Journal of Integrative Neuroscience

J. Integr. Neurosci. **2024**; 23(1): 17
https://doi.org/10.31083/j.jin2301017

Original Research

Puerarin Attenuates Cycloheximide-Induced Oxidative Damage and Memory-Consolidation Impairment in Rats

Kuo-Jen Wu[1,†], Jin-Cherng Lien[1,†], Chi-Rei Wu[2,*]

[1]School of Pharmacy, China Medical University, 40402 Taichung, Taiwan
[2]The Department of Chinese Pharmaceutical Sciences and Chinese Medicine Resources, China Medical University, 40402 Taichung, Taiwan
*Correspondence: crw@mail.cmu.edu.tw (Chi-Rei Wu)
[†]These authors contributed equally.

Academic Editor: Gernot Riedel
Submitted: 9 August 2023 Revised: 27 October 2023 Accepted: 31 October 2023 Published: 16 January 2024

Abstract

Background: Cycloheximide (CXM), an antifungal antibiotic, causes impaired memory consolidation as a side effect partially by disturbing the activities of the central catecholaminergic and cholinergic system. Some reports indicated that puerarin prevented memory impairment in various models in rodents. However, the protective effects of puerarin on the side effects of cycloheximide for memory consolidation impairment have not yet been investigated. **Methods**: The protective effects of puerarin on CXM-induced memory-consolidation impairment, and memory impairment produced by central administration of AF64A neurotoxin, were investigated using a passive avoidance task in rats. A combination of transmitter receptor agonists and antagonists was used to explore the effects of puerarin on nervous system function. The activity of antioxidant defense systems and neurotransmitter systems in the prefrontal cortex and hippocampus were assayed. **Results**: Systemic (25 and 50 mg/kg, i.p.) or central (5 and 10 µg/brain, i.c.v.) administration of puerarin attenuated CXM-induced memory-consolidation impairment produced by 1.5 mg/kg CXM (s.c.) in rats. The improvements produced by 50 mg/kg puerarin were blocked by cholinergic antagonists, a 5-HT_2 receptor agonist, and an adrenergic receptor antagonist. Puerarin (only at 50 mg/kg, i.p.) reversed the CXM-induced alterations of the levels of norepinephrine in the prefrontal cortex and the levels of monoamines in the hippocampus. Puerarin also increased antioxidant-defense-system activities in the prefrontal cortex and hippocampus, which had been decreased by CXM. **Conclusions**: We suggested that the attenuating effects of puerarin on CXM-induced memory-consolidation impairment may be due to decrease oxidative damage and the normalition of the neurotransmitter function in the prefrontal cortex and hippocampus.

Keywords: cycloheximide; neurotransmitters; oxidative damage; passive avoidance task; puerarin

1. Introduction

Memory loss, including anterograde and retrograde amnesia, is the first and major symptom in Alzheimer's disease (AD). Anterograde amnesia is characterized by an inability to learn new things and happens in the early stages of memory loss. Retrograde amnesia is characterized by loss of memory for information acquired before the onset of amnesia and almost always occurs in association with anterograde amnesia. Retrograde amnesia is closely connected to the loss of memory consolidation. It is currently well established that memory consolidation and long-lasting synaptic plasticity require the synthesis of new proteins [1]. Some studies [2,3] have pointed out that antibiotics such as anisomycin, puromycin and cycloheximide (CXM) have the side effect of causing memory loss by blockading protein synthesis. CXM has been shown to produce impairment of memory consolidation and to produce retrograde amnesia in various behavioral paradigms in rodents. Furthermore, memory consolidation is also associated with the activation of receptor-linked enzymes through neurotransmitters such as acetylcholine, dopamine and serotonin, which are responsible for the synthesis of intracellular and intercellular proteins [4,5]. Nabeshima *et al*. [6–8] indicated that CXM treatment caused impairment of memory consolidation partially by reducing cholinergic and catecholaminergic activities, and by increasing the serotonergic activity, in experimental animals. Hence, CXM-induced impairment of memory consolidation serves as a useful model for evaluating the development and mechanism of anti-amnestic drugs [9–11].

Puerarin (PUR), one of the major isoflavonoid bioactive ingredients of *Pueraria lobata* (Willd.) Ohwi, is available in common nutritional supplements to treat cerebrovascular diseases, diabetes, and neurodegenerative disorders such as AD (in animal disease models) [12]. Earlier reports suggested that total isoflavonoids of *Pueraria lobata* improved memory deficits caused by muscarinic receptor blocker scopolamine (SCOP), D-galactose or cerebral artery obstruction in rodents [13,14]. In a purified form, puerarin also attenuated memory deficits caused by D-galactose or cerebral artery obstruction in rodents [15,16]. Furthermore, puerarin improved memory impairment caused by central injection with amyloid β peptide (Aβ) or streptozotocin (STZ) in rodents by restoring

acetylcholinesterase (AChE) and antioxidant-enzyme activity [17–20]. On the other hand, our previous report showed that puerarin attenuated the learning-acquisition deficits and anterograde amnesia induced by the nicotinic receptor blocker mecamylamine (MECA), by the serotonin releaser *p*-chloroamphetamine (PCA), and by the N-methyl-D-aspartate (NMDA) receptor blocker dizocilpine (MK-801), on passive avoidance performance in rats via increasing cholinergic and glutaminergic activity, and decreasing serotonergic neuronal activity [21]. Liu *et al.* [22] showed that puerarin improved lead-induced memory deficits in mice by suppressing oxidative stress and reversing the alterations in transmitter enzymes. So far, the protective effects of puerarin against the side effects of antibiotics on deficits of memory consolidation have not been investigated. This present study investigated the protective effect and mechanism of puerarin on CXM-induced impairment of memory consolidation. Several researchers have indicated that puerarin has the potential to cross the blood-brain barrier (BBB) and become distributed in various areas of the brain [20,23], therefore we first investigated the ameliorating effects of systemically or centrally administered puerarin on CXM-induced impairment of memory consolidation, and on centrally cholinergic neurotoxin AF64A-induced memory deficits, on a passive avoidance task in rats. To explore the role of neuronal system on the effects of puerarin on CXM-induced impairment of memory consolidation, we further investigated whether cholinergic receptor blockers, serotonergic receptor agonists, and adrenergic receptor blockers prevented the ameliorating effects of puerarin. The prefrontal–hippocampal circuit plays a critical role in memory consolidation [24], so we finally clarified the antioxidant and neurochemical mechanism of puerarin on CXM-induced impairment of memory consolidation in the prefrontal cortex and hippocampus by assessing antioxidant defense enzymes and neurotransmitter enzyme activity with microtiter spectrophotometry, and measuring the levels of monoamines using high-performance liquid chromatography with an electrochemical detector (HPLC/ECD).

2. Materials and Methods

2.1 Chemicals

Puerarin (purity >98%), purchased from Wako Pure Chemical Industries. Ltd. (Osaka, Japan), was dissolved in 0.01% ethanol. The following substances were purchased from Sigma-Aldrich (St. Louis, MO, USA): 1-(2,5-dimethoxy-4-iodophenyl)-2 aminopropane (DOI), 3-methoxy-4-hydroxyphenyl glycol (MHPG), 3,4-dihydroxyphenyl acetic acid (DOPAC), 5-hydroxyindoleacetic acid (5-HIAA), 5-hydroxytryptamine (5-HT), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), 5,7-dihydroxytryptamine (5,7-DHT), 6-hydroxydopamine (6-OHDA), 8-hydroxy-2-(di-n-propylamino) tetralin hydrobromide (DPAT), acetylcholine mustard hydrochloride, acetylcholinesterase (AchE), acetylthiocholine iodide (ActCh), cycloheximide (CXM), dopamine (DA), homovanillic acid (HVA), MECA, norepinephrine (NE), phenoxybenzamine hydrochloride (PHEN), propranolol hydrochloride (PROP) and scopolamine hydrobromide (SCOP). The 5,7-DHT and 6-OHDA were dissolved in normal saline containing 0.5% ascorbic acid [25,26]. The AF64A was freshly prepared according to our previous report [27]. DOI, DPAT, MECA, PHEN, PROP, and SCOP were dissolved in normal saline.

