Effects of DX-9386, a Traditional Chinese Preparation, on Passive and Active Avoidance Performances in Mice

Nobuyoshi NISHIYAMA,* Yueping ZHOU, Ken TAKASHINA, and Hiroshi SAITO

Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan. Received June 13, 1994; accepted July 18, 1994

The acute effects of DX-9386, a traditional Chinese medicinal preparation (ginseng, polygala, acorus and hoelen), were studied on learning and memory performances in passive and active avoidance tests in normal, as well as in learning-impaired, mice. A single oral administration of the prescription had no effect on memory registration, consolidation or retrieval or on motor activity in normal mice. DX-9386 reduced the ethanol-induced impairment of memory registration in the step-down test and also tended to ameliorate the scopolamine-induced memory registration deficit in the same test. The preparation reduced the number of spontaneous responses in the active avoidance test. The preparation also prolonged the disappearance of righting reflex induced by pentobarbital. These results suggest that DX-9386 ameliorates the impairment effect of ethanol on learning and memory processes and also exhibits a sedative effect.

Keywords ethanol; learning; memory; step-through test; step-down test; sedative effect

DX-9386, a traditional Chinese medicinal preparation, consists of ginseng (*Panax ginseng* C.A. MEYER), polygala (*Polygala tenuifolia* WILLDENOW), acorus (*Acorus gramineus* SOLAND) and hoelen (*Poria cocos* WOLF) in the ratio of 1:1:25:50 (dry weight). It has long been employed in the clinical treatment of some mental disorders such as brain hypoxia and senile amnesia in China,¹⁾ although few studies on its pharmacological effects on the central nervous system have been carried out. In our previous studies, single oral administration of DX-9386 potentiated the evoked potential induced by subthreshold tetanic stimulation in the rat hippocampal dentate gyrus.²⁾ Moreover, the preparation antagonized the blocking effect of ethanol on the generation of long-term potentiation (LTP) in the same experimental model. LTP in the hippocampus is supposed to be a form of activity-dependent synaptic plasticity that may underlie learning and memory. Therefore, we evaluated the acute effects of DX-9386 on learning and memory performances in passive and active avoidance tests in mice.

MATERIALS AND METHODS

Animals Male Std-ddY mice, 5–6 weeks old (SLC, Japan), were kept under standard temperature- and humidity-controlled ($22 \pm 1^\circ\text{C}$, $55 \pm 2\%$) conditions with food and water *ad libitum*. They were housed 10 animals per cage for 1 week prior to the experiments.

Drugs and Chemicals DX-9386 is composed of ginseng (*Panax ginseng* C.A. MEYER), polygala (*Polygala tenuifolia* WILLDENOW), acorus (*Acorus gramineus* SOLAND) and hoelen (*Poria cocos* WOLF) in the ratio of 1:1:25:50 (dry weight). Aqueous extracts of ginseng root, polygala root and whole hoelen, and an ethanol extract of acorus rhizome were individually prepared and supplied as a mixture of extracts by Daiichi Pharmaceutical Co. Ltd. (Tokyo, Japan). Two doses of the preparation (250 and 500 mg/kg, *p.o.*) were used in all the experiments. DX-9386, at these doses, did not change the general behavior (Irwin's method) and pain sensitivity in normal mice. Scopolamine

hydrobromide, ethanol, chlorpromazine hydrochloride and sodium pentobarbital were the highest purity available from Wako Pure Chemicals Co. Ltd. (Tokyo, Japan). All drug solutions were prepared in physiological saline.

Passive Avoidance Performance. 1. Step-Through Test The step-through test was performed according to the method used in our laboratory.^{3,4)} The apparatus (PA M1, O'Hara & Co., Ltd., Tokyo, Japan) consisted of two compartments separated by a black wall with a hole in the lower middle part. One of the two chambers was illuminated and the other was dark. The test was conducted on two consecutive days at the same time of day. On the first day (learning trial), a mouse was placed in the bright compartment. When it stepped through into the dark compartment, it received an electric shock to its paws (36 V, AC) through a stainless-steel grid floor. The time when the mouse entered the dark chamber was recorded automatically and described as "latency". On the second day (testing trial), the same test procedure was followed and the step-through latency was measured with a ceiling score of 300 s. The latency and the number of mice not entering the dark compartment were recorded in the testing trial.

