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Ameliorating effect of emodin, a constitute of *Polygonatum multiflorum*, on cycloheximide-induced impairment of memory consolidation in rats

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Abstract

The aim of the present study was intended to investigate the ameliorating effects of emodin on memory consolidation via cholinergic, serotonergic and GABAergic neuronal systems in rats. First, we evaluated the ameliorating effects of emodin on cycloheximide (CXM)-induced impairment of passive avoidance response in rats. Secondly, we clarified the role of cholinergic, serotonergic or GABAergic system on the ameliorating effect of emodin by using 5-HT1A receptor partial agonist, 5-HT2 receptor antagonist, GABAB agonist, GABAA antagonist and muscarinic receptor antagonist. Emodin protected the rat from CXM-induced memory consolidation impairment. The beneficial effect of emodin on CXM-induced memory consolidation impairment was amplified by 8-OH-DPAT (5-HT1A receptor partial agonist) and ritanserin (5-HT2 receptor antagonist), but reduced by scopolamine. These results suggested that the beneficial effect of emodin on CXM-induced memory consolidation impairment was amplified by serotonergic 5-HT1A-receptor partial agonist and 5-HT2 receptor antagonist but reduced by muscarinic receptor antagonist.

Keywords: Cholinergic system; Cycloheximide (CXM); Emodin (Emd); Memory consolidation; Passive avoidance task; Scopolamine (SCOP); Serotonergic system

1. Introduction

In general, memory processes are divided into three stages: learning acquisition, memory consolidation and retrieval. According to biochemical studies, memory consolidation needs the participation of protein, especially new protein transcription and synthesis (Goelet et al., 1986). Memory consolidation involves the activation, by neurotransmitters such as acetylcholine, dopamine and serotonin, of receptor-linked enzymes responsible for synthesis of intra- and intercellular messages (McEntee and Crook, 1991; Jodar and Kaneto, 1995). Cycloheximide (CXM), a protein synthesis inhibitor, induced memory consolidation deficits mainly via disturbances in the cholinergic and GABAergic systems and increased serotonergic activity in rodents (Nabeshima et al., 1989a, 1991).

Dried root of *Polygonatum multiflorum* (PM) Thunb. (Polygonaceae), well known as Hersouwu, is one of the most popular traditional medicinal herbs in Taiwan. It is frequently used to treat weakness, backache, knee pain, premature graying of hair in traditional Chinese medicine (Zhao, 2004). PM has been used for a long time as an antiaging agent and possesses hypocholesterolemic, antitumor and vasorelaxant effects (Zhang et al., 1983; Xiao et al., 1993). PM water extract (PME) exhibits a variety of pharmacological effects such as a preventive effect against cognitive deficits induced by Aβ 25–35 in mice (Um et al., 2006), antioxidative action (Chiu et al., 2002), free radical scavenging effect (Chen et al., 1999), the inhibition of monoamine oxidase (MAO) activity (Yang, 1996), improving memory (Chan et al., 2003), etc. The main active constituents of the herb have been reported to be hydroxyanthraquinones, stilbenes, phenolic compounds and their glycosides, etc. (Zheng et al., 1997). Emodin (1,3,8-trihydroxy-6-methylanthraquinone), an anthraquinone derivative from Hersouwu (*Polygonum multiflorum* Thunb.), has protective effect against brain disturbances

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induced by severe cerebral injury (Gu et al., 2000). Emodin has been shown to inhibit lipid peroxidation in rat brain homogenates (Sato et al., 1992). In our previous study, we found that PME ameliorated memory consolidation impairment induced by cycloheximide in rats. However, the effect of emodin on the cycloheximide-induced impairment of memory consolidation has not been studied.

The purpose of the present study is to investigate the effect of emodin on the cycloheximide-induced impairment of memory consolidation in rats. In the present study, the step-through passive avoidance task was used to measure the CXM-induced impairment to evaluate the mechanisms of the emodin on memory consolidation via cholinergic, serotonergic or GABAergic neuronal systems.

2. Materials and methods

2.1. Animal

This experiment was carried out in accordance with The Guidebook For The Care And Use Of Laboratory Animals (2001) published by the Chinese Society for the Laboratory Animal Science, Taiwan, ROC. Male naive Sprague–Dawley rats (200–250 g) were used. Each experimental group included 18 rats. They were fed a standard diet and water *ad libitum* and kept in a regulated environment ($23 \pm 1\,^{\circ}C$), with a 12-h light/12-h dark cycle (8:00–20:00, light). The training trial with the corresponding method was done in the 1 week of the treatment, 60 min after the drug administration.

