Involvement of β-Adrenergic Systems in the Antagonizing Effect of Paeoniflorin on the Scopolamine-Induced Deficit in Radial Maze Performance in Rats

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ABSTRACT—Paeoniflorin, a major constituent of peony root, has been demonstrated to attenuate the radial maze performance deficit produced by scopolamine. In the present study, to investigate the possible involvement of β-adrenergic systems in the paeoniflorin antagonism of the scopolamine deficit, the effects of two β-adrenoceptor antagonists, propranolol and atenolol, on the paeoniflorin effect were examined in male Wistar rats. Paeoniflorin (1 mg/kg, p.o.) significantly attenuated the scopolamine HBr (0.3 mg/kg, i.p.)-induced deficit in the choice accuracy in radial maze performance without changing the running time prolonged by scopolamine. Neither D,L-propranolol HCl, a lipophilic β-antagonist, at 3 mg/kg, i.p. nor atenolol, a hydrophilic β_1-antagonist that is known to hardly ever cross the blood-brain barrier, at 1 mg/kg, i.p. impaired maze performance by itself or aggravated the scopolamine-induced deficit in radial maze performance. Both antagonists, however, completely blocked the antagonizing effect of paeoniflorin on the scopolamine deficit. These data suggest that the β-adrenergic systems, especially peripheral β_1-adrenergic systems, are involved in the antagonizing effect of paeoniflorin on the scopolamine deficit in radial maze performance in rats.

Keywords: Radial maze, Paeoniflorin, Propranolol, Atenolol, Scopolamine

In Chinese and Japanese medicine, peony root has long been used to treat various kinds of dementia as a component of traditional prescriptions. This herb is known to disperse blood stagnancy and relax muscle spasms of both smooth and skeletal muscles (1). Paeoniflorin is a water-soluble substance that was isolated from peony root in 1963 (2). The effects of this substance are reportedly in good accordance with the clinical effects of peony root (3). Recently, Ohta et al. (4) have demonstrated that peony root extract and paeoniflorin dose-dependently attenuate the scopolamine-induced deficit in radial maze performance in rats. Although they have suggested the involvement of non-cholinergic systems in the paeoniflorin effect, the underlying mechanisms are still unclear.

There has been substantial evidence showing that the memory deficits produced by scopolamine are reinstated by cholinomimetic drugs such as oxotremorine, physostigmine and tacrine (5, 6). Recent studies, however, indicate that some adrenergic drugs can attenuate the scopolamine deficits. Amphetamine, for example, reverses scopolamine-induced memory impairments in a variety of tasks (5, 7). A norepinephrine precursor, L-*threo*-DOPS, and a norepinephrine uptake blocker, desipramine, have also been shown to attenuate the scopolamine-induced deficit in radial maze performance (8). Conversely, a β-adrenoceptor antagonist, propranolol, aggravates impairments in water maze and passive avoidance performances following scopolamine (9). These findings indicate that drugs that affect adrenergic functions are able to modulate the effects of scopolamine. On the other hand, it seems established that peripherally administered epinephrine improves memory retention (10). Moreover, it attenuates the memory deficit induced by scopolamine (5, 11) and potentiates the memory-enhancing effects of physostigmine and oxotremorine (12). Since epinephrine does not readily cross the blood-brain barrier, these facts suggest that the peripheral adrenergic system is involved in the modulation of central cholinergic functions. In the

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present study, to test the possible involvement of central and peripheral β-adrenergic systems in the antagonizing effect of paeoniflorin on scopolamine-induced memory deficit, we determined the effects of two β-adrenoceptor antagonists with different lipophilicity, i.e., the lipophilic β-antagonist propranolol and the hydrophilic β_1-antagonist atenolol, on the paeoniflorin effect using an eight-arm radial maze task. Atenolol has a higher selectivity towards β_1-adrenoceptors compared to propranolol and is known to hardly ever cross the blood-brain barrier (13).

MATERIALS AND METHODS

Subjects

The subjects were male Wistar rats obtained from Japan SLC (Hamamatsu) weighing 290–390 g. The rats were 6 weeks old on arrival and were acclimatized to laboratory conditions for 1 week before being used. Four or five animals were housed in a cage with free access to water in an air-conditioned room with a 12-hr light/dark cycle (lights on, 0730–1930). The animals were maintained on a restricted feeding schedule designed to keep their body weight at approximately 85% of the free-feeding level. All training was conducted between 9:00–14:00.

Apparatus

An eight arm radial maze was used. Each arm (50 × 12 cm) 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, Frenchtown, NJ, USA). Guillotine doors surrounded the hub.

