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EFFECTS OF SAN-YU-TANG ON THE CYCLOHEXIMIDE-INDUCED AMNESIA IN RATS

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The effects of San-Yu-Tang (SYT) on cycloheximide (CXM)-induced amnesia were assessed by passive avoidance in rats. Oral SYT administration (0.5 or 1 g/kg for 1 week) significantly prolonged step-through latency in the amnesic animals. This beneficial effect of SYT on CXM-induced amnesia was enhanced by 8-OH-DPAT (a partial agonist of the 5-HT_{1A} receptor), but reduced by scopolamine (a muscarinic antagonist) and p-chloroamphetamine (a 5-HT releaser) and baclofen (a $GAGA_B$ receptor antagonist). These results suggest that SYT enhances memory functions in amnesic rats, at least in part, by increasing the activity of the muscarinic and $GABA_B$ receptors, and decreasing the activity of the serotoninergic system.

Key words: San-Yu-Tang, Memory consolidation, Cholinergic system, Serotoninergic system, GABAergic system, Passive avoidance task.

INTRODUCTION

In general, memory processes are divided into three stages: learning acquisition, memory consolidation, and memory retrieval. According to biochemical studies, memory consolidation needs the participation of protein, especially transcription and synthesis of new protein[1,2]. Memory consolidation involves the activation by neurotransmitters such as acetylcholine, dopamine, and serotonin, of receptor-linked enzymes responsible for synthesis of intra- and inter-cellular messages[3]. Cycloheximide (CXM), a protein synthesis inhibitor, induced memory consolidation deficits[4-6]. In a series of studies, Nabeshima et al. (1991) pointed out that CXM caused memory consolidation

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deficits mainly by disturbing the cholinergic and GABAergic systems and increasing serotoninergic activity in experimental animals[7,8].

San-Yu-Tang (SYT), a traditional Chinese medicine, is composed of Radix Ginseng and Radix Astragali, which possess tonic effects; Radix Rehmanniae, Radix Angelicae Sinensis and Radix Paeoniae alba, which strengthen the blood; and Radix Chuanxiong, which possess ameliorative effect on blood circulation. SYT was modified from Si-Wu-Tang and combined with Radix Ginseng and Radix Astragali. These medications used to treat blood diseases, immunological disorders, hematemesis, and cerebral ischemia[9-14]. The tonic effect of SYT on blood may improve the impairment of learning and memory. Therefore, in the present study, we attempted to investigate the learning and memory ameliorating effect of SYT on CXM-induced amnesia and clarify the role of cholinergic, serotonergic and GABAergic systems by using the passive avoidance response in rats.

MATERIALS AND METHODS

1. Experimental animal

Male Sprague-Dawley rats, weighing 200-250 g, were given food and water *ad libitum* and kept in a regulated environment (23 ± 1 °C), with a 12 h light-dark cycle (8:00-20:00, light). Each experimental group included 12 to 18 rats.

2. Preparation of SYT

SYT was composed of six medicinal plants as shown in Table 1. SYT consists of crude ingredient extracted from 1.6 kg of herbal medicine. Dr. Chung-Chun Chen, Institute of Chinese Pharmaceutical Science, made the botanical identification. The 1.6 kg of herb was boilded with sixteen liters of water for 50 min. The supernatant was collected. We reextracted with 16 L of water for another 50 min and collected this supernatant. These supernatants were centrifuged (5,500 × *g*, 30 min) to remove insoluble ingredients, and concentrated to dryness. The yield ratio was 32.07 %.

Table 1. The ratio of the components in SYT

Components		ratio
1.	Radix Astragali (root of *Astragalus membranaceus* BGE)	3
2.	Radix Ginseng (root of *Panax ginseng* C.A. MEY)	2
3.	Radix Angelicae sinensis (root of *Angelica sinensis* DIELS)	3
4.	Radix Rehmannia (root of *Rehmannia glutinosa* LIBOSCH)	1
5.	Radix Paeonia alba (root of *Paeonia lactiflora* PALL)	2
6.	Radix Chuanxiong (root of *Ligusticum chuanxiong* HORT)	2

3. Drug and administration

CXM, scopolamine hydrobromide (SCOP), *p*-chloro-amphetamine hydrochloride (PCA), bicuculine (BIC) and baclofen (BAC) were all purchased from Sigma Chemical Co. and dissolved in 0.9 % saline. 8-Hydroxy-2-(di-n-propylamino) tetralin hydrobromide (8-OH-DPAT, Sigma Chemical Co.) was dissolved in 0.9 % saline with 0.5 % ascorbic acid. Ritanserin (RIT, Research Biochemicals Inc.) was dissolved in distilled water with lactic acid to adjust pH to about 4. These drugs were administered in a volume of 1 ml/kg body weight. SYT was administered orally to rats once a day for one week. The training trial was done after the last administration for 60 mins. CXM was administered immediately after training trial.