2.2 Animals

Male Sprague-Dawley rats (200–250 g), obtained from BioLASCO Taiwan Co., Ltd. (Taipei, Taiwan), were housed in a temperature- (23 ± 1 °C) and humidity-(60%) regulated environment with a 12 h–12 h light/dark cycle (light phase: 08:00 to 20:00). After a one-week acclimatization period, the rats were divided into groups (n = 8/gp) for use in the following experiments and the behavioral experiments were executed in a double-blind fashion.

2.3 Step-Through Passive Avoidance Test

The procedure was as described in our previous reports [21]. The test was conducted in two days, with the first day being the training trial and the second day being the retention trial. During the training trial, each rat was placed in the light compartment with its back to the guillotine door. The step-through latency (STL) before the rat entered the dark compartment was recorded. When the rat entered the dark compartment, the door was closed and an inescapable footshock (0.8 mA for 2 s) was delivered through an MCU-101 Controller (Muromachi Kikai Co., Tokyo, Japan). After the footshock, the rat was put back into the home cage. The next day (24 h later), the rat was again placed in the light compartment and the STL of the retention trial was recorded. The cut-off time was 300 sec. The experiments were performed between 09:00 and 17:00 hours.

2.4 Experimental Design

The schematic diagram of experimental designs and stages is shown in Fig. 1. For evaluating the mechanism of the attenuating action of puerarin on memory-consolidation impairment, we designed two series of experiments. The first, to evaluate the memory-improving effects of *systemic* treatment with puerarin, was performed using an injection of puerarin (10, 25, 50 mg/kg, i.p.) on CXM-induced impairment of memory-consolidation [21]. The second series, to evaluate the memory-improving effects of *central* treatment with puerarin, was performed using an intracerebroventricular (i.c.v.) injection of puerarin (1, 5, or 10 µg/brain) on rats with CXM-induced or AF64A-induced memory impairment. The detailed experimental performance was described as follows.

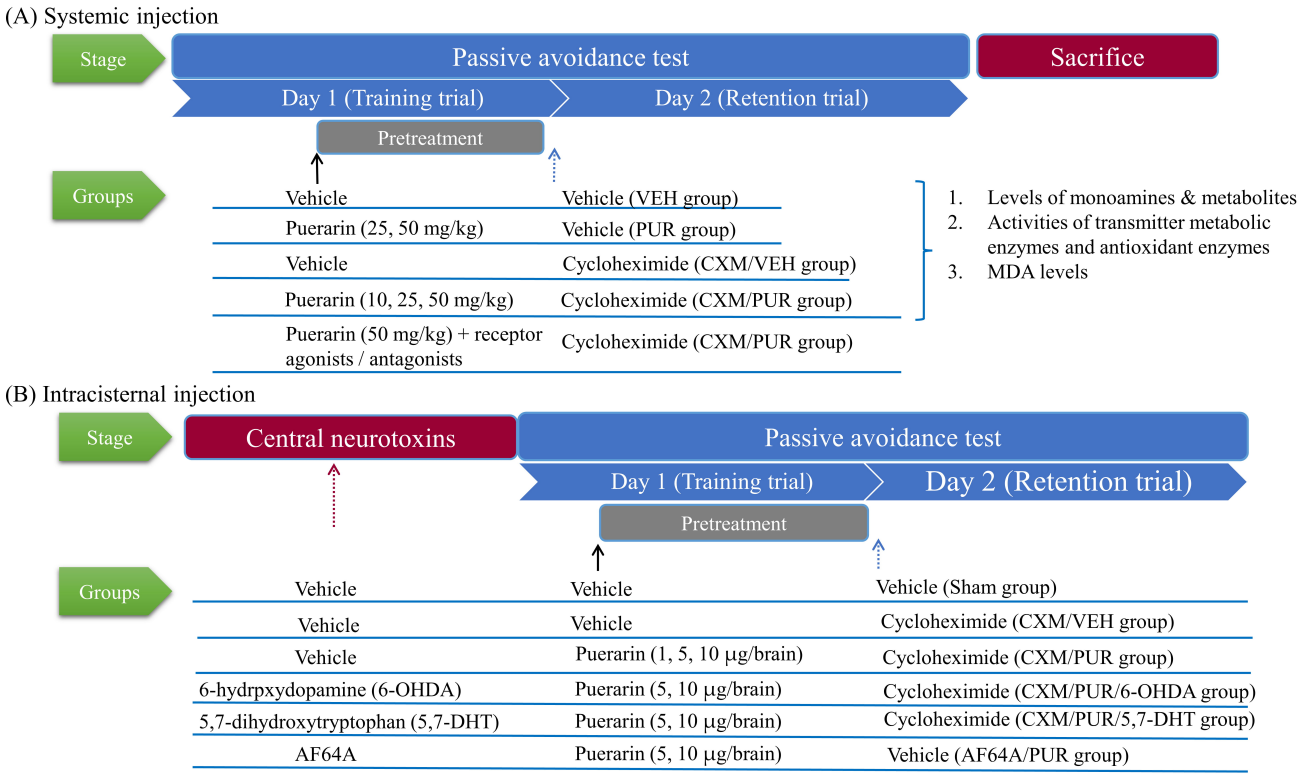

Fig. 1. Schematic diagram of experimental timeline and experimental designs. VEH,Vehicle; CXM, cycloheximide; PUR, Puerarin; MDA, malondialdehyde; 6-OHDA, 6-hydroxydopamine; 5,7-DHT, 5,7-dihydroxytryptamine.

2.5 Intraperitoneal Injection of Puerarin on CXM-Induced Impairment of Memory Consolidation

The first series of experiments was designed to document the ameliorating effects of systemic treatment of puerarin on the impairment of memory consolidation that is produced by CXM treatment. CXM (1.5 mg/kg, s.c.) was administered immediately after the training trial of the passive avoidance task in order to impair memory consolidation [28]. Puerarin (10, 25, or 50 mg/kg, i.p.) was administered to CXM-treated rats 30 min before the training trial, in accordance with our previous report [21] and as per the pharmacokinetic report of Kong *et al.* [23] showing that T_{max} of puerarin in brain, after i.p. administration, is about 30 min. Thereafter, we explored the memory-improving mechanism of puerarin (50 mg/kg, i.p.) on CXM-injected rats by combination it with cholinergic receptor antagonists (SCOP, MECA), serotonergic receptor agonists (DPAT, DOI), or adrenergic receptor antagonists (PHE, PROP). SCOP (0.3 mg/kg), MECA (3 mg/kg), DPAT (0.025 mg/kg), DOI (0.02 mg/kg), PHE (0.01 mg/kg) or PROP (3 mg/kg) were administered by i.p. injection simultaneously with CXM s.c. injection [28].

2.6 Effects of i.c.v. Injection of Puerarin on CXM-Induced or AF64A-Induced Memory Impairment

We investigated whether central treatment with puerarin improved memory impairment in the second series of experiments. For central injection of puerarin, rats were anesthetized with sodium pentobarbital (45 mg/kg, i.p.) and mounted in a stereotaxic frame (Stoelting, Wood Dale, IL, USA). A hole was drilled in a rat's skull (anteroposterior (AP): –0.8 mm, mediolateral (ML): –1.5 mm from bregma) and a cannula (12 mm, 23 ga) was inserted to a depth of 3.6 mm below dura (Paxinos and Watson [29]). Postoperative care was provided for the first 2 h after surgery, including a single sterile saline injection, a single cefamandole injection and a heating pad under the cage. After 14 days of recovery, vehicle or puerarin (20 μL, 1, 5, or 10 μg/brain) was injected into the lateral ventricle with a 30-gauge injection needle attached to a 25-μL Hamilton syringe (Model 702RN, Reno, NV, USA), with a microinfusion pump (KDS310 syringe pump, KD Scientific Inc., Holliston, MA, USA), at a rate of 1 μL/min. After injection, the needle was left in place in the cannula for 2 min to allow puerarin to diffuse into the surrounding tissues of the injection area.