2.2. Drugs

The following drugs were used: emodin, cycloheximide, scopolamine hydrobromide (SCOP), *p*-chloro-amphetamine hydrochloride (PCA), bicuculine (BIC) and baclofen (BAC) were all purchased from Sigma–Aldrich Co. (USA) and dissolved in 0.9% saline. 8-Hydroxy-2-(di-*n*-propylamino) tetralin hydrobromide (8-OH-DPAT, Sigma–Aldrich Co.) was dissolved in 0.9% saline with 0.5% of ascorbic acid. Ritanserin (RIT, Research Biochemicals Inc.) was dissolved in distilled water with lactic acid to adjust pH to about 4. Drug concentrations were prepared in such a way that the necessary dose could be administered in a volume of 1 ml/kg by intraperitoneal injection, emodin (p.o.) was administered 30 min before the training trial. CXM (s.c.) and the other above drug (i.p.) were administered immediately after training to avoid any direct acute effect on behavior in the training or retention trial.

2.3. Passive avoidance test

The step-through passive avoidance task was used to measure the three-memory processes stage depending on drug-treated period. This apparatus consisted of two compartments having a steel rod grid floor (36 parallel steel rods, 0.3 cm in diameter set 1.5 cm apart). One of the compartments (48 cm × 20 cm × 30 cm) was dark, and the other was equipped with a 20 W lamp located centrally at a height of 30 cm as the size, connected through a guillotine door (5 cm × 5 cm). The dark room was used during the experimental sessions that were conducted between 09:00 and 17:00 h.

At the beginning of a training trial, the guillotine door connecting the light and dark compartment was closed. After each rat was placed in the light compartment with its back to the guillotine door, the door was opened and simultaneously the time (step-through latency, STL) taken by the rat to enter the dark compartment was measured with the stopwatch. Once the rat entered the dark compartment, the door was closed. An inescapable scrambled foot shock (1.0 mA, 2 s) was then delivered through the grid floor by MCU-101 Controller (Muromachi Kikai Co., Tokyo). The rat was removed from the dark compartment 5 s after administering the shock. The rat was then put back to its home cage until the retention trial, which was carried out 24 h later. The rat was once again placed in the light compartment, and as in the case of the training trial, the guillotine door was opened and the STL was recorded and used as a measure of retention (Worms et al., 1989). An upper cutoff time of 300 s was set.

To evaluate the effect of various drug combinations on motor activity in passive avoidance task, the same experimental steps were followed as described in the above section exception that rat received no foot shock during the training period. Twenty-four hours later, the retention trial was also carried out and the STL was recorded. The rats were given 1.5 mg/kg CXM in combination with the following drugs.

In order to investigate the action of mechanism of emodin on CXM-induced amnesia in rats, we combined the drugs as following:

(a) Scopolamine HBr (muscarinic receptor antagonist, 0.3 mg/kg, i.p.) administered 30 min before the training trial.
(b) 8-OH-DPAT (5-HT1A receptor partial agonist, 0.025mg/kg, i.p.) administered 30 min before the training trial.
(c) Ritanserin (5-HT2 receptor selective antagonist, 0.25 mg/kg, i.p.) administered 30 min before the training trial.
(d) Bicuculine (GABAA antagonist, 0.025 mg/kg, i.p.) administered 20 min before the training trial.
(e) Baclofen (GABAB receptor antagonist, 0.01 mg/kg, i.p.) administered 20 min before the training trial.

2.4. Statistical analysis

Because the data distribution from the passive avoidance task was truncated at 300 s, nonparametric Kruskal–Wallis analysis followed by two-tailed Mann–Whitney *U*-tests was used to analyze the data. The criterion for statistical significance was $p < 0.05$ in all the above statistical evaluations.

3. Results

3.1. Effects of emodin on CXM-induced amnesia

CXM (1.0 mg/kg) injected immediately after the training session was endowed with amnesic properties. This significantly reduced the STL in the retention test ($p < 0.001$). Mann–Whitney *U*-tests indicated that emodin significantly prevented CXM-