2. Step-Down Test^{3,4)} The test chamber was equipped with a stainless-steel grid floor through which an electric shock (50 V, AC) was given. A platform was placed at one corner of the rectangular box. A mouse learned to stay on the platform to avoid the electric shock from the floor. On the first day (learning trial), a mouse was exposed to a 10 min-learning period in which it was punished by a paw shock when it stepped down from the platform (error). On the second day (testing trial), the same test procedures were performed at the same time of day for 3 min. The number of mice not stepping down to the floor were recorded in the testing trial.

3. Procedures of Drug Administration 1) Effects of DX-9386 on Passive Avoidance Performances in Normal Mice: DX-9386 was given, i) 30 min before the learning trial, ii) immediately after the learning trial, or iii) 30 min before the testing trial, to evaluate its effects on-memory

registration, memory consolidation and memory retrieval, respectively. Twelve mice were assigned to each experimental group.

2) Effects of DX-9386 on Passive Avoidance Performances in Memory-Impaired Mice: Twelve mice were assigned to control, active control, DX-9386 250 and 500 mg/kg groups.

i) DX-9386 was given 30 min prior to the learning trial. Ethanol (30%, 10 ml/kg, *p.o.*) or scopolamine hydrobromide (0.5 mg/kg, *i.p.*) was given 20 min before the learning trial to produce memory registration impairment.

ii) DX-9386 was given 30 min before the learning trial. Electric convulsive shock (ECS; 100 Hz, 20 pulses, 0.4 ms width, 94 mA) was applied to the mice through the ears immediately after the learning trial to impair the memory consolidation process.

iii) DX-9386 was given 30 min prior to the testing trial. Ethanol (40%, 10 ml/kg, *p.o.*) or ECS (100 Hz, 20 pulses, 0.4 ms width, 80 mA) was given 20 min before the testing trial to inhibit the memory retrieval process.

Active Avoidance Performance. 1. Shuttle-Box Test^{5,6)}

The shuttle-box test was performed for 8 consecutive days with 60 trials per day. The chamber of the apparatus (model GT-8332S, O'Hara Co. Ltd.) was equipped with a stainless-steel grid floor connected to an electric stimulator. Two separate sets of infrared beams were located at the lower part of the long sides and a buzzer and lamp were located on the ceiling. The apparatus was computer-controlled and the programmed schedule for one trial was 60 s, of which 40 s was for an interval, 10 s for conditioned stimulation with a warning buzzer and light and 10 s for unconditioned stimulation with an actual electric shock (36 V, AC) concurrently with the warning buzzer and light. When a mouse moved from one side of the apparatus to the other to interrupt both of the infrared beams during the period of conditioned or unconditioned stimulation, the electric shock was either avoided or canceled immediately. The perturbation of the photo beam occurring in the period of conditioned stimulation divided by the total number of trials (*i.e.* 60) was recorded as the conditioned avoidance response (CAR, %), and that occurring in the period of interval was defined as the spontaneous response (SR).

2. Lever-Press Test^{5,6)} Mice were subjected to 8 consecutive days testing, with 60 trials per day in this test. A steel lever was vertically located at one side of the testing chamber (model GT-8332, O'Hara Co. Ltd.). The chamber was also equipped with a warning buzzer, a lamp on the ceiling and a stainless-steel grid floor for giving an electric shock. The program and schedule of the trials were the same as those in shuttle-box test. In lever-press test, however, mice had to press the lever to cancel or avoid the electric shock. The number of times the lever was pressed in the period of conditioned stimulation divided by the total number of the trials (*i.e.* 60) was recorded as the CAR (%), and that occurring in the period of interval was defined as the SR.

3. Procedures for Drug Administration 1) Effects of DX-9386 on Memory Registration in Active Avoidance Performances: The shuttle box or lever press tests were performed for 8 consecutive days at the same time of day.

DX-9386 was administered 15 min prior to the daily session ($n=9$).

2) Effects of DX-9386 on Memory Retrieval in Active Avoidance Performances: After intensive training, the mice achieving a CAR greater than 90% in the shuttle-box test or a CAR greater than 80% in the lever-press test, were selected. The shuttle box or lever-press test was conducted on the next day to determine the basal performance level of the mice. On the following day, 15 min after DX-9386 administration, the shuttle-box or lever-press test was again carried out. Data from the post-DX-9386 administration test were compared with that from the basal performance test.

Measurement of Motor Activity A mouse was put into a testing cage of a round (18 cm in diameter and 18 cm in height) tilting-type apparatus (Ambulometer MA001, O'Hara & Co. Ltd., Tokyo, Japan), and the amount of motor activity was recorded over a 30 min period. The measurement was conducted twice with an interval of 24-h. DX-9386 (250 and 500 mg/kg) was given orally 30 min before the first or second measurements ($n=12$).