These cannula-implanted rats were divided into nine groups: vehicle (Sham), CXM injection (CXM/VEH), pretreatment with puerarin in CXM injection (CXM/PUR), pretreatment with 6-OHDA in CXM injection (6-OHDA), pretreatment with puerarin and 6-OHDA in CXM injection (CXM/PUR/6-OHDA), pretreatment with 5,7-DHT in CXM injection (5,7-DHT), pretreatment with puerarin and 5,7-DHT in CXM injection (CXM/PUR/5,7-DHT), AF64A

entral injection (AF64A), and pretreatment with puerarin in AF64A central injection (AF64A/PUR). The AF64A (3 nmol/brain) was administrated 14 days before the passive avoidance test [27]. The 5,7-DHT (10 µg/2 µL) was delivered to the dorsal raphe nuceli, bilaterally (AP: –7.8 mm, ML: ±0.3 mm, dorsal-ventral (DV): –6.4 mm from bregma) 14 days before the passive avoidance test [25]. The 6-OHDA (4 µg/2 µL) was delivered to the locus coeruleus, bilaterally (AP: –9.8 mm, ML: ±1.3 mm, DV: –7.2 mm from bregma) 14 days before the passive avoidance test [26]. Rats received the i.c.v. injection of vehicle or puerarin (1, 5, 10 µg/brain) 15 min before the training trial [23].

2.7 Preparation of Prefrontal Cortical and Hippocampal Tissues

The day after the retention trial, each rat was euthanized and the brain was separated into the prefrontal cortex and hippocampus on ice, 30 min after injection with vehicle or puerarin (25, 50 mg/kg, i.p.). These tissues were homogenized with ice-cold phosphate-buffered saline. Homogenates were centrifuged at 12,000 rpm for 15 min at 4 °C and the supernatants were collected. The supernatants were stored at –80 °C for subsequent neurotransmitter function and oxidative stress analysis.

2.8 Assessment of Neurotransmitter Function

Neurotransmitter function in the prefrontal cortex and hippocampus was assessed by measuring monoamines (NE, DA, 5-HT) and their metabolite (MHPG, DOPAC, HVA, 5-HIAA) concentrations, and AChE and monoamine oxidases (MAO-A and MAO-B) activity. Biogenic amines and their metabolite concentrations in the prefrontal cortex and hippocampus were measured using HPLC/ECD. Briefly, the above supernatants were again centrifuged with 0.22 µM Ultrafree MC centrifugal filter units (Millipore, Merck KGaA, Darmstadt, Germany) at 14,000 rpm for 10 min at 4 °C. The collected samples were assayed with an HPLC Model PM80 (Bioanalytic system Inc., West Lafayette, IN, USA), a Data Model M746 (Waters Co., Taipei, Taiwan), a Model LC-4C electrochemical detector (Bioanalytic system Inc.) and a Bioanalytic system MF-6026 column. AChE, MAO-A and MAO-B activity in the prefrontal cortex and hippocampus were measured using our previous method [30]. The supernatant or the AChE standard solution was reacted with DTNB for 10 min at 25 °C, and then ACtCh was added as a substrate for color development. The production of 5-thio-2-nitrobenzoic acid was measured at 412 nm with a microplate reader (BioTek Inc., Winooski, VT, USA). AChE activity was expressed as U AChE /mg protein. MAO-A and MAO-B activity was determined by the color produced by the reaction products of rat brain homogenate, horseradish peroxidase, and amplex red, and the substrate (5 mM serotonin for MAO-A or 5 mM benzylamine for MAO-B) for 60 min at 25 °C. The enzyme activity was expressed as a percentage of Sham-group values. The protein content was measured with a Bio-Rad protein assay kit (Bio-Rad Laboratories Inc., Taipei, Taiwan).

2.9 Assessment of Oxidative Stress

Antioxidant enzyme activity (including superoxidase dismutase [SOD], glutathione peroxidase [GPx], glutathione reductase [GR] and catalase) and glutathione (GSH) and malondialdehyde (MDA) levels in the prefrontal cortex and hippocampus were measured. Antioxidant enzyme activity was analyzed with a spectrophotometric microplate reader as specified in our previous report [31]. SOD activity was assayed by the trend of absorbance at 560 nm for the production of nitroblue tetrazolium. Catalase activity was assayed by the decrease in the absorbance at 560 nm for amplex red. GPx and GR activity was measured using a Cayman assay kit (Cayman Chemical Co., Ann Arbor, MI, USA). GPx, SOD and catalase activity was expressed as U/mg of protein. GR activity was expressed as mU/mg of protein. GSH and MDA levels were also determined as described previously [30]. Briefly, the supernatant or GSH standard solution was reacted with the reaction solution including DTNB, NADPH (nicotinamide adenine dinucleotide phosphate), and GR. Then the absorbance was recorded at 405 nm for 5 min with a microplate reader. GSH levels were expressed as nmol/g of protein. The TBARS (thiobarbituric acid reactive substances) assay was used to measure MDA levels in the prefrontal cortex and hippocampus. Briefly, a thiobarbituric acid (TBA) test was performed with the supernatant or MDA standard solution and the absorbance was determined at 532 nm. MDA levels were expressed as nmol/mg of protein.

2.10 Statistical Analysis

All results were expressed as the mean ± standard errors (SEM). The data of the passive avoidance response were analyzed with non-parametric Kruskal Wallis analysis followed by two-tailed Mann Whitney U-tests because the data distribution was truncated at 300 s. The data included monoamines and their metabolite concentrations, AChE, MAO-A and MAO-B activities, antioxidant enzyme activity, and GSH and MDA levels, which were analyzed using 1-way analysis of variance (ANOVA), followed by Dunnett's test. When $p \leq 0.05$, the difference was considered significant.

3. Results

3.1 Effects of Systemic Administration of Puerarin

Our previous report pointed out that systemic administration of puerarin improved learning disabilities induced by MECA, PCA, and MK-801 in a passive avoidance task [21], therefore this study was conducted to evaluate the improving effect of systemic administration of puerarin on CXM-induced impairment of memory consolidation on a passive avoidance task. CXM, a protein inhibitor, impaired

