Effect of Long-Term Administration of Berberine on Scopolamine-Induced Amnesia in Rats

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ABSTRACT—The effect of berberine (BER) on scopolamine (SCOP)-induced amnesia was investigated in a step-through passive avoidance task in rats. It was observed that BER at the doses of 0.1 and 0.5 g/kg after 7-day or 14-day administration significantly improved SCOP-induced amnesia. The anti-amnesic effect of BER after 14-day administration on the SCOP-induced amnesia was significantly augmented by physostigmine or neostigmine, and completely reversed by scopolamine N-methylbromide. These results suggest that the antiamnesic effect of BER after 14-day administration may be related to the increase in the peripheral and central cholinergic neuronal system activity.

Keywords: Berberine, Scopolamine, Passive avoidance

The rhizome of *Coptis chinensis* FRANCH (Ranunculaceae) had been provided to possess analgesic and sedative effects and used to cure amnesia after long-term administration (1). Berberine (BER) is a benzodioxoloquinolizine alkaloid that belongs to the berbane group and is a main active constituent of *Coptis rhizoma* (2). In modern pharmacologic studies, BER has been found to have antiplatelet (3), anticerbral ischemic (4), vasodilatory (5) and antiarhythmic (6) effects and increases ileal contractility by increasing acetylcholine release from postganglionic parasympathetic nerve terminals, increasing acetylcholine retention through an inhibition of cholinesterase activity and blocking α2-adrenoceptors (7).

It is well-known that the cholinergic neuronal system plays an important role in learning and memory in humans and animals (8–10). Scopolamine (SCOP) is a muscarinic antagonist, and it impaired learning and memory in rodents and humans, especially learning acquisition and short-term memory (11–13). Therefore, SCOP is used as a useful model in the screening of antiamnesic drugs (14). Recent studies have shown that several cognitive enhancers can improve the SCOP-induced amnesia, but their effects can be inhibited by a peripherally acting cholinergic muscarinic receptor antagonist such as scopolamine N-methylbromide (M-SCOP), which is substituted by methyl group and does not pass through the brain-blood-barrier (15). On the other hand, recent reports also have shown that adrenalectomy inhibits the improvement effect of cognitive enhancing drugs on SCOP-induced amnesia (16). These results suggest that the memory-improving effects of several cognitive enhancers could be partially related to the peripheral nervous system and adrenal gland (17).

In the present study, we attempted to investigate 1) whether long-term administration of BER attenuates SCOP-induced amnesia, 2) whether its antiamnesic effects are antagonized by M-SCOP (peripheral muscarinic antagonist) but potentiated by physostigmine [central and peripheral acetylcholinesterase (AChE) inhibitor, PHY], neostigmine (peripheral AChE inhibitor, NEO).

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats, weighing 200–250 g, were housed in groups of six with free access to food and water and kept in a regulated environment (23 ± 1 °C), wherein a 12 hr light-dark cycle (8:00 to 20:00, light) was maintained. Each experimental group included 12 to 18 rats.

Passive avoidance response task

The apparatus consisted of a light compartment (48 × 20 × 30 cm) connected by an opening (5 × 5 cm) to a black compartment (48 × 20 × 30 cm), with a steel-rod grid floor (36 parallel steel rods, 0.3 cm in diameter set 1.5 cm apart). A (20 W) lamp was positioned centrally 30 cm above the base of the light compartment. The room was dark during the experimental sessions that were conduct-
During the training trial, the guillotine door connecting the light and dark compartment was kept closed. After each rat was placed in the light compartment with its back to the guillotine door, the door was opened. The time taken by the rat to have four paws on the grid of the dark compartment was measured simultaneously with a stopwatch. Once the rat entered the dark compartment, the door was then closed and an inescapable scrambled footshock (1.0 mA for 2 sec) was delivered through the grid floor by a shock generator (Muromachi Kikai Co., Tokyo). The rat was removed from the dark compartment 5 sec after administering the shock. The rat was then put back into its home cage until the retention trial, which was carried out 24 hr later. The rat was once again placed in the light compartment and as in the case of training trial, the guillotine door was opened and the step-through latency (STL) was recorded and used as a measure of retention (18). An upper cut-off time of 300 sec was set.

In the first series of experiments, SCOP (1.0 mg/kg, i.p.) was administered 30 min before the training trial (19). BER (0.1 and 0.5 g/kg, p.o.) was administered to SCOP-treated rats orally daily for 7 days or 14 days, and the training trial was carried out 1 hr after the last dosage of the 7-day or 14-day treatment. The vehicle-treated rats received the same volume (0.1 ml/100 g body weight).