4. Step-through passive avoidance apparatus and behavior training trial

The 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 × 20 × 30 cm) was equipped with a 20 W lamp located centrally at a height of 30 cm, and the other was dark compartment of same size, connected through a guillotine door (5 × 5 cm)[4-6]. The dark room was used during the experimental sessions that were conducted between 09:00 and 17:00 hours.

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 enters the dark compartment, the door was closed. An inescapable scrambled foot shock (1.0 mA, 2s) was then delivered through the grid floor by the MCU-101 Controller (Muromachi Likai 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 step-through latency (STL) was recorded and used as a measure of retention. An upper cutoff time of 300 s was set.

In order to investigate the action of mechanism of SYT on CXM-induced amnesia in rats, each of the following drugs was administered to rats 30 min before the training trial: SCOP (muscarinic receptor antagonist, 0.3 mg/kg, i.p.); PCA (5HT releaser, 1.0 mg/kg, i.p.); 8-OH-DPAT ($5HT_{1A}$ receptor partial agonist, 0.025 mg/kg, i.p.); and RIT ($5HT_2$ receptor selective antagonist, 0.25 mg/kg, i.p.). The BAC ($GABA_B$ receptor agonist, 0.01 mg/kg, i.p.) and BIC ($GABA_A$ antagonist, 0.025 mg/kg, i.p.) were administered 20 mins before the training trial.

5. Statistics

Because the data distribution from the passive avoidance task was truncated at 300 seconds, nonparametric Kruskal-Wallis analysis followed by two-tailed Mann-Whitney *U*-tests were used to analyze the data. The criterion for

statistical significance was $p < 0.05$ in all the above statistical evaluations.

RESULTS

1. Effects of SYT on CXM-induced memory consolidation impairment

CXM (1.0 mg/kg) injected immediately after the training trial significantly reduced the STL in the retention test ($p < 0.001$). Mann-Whitney U-tests indicated that SYT significantly prevented CXM-induced memory disruption at 1.0 g/kg ($p < 0.01$, Fig. 1).

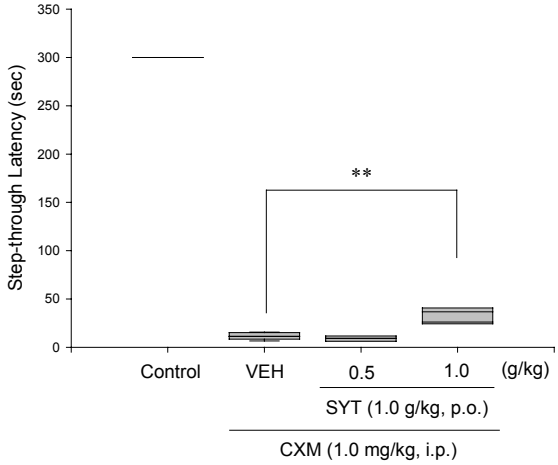

Fig. 1. Effect of San-Yu-Tang (SYT)-induced amelioration from cycloheximide (CXM)-induced amnesia on rats. Each column, centerline in the column and the bars represent the 95 % confidence interval, median and the range of 12-18 rats, respectively. ** $p < 0.01$, compared with the group given CXM in combination with SYT.

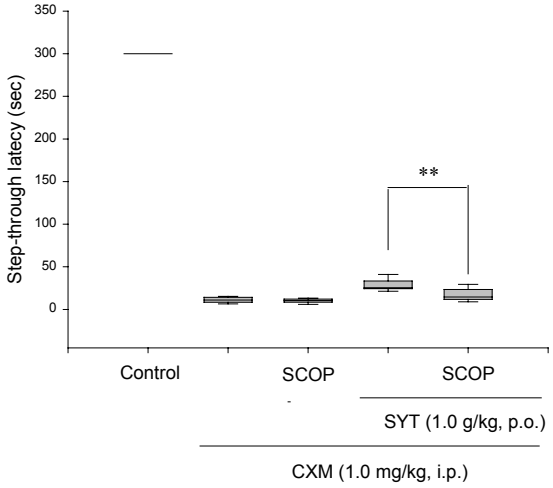

Fig. 2. Effect of scopolamine (SCOP) (0.3 mg/kg) on San-Yu-Tang (SYT)-induced amelioration from cycloheximide (CXM)-induced amnesia on rats. Each column, centerline in the column and the bars represent the 95 % confidence interval, median and the range of 12-18 rats, respectively. ** $p<0.01$, compared with the group given CXM in combination with SYT.