Pentobarbital-Induced Disappearance of Righting Reflex Saline, chlorpromazine (2 mg/kg, *i.p.*) or DX-9386 was administered to mice 15 min prior to the injection of pentobarbital (50 mg/kg, *i.p.*). The duration of the disappearance of the righting reflex was recorded as the sleeping time ($n=10$).

Statistics The latency in the step-through test, the number of errors in the step-down test, the CAR and SR in the shuttle-box and lever-press tests were analyzed using the Mann-Whitney's *U*-test; the number of mice that did not make errors in either the step-through or step-down test were analyzed with Fischer's exact probability test and the motor activity and the duration of disappearance of the righting reflex were analyzed with ANOVA followed by Duncan's multiple range test.

RESULTS

1. Effects of DX-9386 on Passive Avoidance Performance

1) Effects of DX-9386 in Normal Mice: The effects of single oral administrations of DX-9386 were investigated using the step-through and step-down tests. In the memory registration test, the preparation did not affect the latencies to enter the dark compartment (step-through test), the number of errors (step-down test), and the percentage of successful mice (both tests). Furthermore, DX-9386 had no effect on the parameters of the memory consolidation and retrieval tests (data not shown). These results indicate that DX-9386 has no effect on passive avoidance performances in normal mice.

2) Effect of DX-9386 in Memory-Impaired Mice: Oral administration of ethanol 20 min prior to the learning trial caused memory registration impairment in the passive avoidance tests. When DX-9386 was given 10 min before the ethanol treatment, the preparation at a dose of 250 mg/kg increased the number of successful mice in the step-down test (Fig. 1). A higher dose of DX-9386 (500 mg/kg) produced a similar tendency which, however, did not reach statistical significance. The preparation did not affect the latency to enter the shock-compartment or

the percentage of successful mice in the step-through test (data not shown). We also studied the effects of DX-9386 on the scopolamine-induced memory registration deficit. When scopolamine was injected intraperitoneally 20 min before the learning trial, memory registration was significantly impaired. DX-9386 given 10 min prior to the scopolamine tended to increase the number of successful mice in the step-down test (Fig. 2). The preparation had no effect on the scopolamine-induced memory registration impairment in the step-through test (data not shown). Treatment with DX-9386 did not improve the memory consolidation impairment induced by the electro-

convulsive shock nor the memory retrieval disorder caused by 40% ethanol in the passive avoidance tests (data not shown).

2. Effects of DX-9386 on the Active Avoidance Performance in Normal Mice 1) Shuttle-Box Test: The shuttle box test was performed for 8 consecutive days, and the CAR (%) and SR were recorded daily. Control mice acquired this task very rapidly as far as CAR is concerned. Nearly 80% of the trials were performed successfully by the second day. Daily oral administration of DX-9386 did not affect the CAR when compared with the saline-treated control (Fig. 3A). The unconditioned avoidance response

Fig. 1. Effect of DX-9386 on Memory Registration Impairment Induced by 30% Ethanol in the Step-Down Test

Thirty percent ethanol was given orally 20 min prior to the learning trial to induce memory registration impairment. DX-9386, at doses 250 or 500 mg/kg (DX250 or DX500, respectively), was administered 30 min before the learning trial. The testing trial was performed 24 h after the learning trial. The number of mice that did not step down to the electric floor grid in the testing trial is shown. **, $p < 0.01$ vs. control (cont), #, $p < 0.05$ vs. no added DX-9386 (DX0) using Fisher's exact probability test, $n = 12$.

Fig. 2. Effects of DX-9386 on Memory Registration Impairment Induced by Scopolamine in the Step-Down Test

Scopolamine (0.5 mg/kg, i.p.) was injected 20 min before the learning trial to induce memory registration impairment. DX-9386, at doses 250 and 500 mg/kg, was given orally 30 min prior to the learning trial. The number of mice that did not step down to the electric floor grid in the testing trial is shown. The testing trial was performed 24 h after the learning trial. **, $p < 0.01$ vs. control using Fisher's exact probability test, $n = 12$. For abbreviations see Fig. 1.