In the second series of experiments, BER (0.5 g/kg, p.o.) was administered to SCOP-treated rats orally daily for 14 days, and the training trial was carried out 1 hr after the last dosage on the 14th day of treatment. PHY (0.02 mg/kg, i.p.) was administered 20 min before the training trial, NEO (0.02 mg/kg, i.p.) was administered 20 min before the training trial and M-SCOP (0.5 mg/kg, i.p.) was administered 30 min before the training trial.

Motor activity measurements

The motor activity of individual rats was measured with the MK-ANIMEX activity meter (Model SE, Muromachi Kikai Co.). The sensitivity and tuning of the instrument were adjusted to 35 μA to enable plenty of motor behavior, including locomotion, rearing, grooming, sniffing and licking to be jointly recorded. Activity was recorded for 1 hr, starting 5 min after inserting the animal into the test cage. Each rat was used only once, and a total of six rats were used for each treatment (20).

BER (0.1 and 0.5 g/kg, p.o.) was administered orally daily for 14 days, and the recording was carried out 1 hr after the last dosage on the 14th day of treatment. SCOP (1.0 mg/kg, i.p.) was administered 30 min before the recording, and PHY (0.02 mg/kg, i.p.) was administered 20 min before the recording. The vehicle-treated rats received the same volume of vehicle (0.1 ml/100 g body weight).

**Fig. 1.** Effects of berberine (BER) administered to scopolamine (SCOP)-treated rats on SCOP-induced amnesia in rats. A: BER was administered for 7 days B: BER was administered for 14 days. Each column, center line in the column and the bars represent the 95% confidence interval, medium and range of 12–18 rats, respectively. **P<0.01, ***P<0.001 compared with the SCOP group.

**Drugs**

Berberine hydrochloride (BER), scopolamine hydrobromide (SCOP), physostigmine (Eserine, PHY), neostigmine (NEO), scopolamine N-methylbromide (M-SCOP) were purchased from Sigma (St. Louis, MO, USA). All drugs were dissolved in 0.9% saline.

**Statistics**

All data obtained during the passive avoidance task were expressed in terms of medians and interquartile ranges and further analyzed by Kruskal-Wallis non-parametric one-way analysis of variance, followed by Mann-Whitney's U-test. In addition, the data collected during motor activity recording was analyzed by one-way analysis of variance, followed by the Duncan multiple range test. The criterion for statistical significance was P<0.05 in all the above statistical evaluations.

**RESULTS**

**Passive avoidance**

As shown in Fig. 1, SCOP (1.0 mg/kg, i.p.) remarkably
reduced the STL. BER orally administered to SCOP-treated rats daily for 7 days or 14 days at doses of 0.1 and 0.5 g/kg remarkably increased the SCOP-induced STL.

M-SCOP (0.5 mg/kg), which is a peripheral muscarinic receptor blocker and does not shorten the STL in the retention trial itself at this dosage, completely attenuated the anti-amnesic effect of BER (0.5 g/kg) after 14 days of administration in SCOP-induced amnesia (Fig. 2).

**Fig. 2.** Effects of scopolamine methylbromide (M-SCOP, 0.5 mg/kg) on berberine (BER, 0.5 g/kg)-induced recovery from SCOP-induced amnesia in rats. BER was administered for 14 days. Each column, center line in the column and the bars represent the 95% confidence interval, median and range of 12–18 rats, respectively. **P<0.01, compared with the SCOP group. ##P<0.01, compared with the group given SCOP in combination with BER.**

**Fig. 3.** Effects of physostigmine (PHY, 0.02 mg/kg) on berberine (BER, 0.5 g/kg)-induced recovery from scopolamine (SCOP)-induced amnesia in rats. BER was administered for 14 days. Each column, center line in the column and the bars represent the 95% confidence interval, median and range of 12–18 rats, respectively. **P<0.01, compared with the SCOP group. #P<0.05, compared with the group given SCOP in combination with BER.**

**Fig. 4.** Effects of neostigmine (NEO, 0.02 mg/kg) on berberine (BER, 0.5 g/kg)-induced recovery from scopolamine (SCOP)-induced amnesia in rats. BER was administered for 14 days. Each column, center line in the column and the bars represent the 95% confidence interval, median and range of 14–17 rats, respectively. **P<0.01, compared with the SCOP group. #P<0.05 compared with the group given SCOP in combination with BER.**

**Fig. 5.** Effect of berberine (BER) on the motor activity in rats. BER was administered for 14 days. Statistical evaluation by means of one-way ANOVA, followed by the Duncan multiple range test. Each column and vertical bar represent the mean±S.E.M. N=6–8 rats.
PHY (0.02 mg/kg), which is a peripherally and centrally acting AChE inhibitor and does not have a significant anti-amnestic effect on its own at this dosage, augmented the anti-amnesic effect of BER (0.5 g/kg) after 14 days of administration in SCOP-induced amnesia (Fig. 3).