Procedures

The procedure was detailed in our previous report (4). Prior to the 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. On the first day of the adaptation period, two rats at a time were placed on the maze where food pellets were scattered on the floor and in the food cups. From the second day, one rat was placed in the maze and allowed to move freely until all eight arms were chosen. Following this adaptation period, one daily training trial was conducted for each rat. The trial was judged complete when the rat had visited all 8 arms or had spent 10 min in the maze. Entry into an arm that the rat had not previously visited was recorded as a correct response and re-entry was counted as an error. The number of correct responses before committing the first error (No. of initial correct), the number of errors and the running time were used as the measures of radial maze performance. To calculate the running time, the total running time was divided by the total number of choices. Rats that made no errors or only one error at the eighth choice for 5 consecutive days were used for the drug tests.

Drug treatment

The drugs used in these experiments were a muscarinic antagonist, scopolamine hydrobromide (Nacalai Tesque, Kyoto); a β-adrenoceptor antagonist, D,L-propranolol hydrochloride (Sigma, St. Louis, MO, USA); and a peripherally acting β_1-adrenoceptor antagonist, atenolol (Sigma). These drugs were dissolved in saline and intraperitoneally (i.p.) injected 30 min before testing. Paeoniflorin was extracted and purified as described by Shibata et al. (2). Paeoniflorin was dissolved in water and orally administered 90 min before testing. In each experiment, the drugs were tested in a counterbalanced sequence to exclude any order effect.

Statistics

The effects of the drugs on the number of initial correct responses, the number of total errors and the running time were analyzed by the Kruskal-Wallis test followed by the Mann-Whitney *U*-test for multiple comparisons. Differences with a $P < 0.05$ were considered statistically significant.

RESULTS

Effects of propranolol and atenolol on scopolamine disruption of radial maze performance

Neither propranolol (3 mg/kg) nor atenolol (1 mg/kg), by itself, affected radial maze performance with respect to the initial correct responses (Fig. 1), the number of errors and the running time (Table 1). Scopolamine (0.3 mg/kg) significantly decreased the initial correct responses ($P < 0.05$, Fig. 1) and increased the number of errors ($P < 0.05$, Table 1). The running time of scopolamine treated rats was significantly longer than that of the saline group ($P < 0.05$, Table 1). Neither propranolol nor atenolol changed the scopolamine-induced impairment of the initial correct responses (Fig. 1), the number of errors and the running time (Table 1).

Effects of propranolol and atenolol on paeoniflorin attenuation of scopolamine deficit

Paeoniflorin (1 mg/kg) significantly attenuated the scopolamine-induced decrease in the initial correct responses ($P < 0.05$, Figs. 2 and 3) and the increase in the number of errors without changing the running time prolonged by scopolamine (Tables 2 and 3). These results agree well with our previous report (4). Propranolol (3

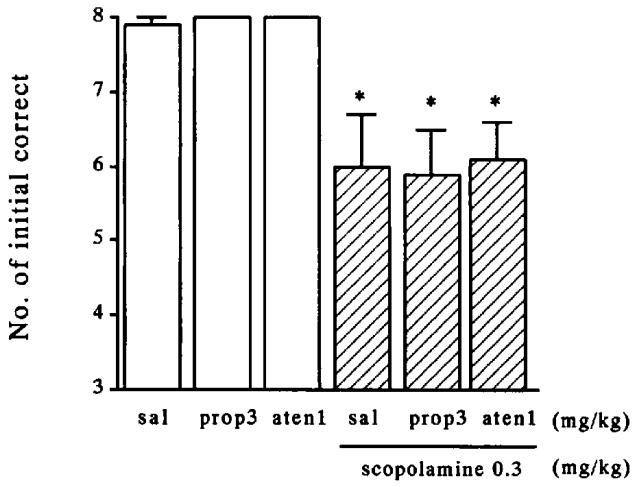

Fig. 1. Effects of propranolol and atenolol on radial maze performance in rats treated with or without scopolamine. Scopolamine HBr, D,L-propranolol HCl and/or atenolol was intraperitoneally injected 30 min before testing. Each datum represents the mean number of initial correct responses with S.E. indicated. *P<0.05 compared with the saline control.

