and its major constituent, paeoniflorin, significantly reinstated the radial maze performance impaired by scopolamine. Our previous study has demonstrated that a Chinese medicine, Shimotsu-to, ameliorates spatial working memory deficits produced by scopolamine in an eight-arm radial maze task and in a T-maze delayed alternation task in rats (13). This medicine consists of Japanese angelica root, peony root, cnidium rhizome, and rehmannia root in the proportions 1 : 1 : 1 : 1. The memory-enhancing effects of Shimotsu-to were observed at a dose of 1 g dried herbs/kg (PO) in both tasks. In the present study, peony root extract significantly attenuated the scopolamine-induced deficit in the radial maze performance at 0.25 g/kg which corresponds to 1 g/kg Shimotsu-to. Although the extract of Japanese angelica root also dose-dependently antagonized the scopolamine disruption of maze performance, the effect was less potent than that of peony root extract. Therefore, these results suggest that the memory-enhancing effect of Shimotsu-to may attribute mainly to the effect of the peony root component.

Several constituents have been isolated from peony root, such as paeoniflorin (9), albiflorin, oxypaeoniflorin, and benzoylpaeoniflorin (5). Among them, paeoniflorin is known to be the main constituent of this herb, and the clinical effects of peony root are reported to be in good accordance with the effects of paeoniflorin (10). The ratio of paeoniflorin has been

TABLE 1

EFFECTS OF PAEONIFLORIN ON THE SCOPOLAMINE-INDUCED DECREASE IN ACh LEVELS IN THE BRAIN

Paeoniflorin (mg/kg)	Scopolamine (mg/kg)	n	ACh Content (nmol/g tissue)		
			Hippocampus	Cortex	Straitum
–	–	7	26.8 ± 0.4	14.3 ± 0.3	102.8 ± 3.8
0.1	–	7	27.0 ± 0.7	13.6 ± 0.5	107.2 ± 3.3
1	–	7	27.7 ± 0.7	13.7 ± 0.4	101.9 ± 4.8
–	0.3	9	22.5 ± 1.3*	10.1 ± 0.9*	85.2 ± 5.2*
0.1	0.3	9	22.4 ± 1.5*	11.0 ± 1.0*	86.5 ± 5.4*
1	0.3	9	23.4 ± 1.4†	11.1 ± 1.1*	99.4 ± 4.3‡

Paeoniflorin was orally administered. One hour thereafter, saline or scopolamine was intraperitoneally injected. Rats were killed by microwave irradiation 45 min after the injection. Each value represents the mean ± SE.

*$p < 0.01$; †$p < 0.05$ compared with saline control.

‡$p < 0.05$ compared with scopolamine alone (Duncan's multiple range test).

reported to be 2-3% of the dried peony root weight (1,14). This compound has very low toxicity, and the LD_{50} (IP) is 9.53 g/kg in mice (10). In the present study, paeoniflorin attenuated scopolamine-induced impairment in the working memory at 1 mg/kg, which was approximately equivalent to 50 mg/kg of peony root extract. This suggests that paeoniflorin is one of the potent active constituents of peony root.

Paeoniflorin did not reinstate the scopolamine-induced decrease in ACh contents in the hippocampus or the cerebral cortex. This suggests that the antagonizing effect of this compound on the scopolamine disruption of maze performance may not be due to direct stimulation of the cholinergic systems. Paeoniflorin, however, partially antagonized the scopolamine decrease in striatal ACh content. Lesions of the anterodorsal caudate nucleus is reported to impair the radial maze performance (6). This suggests the involvement of striatal function in the maintenance of the maze performance. Therefore, we cannot exclude the possibility that paeoniflorin may improve the radial maze performance by activating the striatal cholinergic system.

The mechanisms underlying the memory-enhancing effect of paeoniflorin remain unclear. Paeoniflorin reportedly binds to the glucocorticoid receptor and the mineral-corticoid receptor, and to a lesser extent, the estrogen receptor, sex hormone-binding globulin and cortico-steroid-binding protein (11). Because recent evidence suggests the modulatory effects of endogenous steroids on memory performance (3,4,8), paeoniflorin may be able to interact with these steroidal systems to modulate memory in rats.

Although extracts of Japanese angelica and peony roots as well as paeoniflorin improved the choice accuracy of radial maze performance impaired by scopolamine, none of them affected the running time prolonged by scopolamine. The scopolamine prolongation of running time may be mainly due to peripheral, not central action of this agent, because methyl scopolamine, a peripherally acting scopolamine derivative, reportedly prolongs the running time without affecting the choice accuracy in radial maze performance (2). Therefore, the present findings indicate that these herbal extracts and paeoniflorin antagonize the central, and not the peripheral effect of scopolamine.

In summary, peony root extract and its major constituent, paeoniflorin, significantly attenuated the working memory deficits produced by scopolamine in an eight-arm radial maze task. Paeoniflorin recovered the scopolamine-induced decrease in ACh content in the striatum but not in the hippocampus or cerebral cortex. These data indicate the beneficial effects of these treatments on deficits in the spatial working memory in rats, and suggest that the attenuation might be due to indirect activation of the central cholinergic system.

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