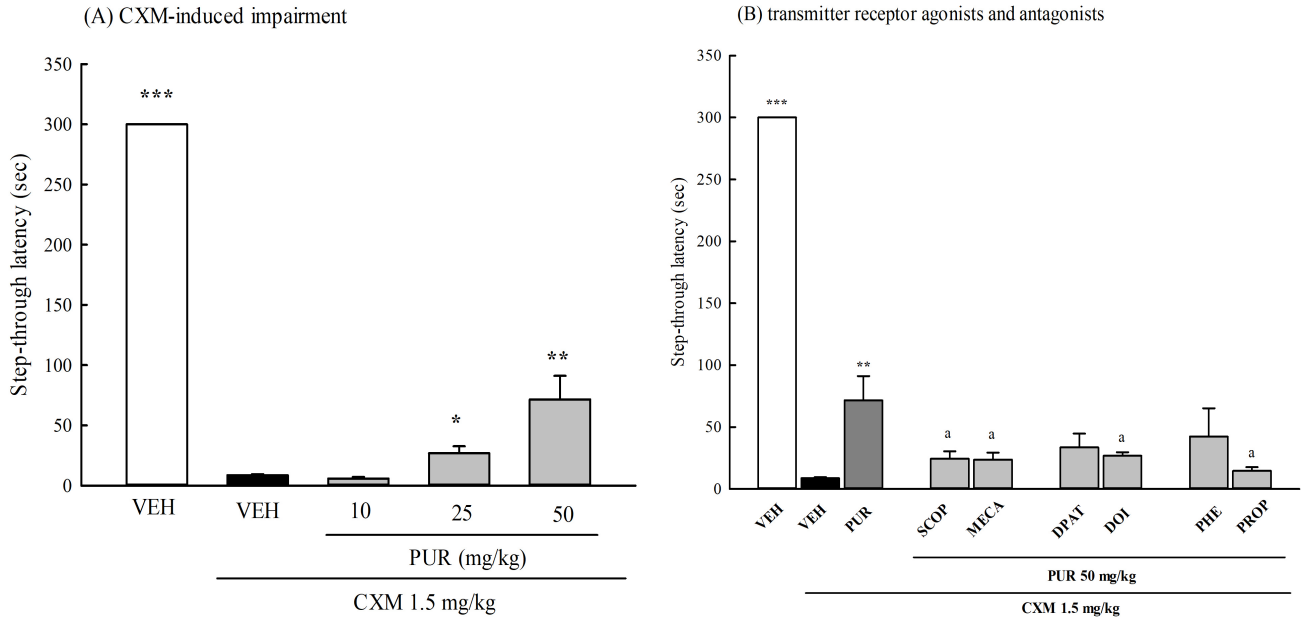

Fig. 2. Effects of puerarin (PUR, 10, 25, 50 mg/kg, i.p.) on cycloheximide (CXM, 1.5 mg/kg, s.c.)-induced passive avoidance response impairment in rats. (A) Absence of transmitter receptor agonists and antagonists. (B) Presence of transmitter receptor agonists and antagonists. Data are expressed as mean ± SEM (n = 8). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with CXM/VEH group. a $p < 0.05$ compared with CXM/PUR group. SCOP, scopolamine; MECA, mecamylamine; DPAT, 8-hydroxy-2-(di-n-propylamino) tetralin hydrobromide; DOI, 1-(2,5-dimethoxy-4-iodophenyl)-2 aminopropane; PHE, phenoxybenzamine hydrochloride; PROP, propranolol hydrochloride; SEM, standard error of the mean.

memory consolidation in rodents when subcutaneously injected immediately after behavioral performance [7]. Our present study confirmed that CXM administration immediately after the training trial impaired memory consolidation in rats, and showed that systemic administration of puerarin (25 or 50 mg/kg, i.p.) before the training trial significantly attenuated the impairment of memory consolidation caused by CXM (Fig. 2A).

Furthermore, the underlying neurochemical mechanisms of the improving effect of systemic administration with puerarin against CXM-induced memory consolidation impairment were clarified by combining the puerarin treatment with cholinergic receptor antagonists such as SCOP and MECA, serotonergic receptor agonists such as DPAT and DOI, and adrenergic receptor blockers such as PHEN and PROP, which, by themselves did not cause memory impairment [8,28]. The present data showed that SCOP, MECA, DOI, and PROP blocked the improving effects of puerarin (50 mg/kg) on the impairment of memory consolidation produced by CXM treatment in rats (Fig. 2B).

3.2 Effects of Intracisternal Administration of Puerarin

Several researchers have shown that puerarin could cross the BBB and become distributed in various areas of the brain [20,23]. We further evaluated the effects of i.c.v. injection of puerarin on the CXM-induced impairment of memory consolidation in rats. Administration of puerarin (5 or 10 µg/brain; i.c.v.) before the training trial significantly attenuated the CXM-induced impairment of memory consolidation (Fig. 3A). Furthermore, we investigated the role of monoaminergic systems in the ameliorating effects of puerarin by destroying central monoaminergic systems with central parenchymal injection of monoaminergic neurotoxins 5,-7-DHT or 6-OHDA. The results (Fig. 3A) showed that bilateral injection with 6-OHDA into the locus coeruleus did not affect CXM-induced impairment of a passive avoidance response, but blocked the puerarin from ameliorating the deficit caused by CXM treatment. However, bilaterally injected 5,7-DHT into the dorsal raphe nuclei did not affect CXM-induced impairment of passive avoidance response or the ameliorating effect of puerarin on the CXM-induced impairment (Fig. 3A).

The basal forebrain cholinergic system (especially the prefrontal cortex and hippocampus) plays an important role in memory. Therefore, we further evaluated the ameliorating effect of puerarin on AF64A-induced memory impairment in rats. Administration of puerarin (10 µg/brain, i.c.v.) before the training trial significantly attenuated AF64A-induced memory impairment (Fig. 3B).

3.3 Effects of Systemic Administration of Puerarin on Neurotransmitter Functions

The results indicated that the ameliorating effect of puerarin on CXM-induced impairment of memory consolidation are related to the catecholaminergic system of the brain, we further measured the monoamine levels (includ-

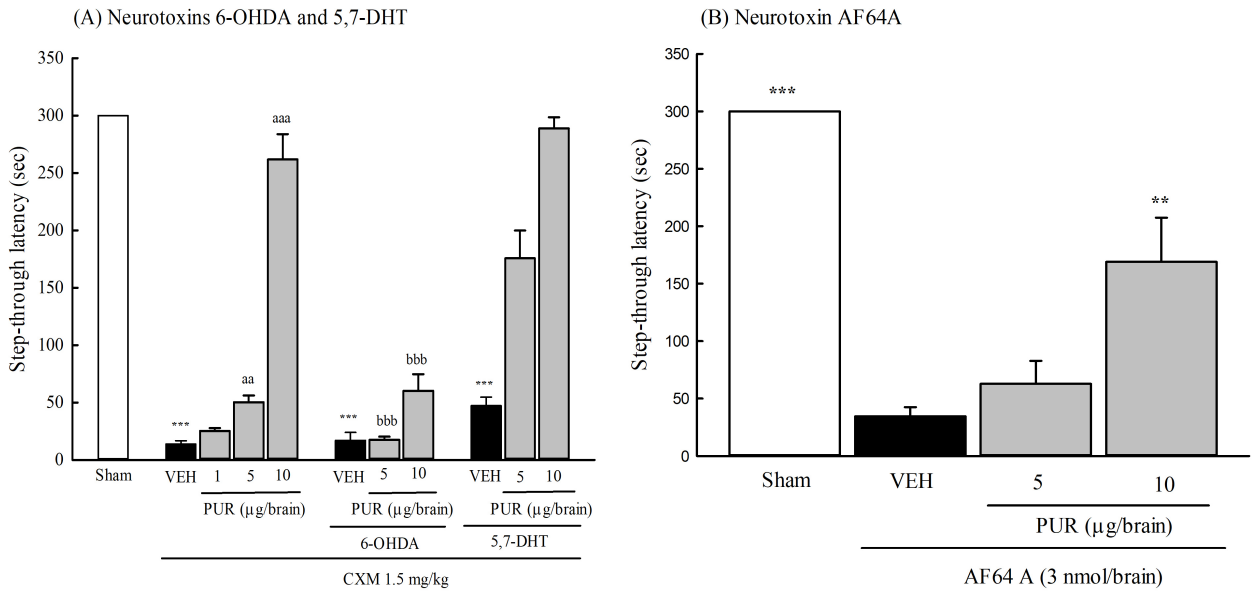

Fig. 3. Effects of puerarin (PUR, 5, 10 μg/brain, i.c.v.) on cycloheximide (CXM, 1.5 mg/kg, s.c.)-induced or AF64A-induced (B) passive avoidance response impairment in rats. (A) Absence or presence of 6-hydroxydopamine (6-OHDA) or 5,7-dihydroxytryptamine (5,7-DHT). (B) AF64A. Data are expressed as mean ± SEM (n = 8). ** $p < 0.01$, *** $p < 0.001$ compared with Sham group (A) or AF64A/VEH group (B). aa $p < 0.01$, aaa $p < 0.001$ compared with CXM/VEH group. bbb $p < 0.001$ compared with CXM/PUR group. SEM, standard error of the mean.

Table 1. Effects of puerarin (25, 50 mg/kg, i.p.) on monoamines and metabolites levels in prefrontal cortex and hippocampus of vehicle (VEH)- and cycloheximide (CXM)-treated rats.