Fig. 3. Effects of DX-9386 on Memory Registration in the Shuttle-Box and Lever-Press Tests in Normal Mice

The mean values of the conditioned avoidance response (CAR, %) in the shuttle box (A) and lever press (B) tests, and spontaneous response (SR, counts/h) in the shuttle box (C) and lever press (D) tests are shown. DX-9386 was given daily 15 min prior to the session. O, control; ▲, DX-9386 250 mg/kg; ▼, DX-9386 500 mg/kg. **, $p < 0.01$ vs. control using the Mann-Whitney U -test, $n = 9$.

TABLE I. Effects of DX-9386 on Memory Retrieval in the Active Avoidance Tests

		Control	DX-9386	
			250 mg/kg	500 mg/kg
Shuttle-box test				
CAR	pre	95.1 ± 0.9	95.2 ± 0.7	94.8 ± 1.0
	post	95.0 ± 0.9	94.2 ± 1.0	95.0 ± 0.7
SR	pre	95.8 ± 24.5	103.8 ± 32.1	105.8 ± 36.8
	post	90.9 ± 23.2	88.5 ± 27.3	69.0 ± 10.5**
Lever-press test				
CAR	pre	82.5 ± 1.8	80.5 ± 2.1	83.8 ± 2.5
	post	78.1 ± 3.3	75.3 ± 4.1	76.1 ± 4.8
SR	pre	198.5 ± 31.5	225.3 ± 38.2	226.8 ± 30.2
	post	172.3 ± 28.9*	214.8 ± 35.6	148.3 ± 21.5*

After training in the shuttle-box or lever-press test, mice that had learned well were selected ($n=6$ and 7 , respectively). The basal performance levels were determined (pre) and then the CAR (%) and SR (count/h) were measured 15 min after oral administration of the preparation (post). *, $p<0.05$, **, $p<0.01$ vs. corresponding values of "pre" data (paired t -test).

Fig. 4. Effect of DX-9386 on Pentobarbital-Induced Disappearance of Righting Reflex

Pentobarbital (50 mg/kg, i.p.) was injected 15 min after the administration of DX-9386 or chlorpromazine (2 mg/kg, i.p., as a positive control). The duration of the period without righting reflex was expressed as a mean $\pm$ S.E.M. Control, saline; CPZ, chlorpromazine; DX250, DX-9386 250 mg/kg; DX500, DX-9386 500 mg/kg, *, $p<0.05$; **, $p<0.01$ vs. control in ANOVA followed by Duncan's multiple range test, $n=10$.

and the number of errors were not altered by the preparation (data not shown). SR gradually increased in the control mice over the sessions. DX-9386, at a dose of 500 mg/kg, strongly inhibited this increase in SR (Fig. 3C).

Mice exhibiting a CAR greater than 90% were selected and DX-9386 was administered as single doses to investigate the effects of the preparation on memory retrieval. It did not affect the level of CAR when compared with the pre-administration value, but decreased the SR significantly at 500 mg/kg (Table I).

2) Lever-Press Test: The lever-press test was performed for 8 consecutive days, and the CAR and SR were recorded daily. Control mice did not acquire this task very well in terms of CAR, and the maximum level of CAR in control mice was about 20%. Daily oral administration of DX-9386 did not affect the CAR during the sessions when compared with the saline treated controls (Fig. 3B). Unconditioned avoidance response and the number of errors were not altered by the preparation (data not shown). The SR gradually increased in the control animals

during the sessions. DX-9386 tended to decrease the SR of control mice, but this was not statistically significant (Fig. 3D). The preparation did not affect memory retrieval in the lever press-test, but the SR fell markedly at 500 mg/kg when compared with the pre-administration value (Table I).

3) Effects of DX-9386 on Motor Activity: DX-9386, at doses of 250 and 500 mg/kg, did not affect the motor activity measured with a tilting-type ambulometer, either immediately or 24 h after the administration (data not shown).

4) Effect of DX-9386 on the Pentobarbital-Induced Disappearance of Righting Reflex: As shown in Fig. 4, the duration of the disappearance of the righting reflex induced by pentobarbital was significantly prolonged by administration of DX-9386 in a dose-dependent manner. Chlorpromazine, an active control, also markedly prolonged it.

DISCUSSION

In Chinese traditional medicine it is conventional to combine different herbs in order to achieve a variety of treatment purposes simultaneously or to enhance a single effect without causing severe side effects. The pharmacological actions of the different ingredients in the DX-9386 preparation have been reported.^{7,8)} This paper mainly described the action of DX-9386, as a whole, on the central nervous system, particularly, on memory and learning processes.