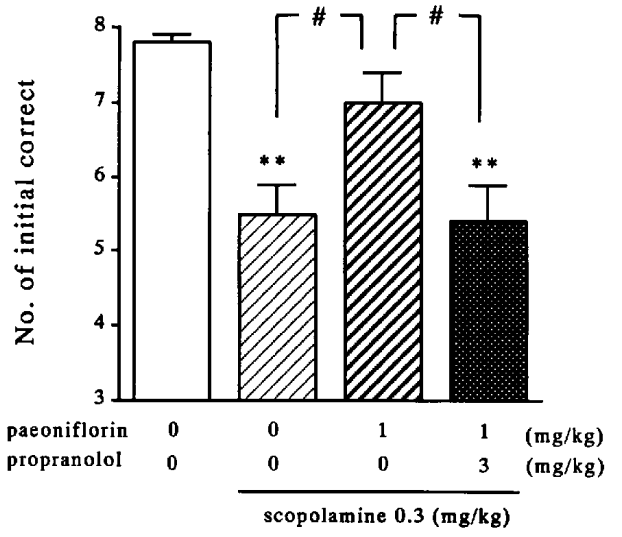

Fig. 2. Effect of propranolol on paeoniflorin attenuation of the scopolamine-induced deficit in radial maze performance in rats. Paeoniflorin was orally administered 90 min before testing. Scopolamine HBr and/or D,L-propranolol HCl was intraperitoneally injected 30 min before testing. Each datum represents the mean number of initial correct responses with the S.E. indicated. **P<0.01, compared with the saline control. #P<0.05, compared with the scopolamine plus paeoniflorin group.

Table 1. Effects of propranolol and atenolol on radial maze performance in rats with or without scopolamine treatment

Drug treatment (mg/kg)	N	No. of errors	Running time (sec)
Saline	8	0.1±0.1	8.8±1.3
Propranolol, 3	8	0.0±0.0	6.4±0.4
Atenolol, 1	8	0.0±0.0	7.0±0.5
Scopolamine, 0.3	8	3.1±0.9*	21.2±3.1*
Scopolamine, 0.3 +Propranolol, 3	8	3.0±1.2*	19.2±2.4**
Scopolamine, 0.3 +Atenolol, 1	8	4.0±1.5*	15.3±1.8*

Scopolamine HBr and/or D,L-propranolol HCl or atenolol was intraperitoneally administered 30 min before testing. Each value represents the mean±S.E. *P<0.05, **P<0.01, compared with the saline control.

mg/kg) significantly antagonized the effect of paeoniflorin on the number of initial correct responses (P<0.05, Fig. 2) and the number of errors (P<0.05, Table 2). The peripherally acting β_1-antagonist atenolol also significantly reversed the paeoniflorin-induced increase in the number of initial correct responses (P<0.05, Fig. 3) and the decrease in the number of errors (P<0.05, Table 3). None of these antagonists affected the running time (Tables 2 and 3).

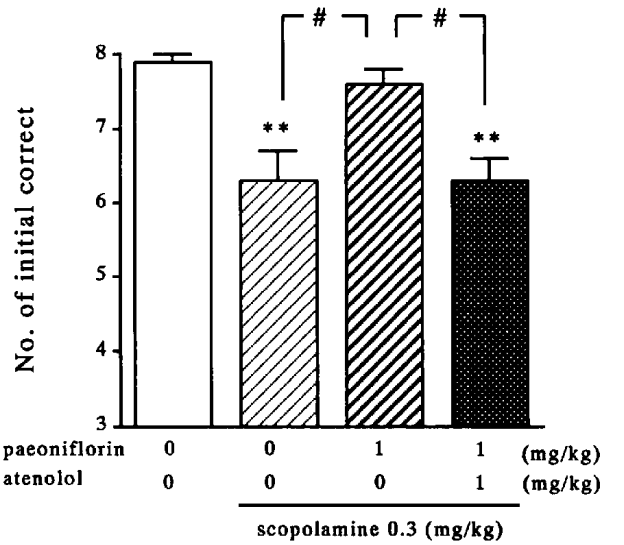

Fig. 3. Effect of atenolol on paeoniflorin attenuation of the scopolamine-induced deficit in radial maze performance in rats. Paeoniflorin was orally administered 90 min before testing. Scopolamine HBr and/or atenolol was intraperitoneally injected 30 min before testing. Each datum represents the mean number of initial correct responses with the S.E. indicated. **P<0.01, compared with the saline control. #P<0.05, compared with the scopolamine plus paeoniflorin group.

Table 2. Effects of propranolol on paeoniflorin antagonism of the scopolamine-induced deficit in radial maze performance in rats

Drug treatment (mg/kg)	N	No. of errors	Running time (sec)
Saline	11	0.2±0.1	8.1±0.6
Scopolamine, 0.3	11	3.7±0.9**,##	18.5±2.4**
Scopolamine, 0.3 +Paeoniflorin, 1	11	0.6±0.2	13.7±1.5*
Scopolamine, 0.3 +Paeoniflorin, 1 +Propranolol, 3	11	5.7±1.3**,##	19.6±2.2**

Scopolamine HBr and/or D,L-propranolol was intraperitoneally injected 30 min before testing. Paeoniflorin was orally administered 90 min before testing. Each value represents the mean±S.E. *P<0.05, **P<0.01, compared with the saline control. ##P<0.01, compared with the scopolamine plus paeoniflorin group.