	MHPG	NE	DOPAC	HVA	DA	5-HIAA	5-HT
Monoamines and metabolites in prefrontal cortex (ng/mg)							
VEH	7503.6 ± 443.9	844.7 ± 46.1	548.6 ± 36.6	236.6 ± 20.2	1308.4 ± 99.4	615.9 ± 25.1	603.5 ± 33.8
Puerarin 25 mg/kg	7159.5 ± 238.3	830.5 ± 76.0	465.7 ± 27.6*	250.5 ± 17.6	1155.3 ± 62.8	598.5 ± 23.4	488.4 ± 46.2
Puerarin 50 mg/kg	7464.9 ± 609.8	918.0 ± 74.5	390.9 ± 38.3*	219.9 ± 11.0	1265.9 ± 68.0	613.4 ± 23.3	624.5 ± 39.3
CXM 1.5 mg/kg	7261.2 ± 282.1	650.5 ± 44.4**	573.5 ± 48.2	348.7 ± 23.3**	1094.7 ± 48.8*	750.6 ± 54.1	598.48 ± 34.9
CXM + puerarin 25 mg/kg	6974.5 ± 298.2	658.1 ± 30.9	422.7 ± 44.9^{a}	243.1 ± 17.6^{aa}	1079.3 ± 35.5	655.7 ± 44.5	577.6 ± 23.1
CXM + puerarin 50 mg/kg	6395.2 ± 404.0	804.5 ± 45.4^{a}	403.8 ± 27.2^{a}	239.6 ± 16.0^{aa}	1200.2 ± 72.2	645.8 ± 46.2	646.8 ± 44.8
Monoamines and metabolites in hippocampus (ng/mg)							
VEH	996.1 ± 65.9	354.7 ± 17.5	61.7 ± 6.7	111.2 ± 9.8	30.7 ± 6.0	294.0 ± 26.2	189.5 ± 16.3
Puerarin 25 mg/kg	1104.9 ± 89.2	333.3 ± 14.3	57.3 ± 5.8	89.3 ± 2.5*	30.5 ± 2.1	296.6 ± 18.9	180.9 ± 13.7
Puerarin 50 mg/kg	1005.6 ± 26.1	426.4 ± 34.4	50.4 ± 5.1	101.4 ± 7.8	57.2 ± 12.3*	356.4 ± 24.5	237.2 ± 17.5
CXM 1.5 mg/kg	674.9 ± 36.8**	158.1 ± 14.2***	32.0 ± 4.5**	62.1 ± 3.4***	15.9 ± 1.1***	200.8 ± 11.0**	134.2 ± 15.2*
CXM + puerarin 25 mg/kg	723.6 ± 41.4	211.7 ± 20.4	33.5 ± 2.9	70.0 ± 4.0	17.9 ± 1.0	233.4 ± 14.2	125.7 ± 11.0
CXM + puerarin 50 mg/kg	874.3 ± 35.0^{aa}	334.6 ± 27.8^{aaa}	33.9 ± 3.5	79.9 ± 6.1	30.7 ± 2.3^{aaa}	260.9 ± 11.1^{aa}	198.3 ± 17.2^{a}

Data were expressed as mean ± SEM for eight rats. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with VEH group. a $p < 0.05$, aa $p < 0.01$, aaa $p < 0.001$ compared with CXM group. MHPG, 3-methoxy-4-hydroxyphenyl glycol; NE, norepinephrine; DOPAC, 3,4-dihydroxyphenyl acetic acid; HVA, homovanillic acid; DA, dopamine; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine.

ing their metabolites) and transmitter enzyme activity (including AChE, MAO-A and MAO-B) in the prefrontal cortex and hippocampus to clarify the role of neurotransmitter function in the ameliorating effects of puerarin. CXM treatment decreased DA and NE levels in the prefrontal cortex, and also decreased monoamine levels (including DA, NE and 5-HT) and their metabolite levels (including MHPG, DOPAC, HVA and 5-HIAA) in the hippocampus (Table 1). Monoamine turnover rates, which were calculated from the above data (monoamine levels *vs* their metabolite levels), showed that CXM treatment produced increased both NE and DA turnover rates in the prefrontal cortex; but only increased the NE turnover rate in the hippocampus (Fig. 4). Puerarin treatment restored the decreased NE levels in the prefrontal cortex and the decreased monoamine levels in the hippocampus that had been produced by CXM treatment,

but only at the 50 mg/kg puerarin dose (Table 1). Puerarin administration at 25 and 50 mg/kg decreased DOPAC and HVA levels in the prefrontal cortex that had been increased by CXM, but only at the 50-mg/kg dose did puerarin increase the lowered levels of MHPG and 5-HIAA in the hippocampus (Table 1). Puerarin increased the DA levels in the hippocampus of vehicle-treated rats only at the 50-mg/kg dose (Table 1). Puerarin decreased the higher NE and DA turnover rates in the prefrontal cortex and hippocampus only at 50 mg/kg (Fig. 4). When puerarin was administered to vehicle-treated rats, it decreased the DA turnover rates in the prefrontal cortex and hippocampus, but only at the 50 mg/kg dose (Fig. 4).

CXM increased MAO-A activity in the prefrontal cortex and AChE, MAO-A and MAO-B activity in the hippocampus (Fig. 5). At 25 and 50 mg/kg, puerarin inhibited MAO-A and MAO-B activity in the prefrontal cortex of CXM-treated rats, but it inhibited the CXM-elevated levels of AChE and MAO-B activity in the hippocampus only at 50 mg/kg (Fig. 5). When puerarin was administered to vehicle-treated rats, it decreased AChE and MAO-B activity in the prefrontal cortex and hippocampus only at 50 mg/kg. However, puerarin decreased only hippocampal MAO-A activity in vehicle-treated rats at 50 mg/kg (Fig. 5).

3.4 Effects of Systemic Administration with Puerarin on Oxidative Stress Parameters

Due to fact that puerarin has radical scavenging and antioxidant properties [32,33], we also measured the antioxidant enzyme activity and GSH levels in the prefrontal cortex and hippocampus to clarify the role of oxidative stress on the attenuating effects of puerarin on CXM-induced impairment of memory consolidation. CXM treatment decreased GSH-recycle system activity including GSH, GPx, and GR in the prefrontal cortex and hippocampus (Fig. 6). We further found that CXM decreased SOD and catalase activity, and increased MDA levels, in the prefrontal cortex and hippocampus (Fig. 7). Puerarin, at 50 mg/kg, restored GSH-recycle system activity and antioxidant enzyme activity in the prefrontal cortex and hippocampus that were decreased by CXM treaatment, and it also decreased the higher MDA levels that had been produced by CXM treatment (Figs. 6,7). In Sham rats, puerarin did not affect GSH-recycle system activity or antioxidant enzyme activity in the prefrontal cortex or hippocampus at any dose (Figs. 6,7).

4. Discussion

CXM, an antifungal antibiotic, interferes with the translocation step in protein synthesis of eukaryotic cells to block the synthesis of new proteins needed in memory formation, and thereby causes retrograde amnesia. Based on the neural basis of memory consolidation, the cholinergic, serotonergic, and catecholaminergic systems in the brain play an important role [34–36]. Nabeshima *et al.* [7,8] demonstrated that CXM treatment produced a memory-consolidation deficits partially by decreasing cholinergic and catecholaminergic activity and by increasing serotonergic activity in experimental animals. Our previous report indicated that puerarin can attenuate drug-induced learning acquisition by increasing cholinergic activity and by decreasing serotonergic activity [21]. Other researchers have also suggested that puerarin ameliorate the behavioral deficits in chronic stress and ischemia/reperfusion by normalizing serotonergic activity and activating nicotinic acetylcholinergic activity [22,37]. This present study found that puerarin, after systemic administration, attenuated CXM-induced impairment of memory consolidation, and that cholinergic antagonists, 5-HT_2 receptor agonist, and a β-adrenergic blocker prevented the amelioration. Furthermore, the integrity of inputs from basal forebrain acetylcholinergic, mesocorticolimbic dopaminergic and raphe serotonergic neurons to the hippocampus are critical for memory function [34–36]. The basal forebrain cholinergic system mainly projects to the prefrontal cortex and hippocampus, and plays an important role in memory consolidation. AF64A, a central cholinergic neurotoxin, induces deficits of cognitive performance such as in the passive avoidance task [38,39]. The present study showed that puerarin, after i.c.v. administration, attenuated AF64A-induced memory impairment. Other reports indicated that puerarin ameliorated cognitive dysfunction in cerebral artery occlusion (vascular dementia) in rats, STZ-induced sporadic AD mice, Aβ-induced familial AD mice, and APP/PS1 transgenic mice (familial AD) [16,17,20,40]. Hence, we suggested that puerarin has potential as a therapeutic compound for the anterograde and retrograde amnesia of various dementias. We further found that puerarin, after intracerebroventricular administration, attenuated CXM-induced impairment of memory consolidation, and bilateral injection of catecholaminergic toxin 6-OHDA into the locus coeruleus, but not serotonergic toxin 5,7-DHT into the dorsal raphe nucleus, blocked the attenuating effect. Hence, we confirmed that the improving effects of puerarin after *systemic* administration, on CXM-induced impairment are consistent with those of puerarin after *central* administration, which is in line with the finding of some researchers that puerarin can cross the BBB and become distributed in various areas of the brain [20,23]. Therefore, we further suggested that puerarin, after systemic/intracisternal administration, attenuated chemically-induced impairment of learning acquisition and memory consolidation in rats, and the beneficial effect of puerarin may be mainly related to the modulation of the activities of central cholinergic and monoaminergic systems, which were dependent on the integrity of central catecholaminergic and acetylcholinergic functions. We further found that central administration of puerarin has a better effect than systemic administration. The effect of intracerebroventricular injection of 5 µg/brain is approximately equivalent to the effect of intraperitoneal injection of 50 mg/kg. We think