2. Roles of cholinergic and serotonergic systems in the memory ameliorating effects of SYT on CXM-induced amnesia

As shown in Fig. 2, SCOP (1.0 mg/kg) reduced the SYT (1.0 g/kg) – induced amelioration from CXM (1.0 mg/kg) in rodents ($p < 0.01$). PCA, a 5-HT releaser, injected before the training trial at 1.0 mg/kg markedly counteracted the memory ameliorating effect of SYT at 1.0 g/kg on CXM-induced impairment of passive avoidance response ($p < 0.001$, Fig. 3). 8-OH-DPAT, a selective 5-HT_{1A} partial agonist, injected before the training trial at 0.025

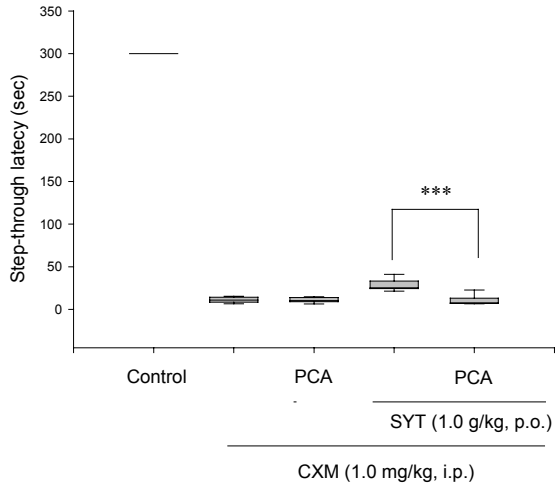

Fig. 3. Effect of p-chloroamphetamine (PCA) (1.0 mg/kg) on San-Yu-Tang (SYT)-induced amelioration from cycloheximide (CXM)-induced amnesia on rats. Each column, centerline in the column and the bars represent the 95 % confidence interval, median and the range of 12-18 rats, respectively. *** $p < 0.001$, compared with the group given CXM in combination with SYT.

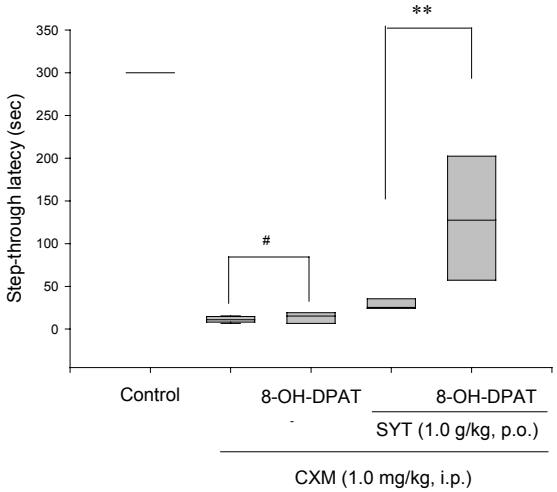

Fig. 4. Effect of 8-OH-DPAT (0.025 mg/kg) on San-Yu-Tang (SYT)-induced amelioration from CXM-induced amnesia on rats. Each column, centerline in the column and the bars represent the 95 % confidence interval, median and the range of 12-18 rats, respectively. ** $p < 0.01$, compared with the group given CXM in combination with SYT. # $p < 0.05$, compared with the group given CXM.

mg/kg enhanced the memory ameliorating effect of SYT at 1.0 g/kg on CXM-induced impairment of passive avoidance response ($p < 0.01$, Fig. 4). RIT, a selective 5-HT_2-receptor antagonist, injected before the training trial at 0.25 mg/kg did not reduce the memory ameliorating effect of SYT (1.0 g/kg) on CXM-induced impairment of passive avoidance response (Fig. 5).