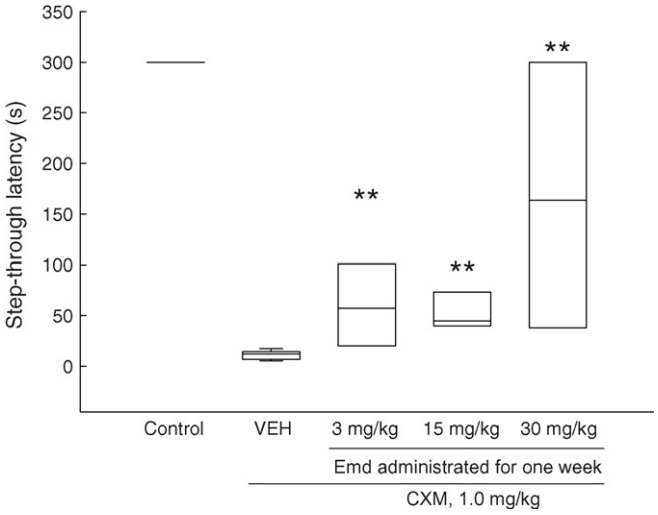

Fig. 1. Effects of emodin (Emd) dose dependently prevent the CXM-induced impairment of passive avoidance response in rats. Each column, centerline in the column and the bars represent the 95% confidence interval, median and range of 18 rats, respectively. $^{**}p<0.01$, compared with the CXM group.

induced memory disruption at 3.0, 15, 30 mg/kg ($p<0.01$, Fig. 1).

3.2. Cholinergic and serotonergic system roles in the memory ameliorating effects of emodin on CXM-induced amnesia

In the learning acquisition, SCOP (1.0 mg/kg) reduced the emodin (15 mg/kg)-induced recovery from CXM (1.0 mg/kg) in rodents ($p<0.01$, Fig. 2). 8-OH-DPAT (0.025 mg/kg) injected before the training trial enhanced the memory ameliorating effect of emodin (15 mg/kg) on CXM-induced amnesia ($p<0.01$, Fig. 3). RIT (0.25 mg/kg) injected before the training trial enhanced the memory ameliorating effect of emodin (15 mg/kg) on CXM-induced amnesia ($p<0.01$, Fig. 4).

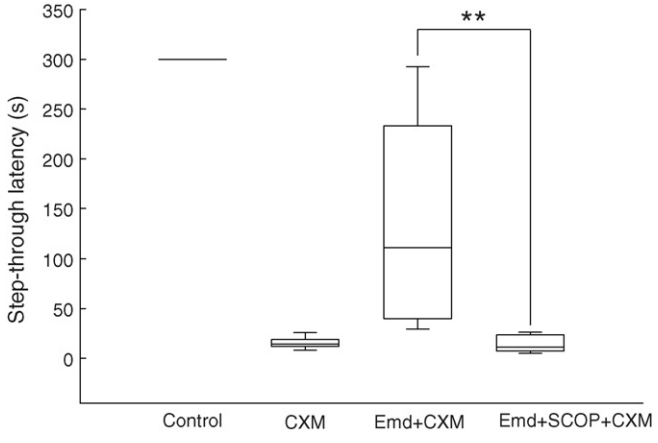

Fig. 2. Effects of scopolamine (SCOP, 0.3 mg/kg) on emodin-induced recovery from cycloheximide (CXM)-induced amnesia on rats. Each column, centerline in the column and the bars represent the 95% confidence interval, median and the range of 18 rats, respectively. $^{**}p<0.01$, compared with the group given CXM in combination with Emd.

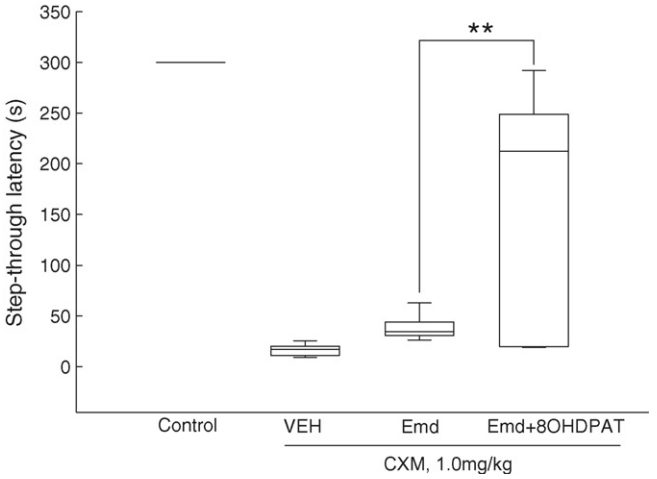

Fig. 3. Effects of 8-hydroxy-2-(di-*n*-propylamino) tetralin hydrobromide (8-OH-DPAT, 0.025 mg/kg) on emodin-induced recovery from cycloheximide (CXM)-induced amnesia on rats. Each column, centerline in the column and the bars represent the 95% confidence interval, median and the range of 18 rats, respectively. $^{**}p<0.01$, compared with the group given CXM in combination with Emd.