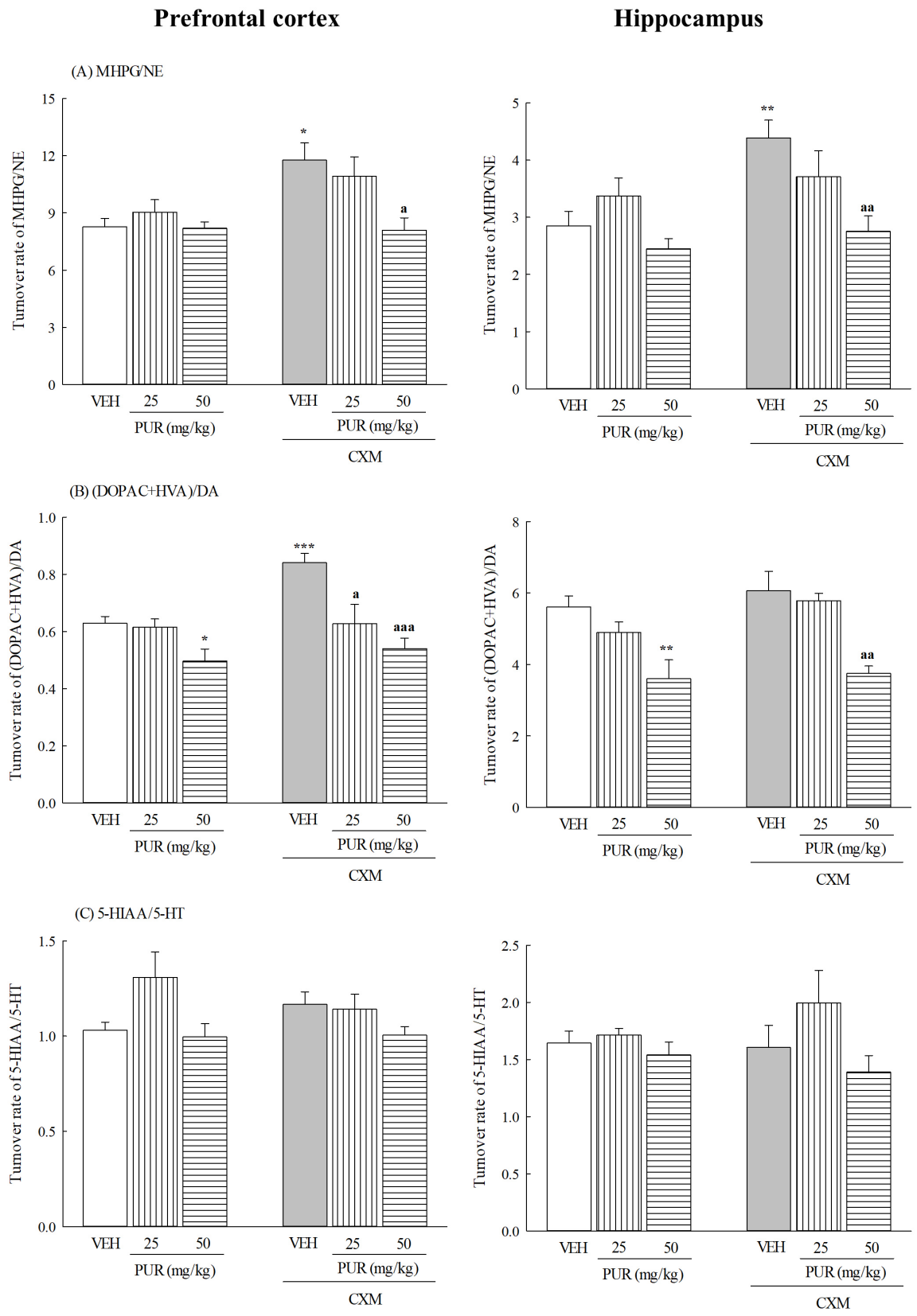

Fig. 4. Effects of puerarin (PUR, 25, 50 mg/kg, i.p.) on monoamine turnover rates in the prefrontal cortex and hippocampus of vehicle (VEH)- and cycloheximide (CXM, 1.5 mg/kg, s.c.)-treated rats. (A) MHPG/NE. (B) (DOPAC+HVA)/DA. (C) 5-HIAA/5-HT. Data are expressed as mean ± SEM (n = 8). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with VEH group. a $p < 0.05$, aa $p < 0.01$, aaa $p < 0.001$ compared with CXM/VEH group.