3. Role of GABAergic system in the memory ameliorating effects of SYT on CXM-induced amnesia

BIC at 0.025 mg/kg did not significantly reduce the ameliorating effect of SYT (1.0 g/kg) on CXM-induced

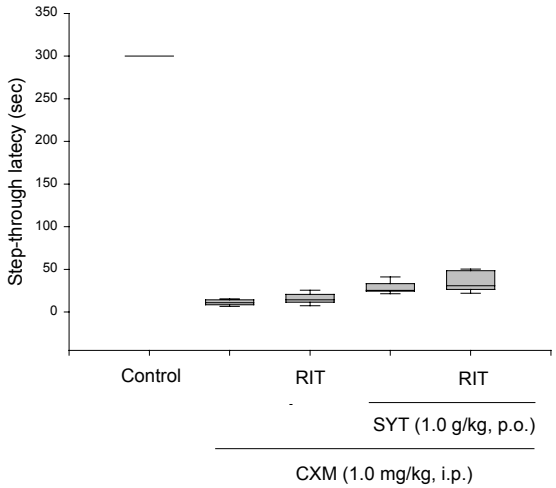

Fig. 5. Effect of ritenserin (RIT) (0.25 mg/kg) on San-Yu-Tang (SYT)-induced amelioration from CXM-induced amnesia on rats. Each column, centerline in the column and the bars represent the 95 % confidence interval, median and the range of 12-18 rats, respectively.

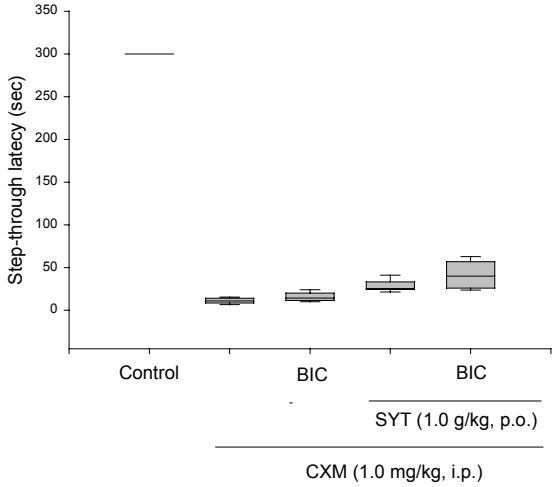

Fig. 6. Effect of bicucurine (BIC) (0.025 mg/kg) on San-Yu-Tang (SYT)-induced amelioration from cycloheximide (CXM)-induced amnesia on rats. Each column, centerline in the column and the bars represent the 95 % confidence interval, median and the range of 12-18 rats, respectively.

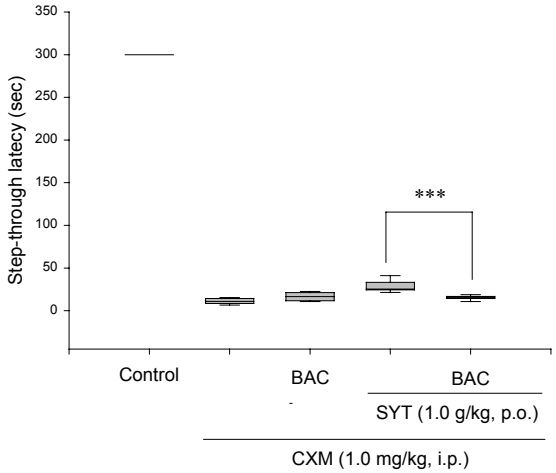

Fig. 7. Effect of baclofen (BAC) (0.01 mg/kg) on San-Yu-Tang (SYT)-induced amelioration from cycloheximide (CXM)-induced amnesia on rats. Each column, centerline in the column and the bars represent the 95 % confidence interval, median and the range of 12-18 rats, respectively. *** $p < 0.001$, compared with the group given CXM in combination with SYT.

impairment of passive avoidance response (Fig. 6). BAC, the selective agonist of $GABA_B$ receptors, injected before the training trial at 0.01 mg/kg decreased the ameliorating effect of SYT (1.0 g/kg) on CXM-induced impairment of passive avoidance response ($p < 0.01$, Fig. 7).