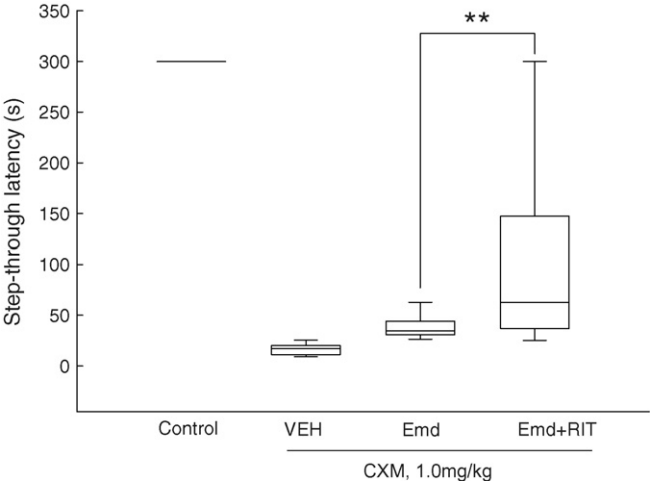

Fig. 4. Effect of ritanserin (RIT, 0.25 mg/kg) on emodin-induced recovery from cycloheximide (CXM)-induced amnesia on rats. Each column, centerline in the column and the bars represent the 95% confidence interval, median and the range of 18 rats, respectively. $^{**}p<0.01$, compared with the group given CXM in combination with Emd.

3.3. GABAergic system roles in the memory ameliorating effects of emodin on CXM-induced amnesia

BIC (0.025 mg/kg) did not significantly reduce the ameliorating effect of emodin (15 mg/kg) on CXM-induced amnesia (Fig. 5). BAC (0.01 mg/kg) injected before the training trial was not able to decrease the ameliorating effect of emodin (15 mg/kg) on CXM-induced amnesia (Fig. 6).

4. Discussion

CXM, a protein synthesis inhibitor, induced impairment of memory consolidation in the passive avoidance apparatus in rats. Emodin (3–30 mg/kg) significantly prevented the efficacy dose

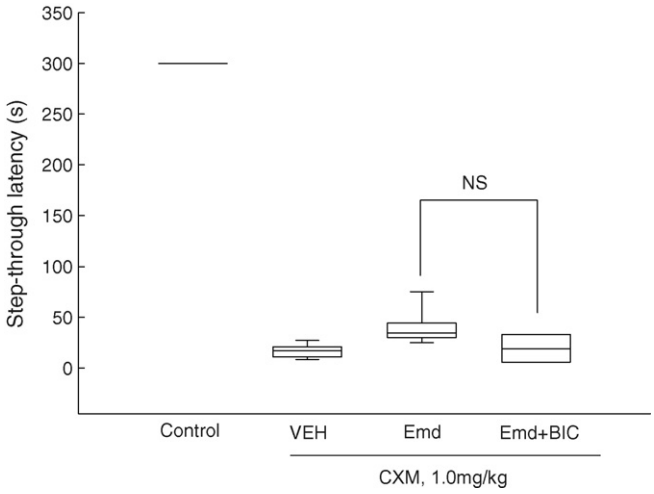

Fig. 5. Effects of bicuculine (BIC, 0.025 mg/kg) on emodin-induced recovery from cycloheximide (CXM)-induced amnesia on rats. Each column, centerline in the column and the bars represent the 95% confidence interval, median and the range of 18 rats, respectively. NS, not significant.

of CXM-induced amnesia in rat. Emodin might be counteractive the protein synthesis inhibition in memory consolidation. CXM-induced amnesia has been found to act through the disturbance in the cholinergic neuronal system and the increase in the serotonergic neuronal system (Davis et al., 1984; Nabeshima et al., 1989a,b).

Memory consolidation can be influenced by various drugs that are affecting the cholinergic, noradrenergic, dopaminergic, serotonergic, GABAergic systems (Decker and McGaugh, 1989; McGaugh, 1989). This present study used SCOP, 8-OH-DPAT, RIT, BIC and BAC to realize the roles of emodin in cholinergic, serotonergic and GABAergic system in learning acquisition and memory consolidation.

Cycloheximide induced memory consolidation deficits via disturbances in the cholinergic system in rodents. In the present study, we found that SCOP (0.3 mg/kg), the muscarinic receptor antagonist shorten the STL of the retention trial; this result was consistent with other report (Hsieh et al., 1999). SCOP (0.3 mg/kg) also significantly attenuated the prevention effect of emodin on CXM-induced amnesia. Therefore, our present data suggest that the ameliorating effect of emodin on memory consolidation could be related to increase in the cholinergic neuronal system via activating muscarinic receptor.