Table 3. Effects of atenolol on paeoniflorin antagonism of the scopolamine-induced deficit in radial maze performance in rats

Drug treatment (mg/kg)	N	No. of errors	Running time (sec)
Saline	8	0.1±0.1	7.1±0.7
Scopolamine, 0.3	8	2.9±0.7**,#	16.3±1.8**
Scopolamine, 0.3 +Paeoniflorin, 1	9	0.8±0.5	20.5±5.5**
Scopolamine, 0.3 +Paeoniflorin, 1 +Atenolol, 1	8	3.4±0.9**,#	19.6±2.2**

Scopolamine HBr and/or atenolol was intraperitoneally injected 30 min before testing. Paeoniflorin was orally administered 90 min before testing. Each value represents the mean±S.E. **P<0.01, compared with the saline control. #P<0.05, compared with the scopolamine plus paeoniflorin group.

DISCUSSION

Our previous study has demonstrated that paeoniflorin (0.01 – 1 mg/kg) attenuates the scopolamine disruption of radial maze performance in a dose-dependent manner, and that this agent is most effective at 1 mg/kg (4). The present result that 1 mg/kg paeoniflorin antagonized the scopolamine-induced impairment in choice accuracy is in good agreement with our previous report. Moreover, paeoniflorin did not change the running time prolonged by scopolamine. This finding suggests that paeoniflorin may antagonize the central, and not the peripheral effect of scopolamine, since the running time can be prolonged not only scopolamine but also by methylscopolamine, a peripherally acting scopolamine derivative, which does not affect the choice accuracy of radial maze performance.

In the present study, 3 mg/kg propranolol completely blocked the paeoniflorin attenuation of the scopolamine deficit. This fact indicates that β-adrenergic systems are involved in the paeoniflorin effect. More interestingly, the hydrophilic β_1-antagonist atenolol at 1 mg/kg also completely blocked the paeoniflorin effect. Atenolol is known to hardly ever cross the blood-brain barrier because of its high hydrophilicity (13). Acutely administered radiolabelled atenolol was reportedly undetectable by autoradiography in rat brain (14), and a recent study using positron emission tomography has revealed that the passage of atenolol into the brain is very limited (15). Taken together, the present finding suggests the involvement of β-adrenergic systems, especially peripheral β_1-adrenergic systems, in the paeoniflorin effect. However, since propranolol blocks not only peripheral β_1-adrenoceptors but also peripheral β_2- or central β-adrenoceptors, we cannot exclude the possible involvement of these receptor systems in the paeoniflorin effect.

Decker et al. (9) have reported that propranolol augments scopolamine-induced impairments in water maze learning and passive avoidance performance. In the present study, both 3 mg/kg propranolol and 1 mg/kg atenolol did not potentiate the disruptive effect of scopolamine with respect to all three measures of radial maze performance. However, both antagonists at higher doses exhibited the tendency to aggravate the scopolamine deficit in radial maze performance, and the highest dose (6 mg/kg) of atenolol significantly increased the number of errors compared to scopolamine alone (unpublished data, H. Ohta et al.). None of these treatments by themselves impaired the maze performance in normal rats. These facts suggest that tonic activity of the β-adrenergic systems may modulate the effects of scopolamine.

The septohippocampal cholinergic system has been demonstrated to play an important role in radial maze performance (16). The fact that norepinephrine release in the septal region increases ACh turnover in the hippocampus (17) suggests the possibility that central adrenoceptor stimulation may enhance the cholinergic function in this system. However, the mechanisms for how the peripheral β-adrenergic system modulates central cholinergic function remain unclear. One possible mediator might be adrenocorticotropine (ACTH). The peripheral β-adrenergic system is suggested to modulate the plasma concentration of ACTH (18), which has been reported to affect memory performance (19). On the other hand, recent evidence suggest that glucose acts as a common mediator of some cognitive enhancers (see 20, for review). Glucose attenuates scopolamine-induced amnesia in the inhibitory avoidance task (8) and an operant bar pressing task (21). In addition, glucose diminishes both the increase in high-affinity choline uptake (21) and the decrease in striatal

ACh content (22) by cholinergic antagonists. Since sympathoadrenal activity regulates glucose release from hepatic stores, it is likely that changes in peripheral adrenergic activity modulates central cholinergic function through the mediation of glucose.

In summary, oral administration of paeoniflorin attenuated the scopolamine-induced deficit in radial maze performance in rats. The attenuating effect of paeoniflorin was completely blocked by either a centrally and peripherally acting β-adrenoceptor antagonist, propranolol, or a peripherally acting β_1-selective adrenoceptor antagonist, atenolol. These results suggest that β-adrenergic systems, especially peripheral β_1-adrenergic systems, are involved in the paeoniflorin effect.

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