DISCUSSION

CXM-induced impairment of passive avoidance response had been found to act through the disturbance in the cholinergic neuronal system and the increase in the serotonergic neuronal system[4-6]. CXM, a protein synthesis inhibitor, induced impairment of memory consolidation in the passive avoidance test in rats. The acute administration of SYT (1.0 g/kg) did not affect the CXM-induced amnesia in rats (data not show). However, SYT after seven-day administraion significantly attenuated the CXM-induced amnesia in rats. The present data suggested that SYT after seven-day administraion could ameliorate CXM-induced impairment of passive avoidance response. Furthermore, the impairment of memory consolidation induced by CXM can be influenced by various drugs that affecting the cholinergic, serotonergic, and GABAergic systems[15-17]. Therefore, this present study used the SCOP, PCA, 8-OH-DPAT, RIT, BIC and BAC to realize the beneficial effect of SYT on CXM-induced amnesia in cholinergic, serotonergic and GABAergic system.

PCA, the serotonin releaser, administered at 1.0 mg/kg could not shorten the STL in the retention test itself[18,19], but counteracted the ameliorating effect of SYT on CXM induced amnesia. This data indicated that SYT might ameliorate amnesia induced by CXM in passive avoidance task via inhibiting the 5-HT release from nerve terminals. Furthermore, to verify the role of serotonin receptor in this passive avoidance task, serotonin receptor agonist and antagonist were tested in the study. 5-HT receptors were classified into several subtypes: 5-HT_1 and 5-HT_2 receptor

subtypes had been reported to play an important role in learning and memory[20]. In the present study, we also found that 8-OH-DPAT, the presynaptic 5-HT_{1A} receptor partial agonist at 0.025 mg/kg, prolonged the STL of the retention trial; this result was consistent with other reports[6,18,19]. 8-OH-DPAT at this dose also significantly enhanced the beneficial effect of SYT on CXM-induced amnesia. Therefore, SYT prevented CXM-induced amnesia might be via the presynaptic 5-HT_{1A}-receptor. RIT (0.25 mg/kg), the 5-HT_2-receptor antagonist, could not augument the ameliorating effect of SYT. Therefore, our present data suggested that the ameliorating effect of SYT on memory consolidation could be related to the decrease in the serotonergic neuronal system activity via activating presynaptic 5-HT_{1A}-receptor or blocking the serotonin release.

Besides cholinergic and serotonergic mechanisms, central GABAergic systems also participates in the process of learning and memory[21-24]. The central action of GABA was mediated by specific receptors classified as $GABA_A$ and $GABA_B$. The $GABA_A$ receptor antagonist BIC given at 0.025 mg/kg could not shortened the STL of the retention trial[18,19]. BIC could not affect the ameliorating effect of SYT. BAC, a potent $GABA_B$ agonist[21] had been demonstrated to interfere with memory consolidation or retention in rodents[22-24]. The present data showed that BAC (0.01 mg/kg) was very significantly reduced the ameliorating effect of SYT on the CXM-induced amnesia. These results indicated that CXM-induced amnesia could be via inhibiting the activity of $GABA_B$ receptor but not $GABA_A$ receptor.

We were 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 retention test (data not shown).

Taking all these observations into account, we proposed that SYT ameliorated the rats from the CXM-induced amnesia. The ameliorating mechanisms of SYT on CXM-induced amnesia may be via blocking the seotonin release, inhibiting the $GABA_B$ receptor or activating the presynaptic 5-HT_{1A} receptor. However, 5-HT receptors had other subtypes such as 5-HT_3 and 5-HT_4 receptors. Recent studies point out that 5-HT_3 and 5-HT_4 receptors also play an important role in learning and memory[25,26]; the role of 5-HT_3 and 5-HT_4 receptors in the ameliorating effect of SYT on CXM-induced amnesia will be further studied in the future.

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聖愈湯改善環己醯胺誘發大鼠之健忘

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本研究以被動迴避學習反應儀探討聖愈湯對環己醯胺誘發大鼠健忘之影響。

結果顯示，每天口服聖愈湯 0.5 或 1.0 g/kg 一次，連續給予一星期，可顯著延長健忘動物被動迴避反應期，聖愈湯改善環己醯胺誘發大鼠之健忘可被 8-OH-DPAT（5-HT1A 部份致效劑）增強；但被 SCOP（蕈毒鹼拮抗劑）、PCA（5-HT 釋放劑）及 BAC（GABA-B 接受器拮抗劑）減弱。

綜合以上結果顯示，聖愈湯增強健忘大鼠的記憶功能可能與增強蕈毒鹼接受器及 GABA-B 接受器活性及降低 5-HT 系統活性有關。

關鍵詞：聖愈湯，記憶鞏固，膽鹼系統，5-HT 系統，GABA 系統，被動迴避儀。

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