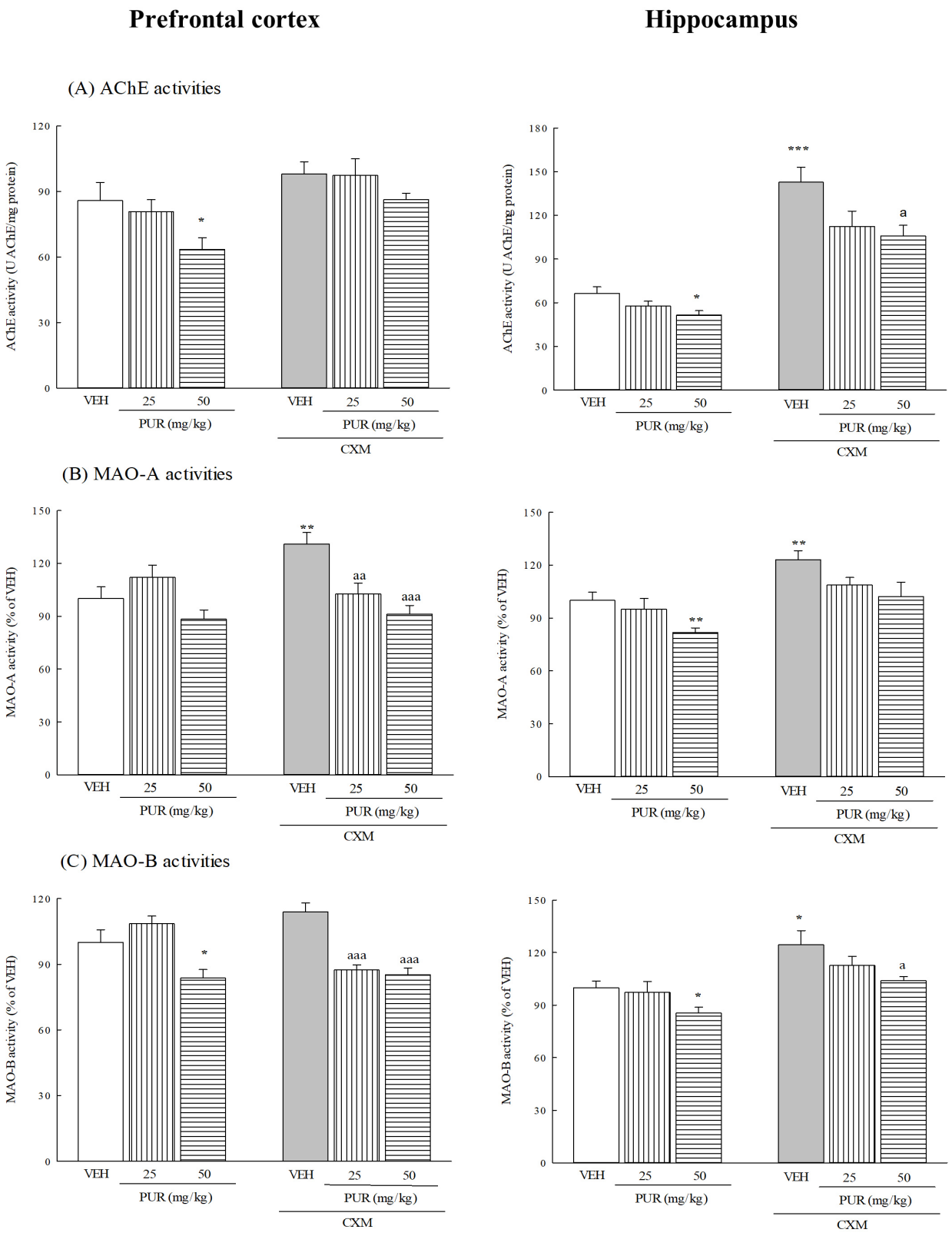

Fig. 5. Effects of puerarin (PUR, 25, 50 mg/kg, i.p.) on the activities of neurotransmitters degrading enzymes in the prefrontal cortex and hippocampus of vehicle (VEH)- and cycloheximide (CXM, 1.5 mg/kg, s.c.)-treated rats. (A) AChE. (B) MAO-A. (C) MAO-B. Data are expressed as mean ± SEM (n = 8). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with VEH group. a $p < 0.05$, aa $p < 0.01$, aaa $p < 0.001$ compared with CXM/VEH group. AChE, acetylcholinesterase; MAO, monoamine oxidase.

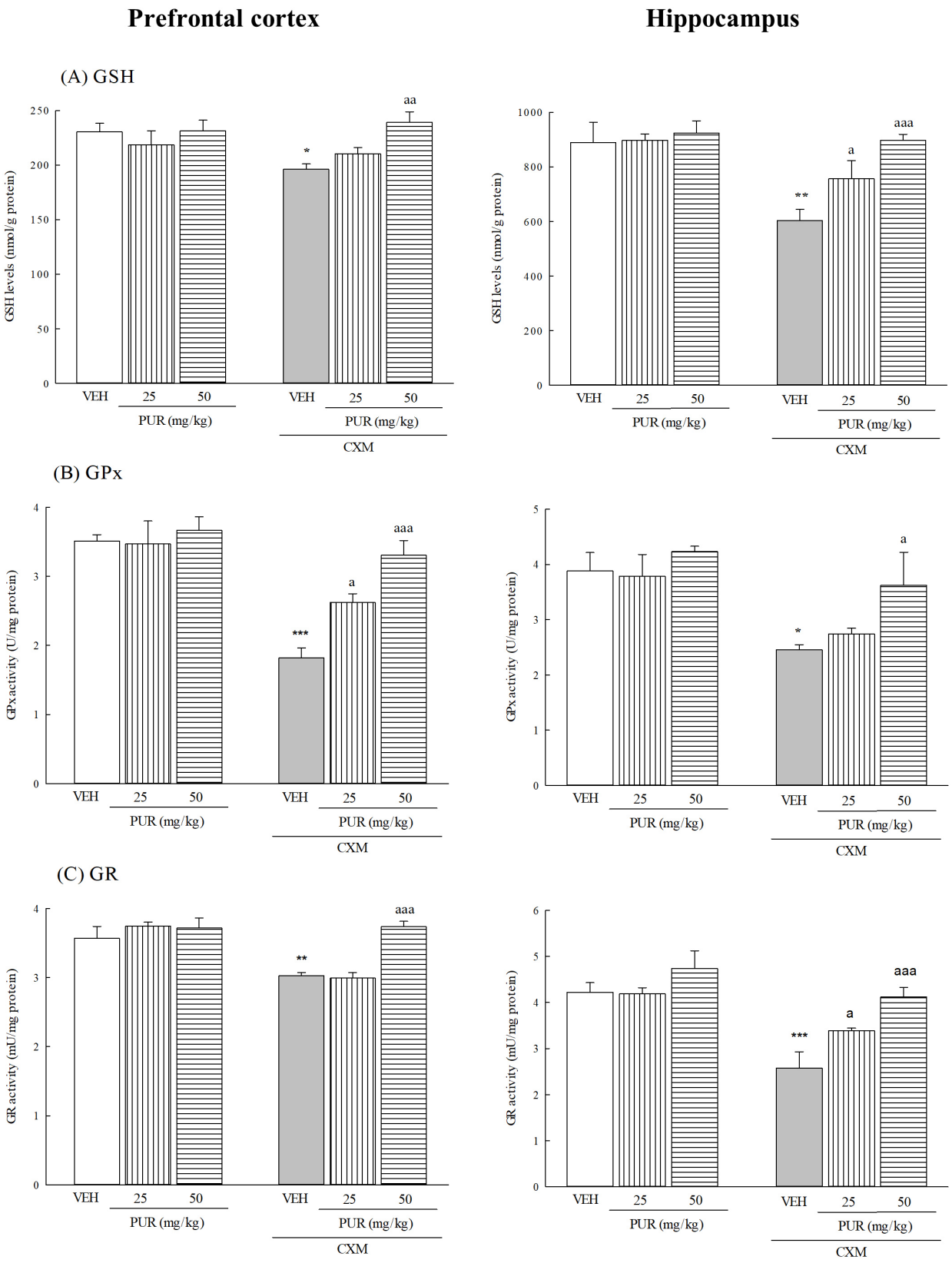

Fig. 6. Effects of puerarin (PUR, 25, 50 mg/kg, i.p.) on glutathione (GSH) recycle system activities in the prefrontal cortex and hippocampus of vehicle (VEH)- and cycloheximide (CXM, 1.5 mg/kg, s.c.)-treated rats. (A) GSH levels. (B) glutathione peroxidase (GPx) activities. (C) glutathione reductase (GR) activities. Data are expressed as mean ± SEM (n = 8). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with VEH group. a $p < 0.05$, aa $p < 0.01$, aaa $p < 0.001$ compared with CXM/VEH group.