As far as passive avoidance performance was concerned, treatment with DX-9386 did not affect memory registration, consolidation or retrieval which are considered to make up the complete memory processes,⁹⁻¹¹⁾ suggesting that the preparation did not alter the memory processes under normal conditions. These results were consistent with those of the active avoidance performance, in which normal mice were used.

In the memory impairment model, DX-9386 at a dose of 250 mg/kg improved the memory registration deficit induced in mice by 30% ethanol and also tended to improve the memory registration impairment induced by scopolamine, while the preparation did not affect the memory retention process inhibited by ECS or the memory retrieval impairment induced by 40% ethanol. These results suggest that the drug preferentially improves the memory registration process rather than the other processes, and also that ethanol inhibited the memory registration and retrieval processes by different mechanisms. Although a higher dose of DX-9386 also tended to alleviate the disturbed learning registration caused by either ethanol or scopolamine, no obvious dose-dependent result was obtained. The preparation at a dose of 500 mg/kg antagonized the blocking effect of ethanol (2 ml/kg, *p.o.*) on the generation of long-term potentiation in the hippocampal dentate gyrus of the rat.²⁾ We recently found that 250, but not 125, mg/kg DX-9386 had this effect in the same experimental system (unpublished observation). To see the effect of a lower dose of the preparation (*i.e.* 125 mg/kg) on leaning behavior it would be necessary to determine the minimum effective dose of

DX-9386. Ginseng, a component of DX-9386, has been reported to improve memory processes.^{12,13)} We have already confirmed that acorus, another constituent of the prescription, improves the memory impairment caused by ethanol (unpublished observation). The beneficial effect of DX-9386 in the memory registration deficit model may reflect the combined effects of distinct ingredients in the preparation. DX-9386 improved the learning performance only in the step-down test and not in the step-through test, which suggests that the step-through test involves a different mechanism from the step-down test. The precise effects of DX-9386 on the memory process in the step-down test remain to be elucidated.

DX-9386 decreased the SR in the later sessions during the memory registration process in the shuttle-box test. SRs were also decreased in the memory retrieval performance of the shuttle-box test and lever-press test in the drug-treated group. These results indicate that the preparation has a tranquilizing effect especially when animals are abnormally excited by the electric foot shock. One important point is that the drug did not affect motor activity under normal conditions. DX-9386 prolonged the duration of the disappearance of the righting reflex induced by pentobarbital. This result also indicates that the preparation possesses a tranquilizing or sedative action which is in agreement with the decrease in SR under stressful conditions. We have already found that acorus, a component of the prescription, also prolonged the period without righting reflex (unpublished observation).

Although the mechanisms of action of DX-9386 remain speculative, the present results suggest that the preparation affects the learning and memory processes in the central nervous system to improve the impairment of memory registration in the step-down test and that the prescription also has a sedative effect.

REFERENCES

- 1) "Dictionary of Traditional Chinese Medicine," Vol. 1, Suzhou New Medical College, ed., Shanghai People's Publication House, Shanghai, 1977, p. 612.
- 2) Y. Zhang, H. Saito, N. Nishiyama, K. Abe, *Biol. Pharm. Bull.*, **17**, 1337 (1994).
- 3) K. Nishizawa, H. Saito, N. Nishiyama, *Jpn. J. Pharmacol.*, **54**, 375 (1990).
- 4) A. Ishihara, H. Saito, H. Ohta, N. Nishiyama, *Jpn. J. Pharmacol.*, **57**, 329 (1991).
- 5) N. Nishiyama, Y. L. Wang, H. Saito, *Shoyakugaku Zasshi*, **46**, 62 (1992).
- 6) Y. P. Zhou, H. Saito, N. Nishiyama, *Shoyakugaku Zasshi*, **46**, 103 (1992).
- 7) "Dictionary of Traditional Chinese Medicine," Vol. 2, Suzhou New Medical College, ed., Shanghai People's Publication House, Shanghai, 1986, p. 1028.
- 8) "Dictionary of Traditional Chinese Medicine," Vol. 2, Suzhou New Medical College, ed., Shanghai People's Publication House, Shanghai, 1986, p. 1596.
- 9) I. Izuquiedo, *Trends Pharmacol. Sci.*, **10**, 174 (1989).
- 10) D. Kucharski, *Science*, **238**, 786 (1987).
- 11) F. J. Hock, *Neuropsychobiology*, **17**, 145 (1987).
- 12) H. Saito, *Taisya*, **10**, 556 (1973).
- 13) V. D. Petkov, A. H. Moshamof, *Am. J. Clin. Med.*, **15**, 19 (1987).