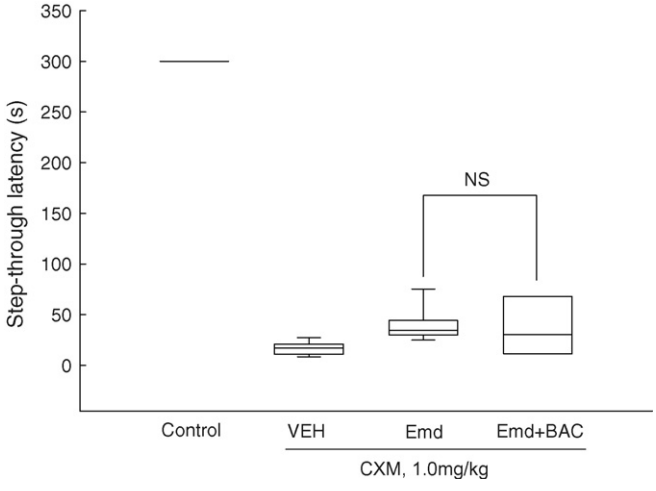

Fig. 6. Effects of bacofen (BAC, 0.01 mg/kg) on emodin-induced recovery from cycloheximide (CXM)-induced amnesia on rats. Each column, centerline in the column and the bars represent the 95% confidence interval, median and the range of 18 rats, respectively. NS, not significant.

Activation of the serotonergic systems produces memory deficit, whereas inhibition of these system enhance memory. 5-HT receptors were classified into several subtypes: 5-HT1 and 5-HT2 receptor subtypes had been reported to play an important role in learning acquisition (Matsuno et al., 1993). In the present study, we also found that 8-OH-DPAT (0.025 mg/kg), the 5-HT1A receptor agonist prolonged the STL of the retention trial; this result was consistent with other reports (Nabeshima et al., 1989b; Hsieh et al., 1999). 8-OH-DPAT (0.025 mg/kg) also significantly enhanced the prevention effect of emodin on CXM-induced amnesia. On the other hand, the increase in serotonergic activity by 5-HT2 receptors may be contributed to CXM-induced amnesia (Nabeshima et al., 1989b). RIT (0.25 mg/kg), the 5-HT2-receptor antagonist significantly progressed the ameliorating effect of emodin when combined the RIT with CXM-induced amnesia. Therefore, our present data suggest that the ameliorating effect of emodin on memory consolidation could be related to decrease in the serotonergic neuronal system via activating presynaptic 5-HT1A, inhibiting postsynaptic 5-HT2 receptors.

GABA affects cognitive processes, such as learning and memory (Swartzwelder et al., 1987; Castellano et al., 1989; Bowery, 1993). Activation of GABAergic receptors impairs memory, while inhibition of these receptors tends to enhance memory. The central action of GABA exerts its numerous actions through interaction with two pharmacologically distinct receptor subtypes: GABAA and GABAB. BIC, the GABAA receptor antagonist (0.025 mg/kg) could not shorten the STL of the retention trial (Hsieh et al., 1999). Emodin could not maintain the memory enhancement when combined with BIC and CXM-induced amnesia. BAC (0.01 mg/kg), a potent GABAB agonist (Bowery et al., 1980) had been demonstrated to interfere with memory consolidation or retention in rodents (Swartzwelder et al., 1987; Castellano et al., 1989; Bowery, 1993). In the present data, it was shown that BAC did not have effect on the beneficial effect of emodin on the CXM-induced amnesia. The findings of the present experiment do not reveal the mechanism through the GABAergic system.

We also studied the effects of various drugs combinations on motor activity in passive avoidance task. During the training period without foot shock, the STL of various drugs combinations did not alter in training trial and testing trial (data not shown).

However, 5-HT receptors had other subtypes such as 5-HT3 and 5-HT4 receptors. Recent studies point out that 5-HT3 and 5-HT4 receptors also play an important role in learning and memory (Brambilla et al., 1993; Eglen et al., 1995); the role of 5-HT3 and 5-HT4 receptors in the ameliorating effect of emodin on CXM-induced amnesia will be further studied in the future.

Taken together, these results suggested that emodin prevented the rats from the CXM-induced amnesia. The ameliorating mechanisms of emodin on CXM-induced amnesia may be via blocking the serotonin release or activating the presynaptic 5-HT1A receptor and muscarinic receptor.

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