NEO (0.02 mg/kg), which is a peripherally acting AChE inhibitor and does not have a significant anti-amnestic effect on its own at this dosage, augmented the anti-amnesic effect of BER (0.5 g/kg) after 14 days of administration in the SCOP-induced amnesia (Fig. 4).

Motor activity

The motor activity of rats administered with 0.1 or 0.5 g/kg BER after 14-day administration was not decreased as compared with the control group (Fig. 5). The motor activity of rats administered with 0.5 g/kg BER for 14 days in combination with SCOP was not decreased as compared with the SCOP group (Fig. 6).

DISCUSSION

SCOP-induced amnesia has been proposed as a short-term amnesia model representative of dementia and useful for drug screening in animals and in humans (21, 22). SCOP, a muscarinic receptor antagonist, could induce the impairment of the passive avoidance response in rodents via the decrease in the central cholinergic neuronal activity (11). In the present study, it was observed that SCOP also significantly shortened the STL in the passive avoidance task. BER at a dose of 0.1 and 0.5 g/kg after acute administration did not significantly affect the STL, which was shortened by SCOP in passive avoidance task (data not shown). However, it prolonged the STL, which was shortened by SCOP after 7 days or 14 days of administration.

On the other hand, the effect on sensory, motivational or motor systems can in turn affect the acquisition of the avoidance response. Several aspects argue against the possibility that the passive avoidance response in drug-treated animals can be related to the sensitivity to nociceptive stimuli or motor alteration during training. SCOP at 1.0 mg/kg could slightly enhance motor activity, but the effect was non-significant. In addition, 0.1 mg/kg SCOP did not alter the animals’ electric pain threshold (data not shown). Consistent with the report by Elord and Buccafusco, SCOP-induced impairment of passive avoidance response is mainly due to the deficit in learning acquisition (11). It was noted that BER after 14 days of administration did not change the motor activity. Furthermore, BER after 14 days of administration also did not change the SCOP-induced motor activity. The present data demonstrated that the improving effect of BER after 14-day administration could be only related to the memory-related process.

A recent study indicated that several cognitive enhancers improve the SCOP-induced amnesia by partially affecting the peripheral nervous system (17). In order to understand whether the anti-amnesic effect of BER after 14-day administration acted through the central or peripheral nervous system, we combined it with M-SCOP, PHY and NEO. M-SCOP, a peripherally acting muscarinic receptor antagonist, is known to reduce the activity of the cholinergic neuronal system in the peripheral nervous system and significantly attenuate the nootropic-reversed STL shortened by SCOP (15). In our present study, M-SCOP at 0.5 mg/kg did not shorten the STL in the retention trial itself, but completely attenuated the anti-amnesic effect of BER after 14 days of administration. PHY, a central and peripheral AChE inhibitor, is known to increase the activity of systemic acetylcholine neuronal system and significantly strengthened STL shortened by SCOP (23). PHY did not have a significant anti-amnestic effect on its own at our used dose and significantly potentiated the anti-amnesic effect of BER after 14 days of administration in the SCOP-induced amnesia. NEO, a peripheral AChE inhibitor, is known to increase the activity of the peripherally acting acetylcholine neuronal system and significantly strengthened the STL shortened by SCOP (24). NEO also did not have a significant anti-amnestic effect on its own at our used dose and significantly augmented the anti-amnesic effect of BER after 14 days of administration. Therefore, the anti-amnesic effect of BER could mainly involve the
Peripheral nervous system, but not the central nervous system.

Recent data suggest glucose as a possible mediator of physiological responses affecting memory process (25, 26). Some cognition enhancing drugs may influence learning and memory by increasing the availability of glucose for uptake and utilization in the brain (17). A variety of compounds exert their memory enhancing effects by inducing the release of epinephrine from the adrenal medulla which in turn releases glucose from hepatic stores. The increased glucose is actively transported into the brain where it is able to enhance energy production and the activity of the cholinergic system which have been most implicated in memory processes (27). A variety of evidence suggests that NEO may have antagonized the SCOP-induced amnesia via the release of epinephrine from the adrenal medulla. The released epinephrine, in turn, may have resulted in the release of glucose that enhanced memory via augmentation of cholinergic neurotransmission (28). Furthermore, $\alpha_2$-adrenoceptor antagonists potentiate AChE inhibitor effects on passive avoidance learning in rats (29). BER has $\alpha_2$-adrenoceptor blockade effect (7). Therefore, the antiamnesic effect of BER after 14 days of administration may partially be due to the increase in the release of epinephrine via blocking $\alpha_2$-adrenoceptor. The intact action mechanism will be worthy of investigation in the future.

Taking all these observations into consideration, we propose that long-term administration of BER could improve the SCOP-induced amnesia. The antiamnesic effect of BER after 14 days of administration may be related to the increase in the systemic cholinergic neuronal activity.

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