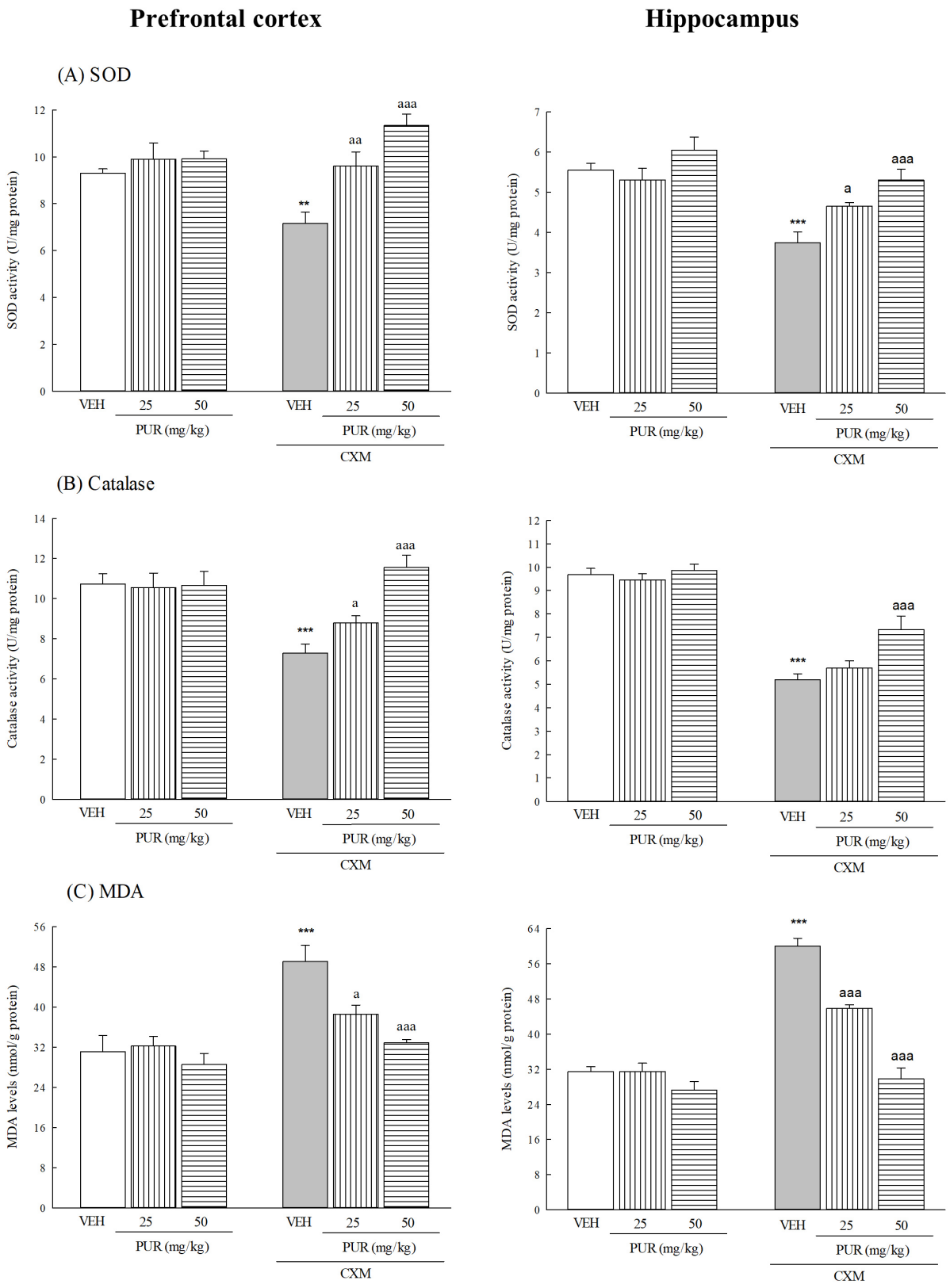

Fig. 7. Effects of puerarin (PUR, 25, 50 mg/kg, i.p.) on antioxidant enzyme activities and malondialdehyde (MDA) levels in the prefrontal cortex and hippocampus of vehicle (VEH)- and cycloheximide (CXM, 1.5 mg/kg, s.c.)-treated rats. (A) Superoxidase dismutase (SOD) activities. (B) Catalase activities. (C) MDA levels. Data are expressed as mean ± SEM (n = 8). ** $p < 0.01$, *** $p < 0.001$compared with VEH group. a $p < 0.05$, aa $p < 0.01$, aaa $p < 0.001$ compared with CXM/VEH group.

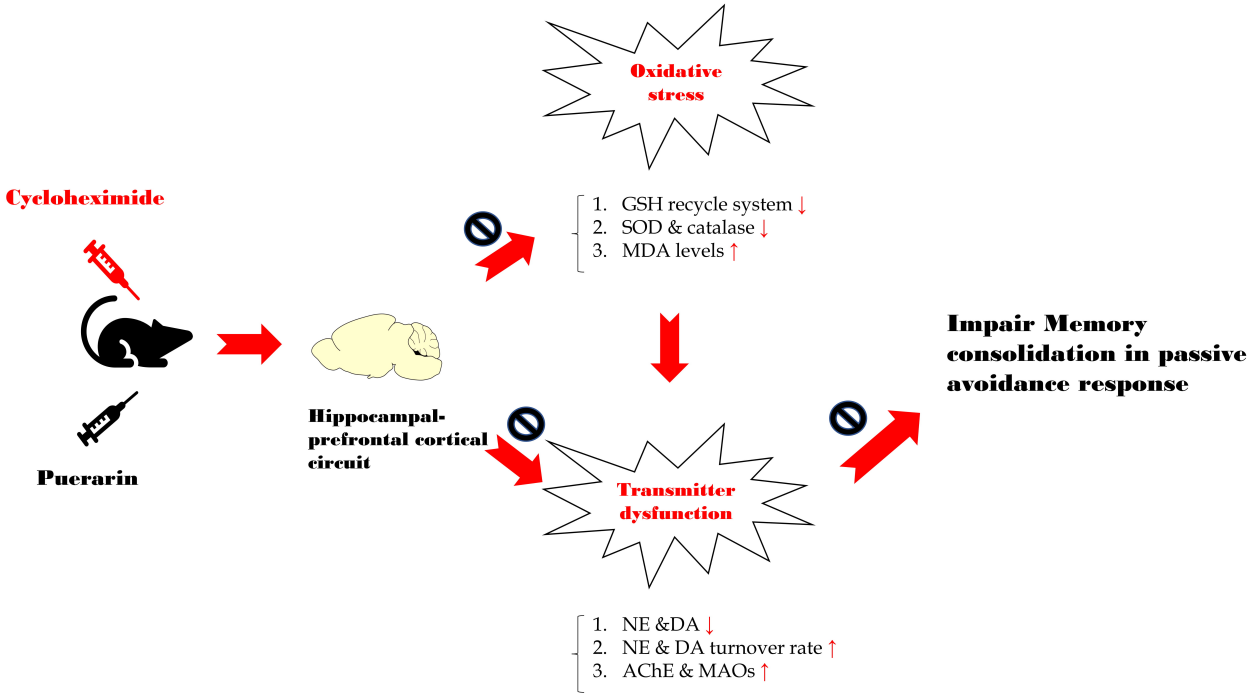

Fig. 8. Schematic representation of the protective mechanism of puerarin in cycloheximide-induced memory consolidation in passive avoidance test. GSH, glutathione; SOD, superoxidase dismutase; MDA, malondialdehyde.

there are two possible reasons: (1) as Kong *et al.* [23] found, ppuerarin after intraperitoneal injection was more distributed in the cortical area, but after cerebroventricular injection puerarin was distributed in areas adjacent to the injection site, such as the hippocampus; (2) puerarin is a glycoside that is easily hydrolyzed into the more active aglycone daidzein in the brain. According to previous reports [41,42], daidzein administered at 1.5–4.5 mg/kg produces memory-improving effects.

Hippocampal-prefrontal cortex circuity plays an important role in cognitive function and memory consolidation [24]. This present investigation showed that CXM treatment mainly decreased all monoamines and their metabolite levels in the hippocampus, but only decreased NE and DA levels in the prefrontal cortex. CXM treatment mainly increased AChE, MAO-A and MAO-B activity in hippocampus, but only increased MAO-A activity in prefrontal cortex. Early reports indicated that CXM treatment inhibited monoamine synthesis and decreased choline acetyltransferase (ChAT) in mouse whole brains [43,44]. The MAO inhibitors pargyline and pheniprazine produced recovery from CXM-induced impairment of memory consolidation [45]. We therefore suggest that CXM treatment impairs memory consolidation partially by decreasing the activity of cholinergic and monoaminergic systems by disrupting the synthesis and metabolism of acetylcholine and monoamines (especially NE and DA) in the hippocampal-prefrontal cortex circuity, especially in the hippocampus. However, some researchers have pointed out that protein-synthesis inhibitors impair long-term potentiation through the nitric oxide-dependent signaling pathway to inhibit neurotransmitter release and cause impairment of memory consolidation [46,47]. The inhibition of neurotransmitter release by protein-synthesis inhibitors may be related to S-nitrosylation enhancement and abnormal farnesylation, which were blocked by GSH [46]. On the other hand, CXM is considered to be a neurotoxin because an early report indicated that CXM (at >1 μM concentration) caused significant oxidative stress and neuronal death in rat primary hippocampal cells [48]. The present results showed that CXM treatment, at the used dosage (1.5 mg/kg, s.c.), caused oxidative damage (lipid peroxidation) and decreased antioxidative-defense-system activities in the prefrontal cortex and hippocampus, thereby causing neuronal damage in the hippocampus and prefrontal cortex via oxidative stress, yielding a decrease in cholinergic and monoaminergic function and consequent impairment of memory consolidation. Some reports indicated that puerarin restored ChAT activity in ovariectomized guinea pigs, inhibited AChE activities in STZ-induced diabetic rats and Aβ-induced AD rats, and normalized serotonergic activity in chronic stress mice [18,20,49,50]. The present results showed that puerarin (50 mg/kg, i.p.) restored hippocampal monoamines levels that had decreased due to CXM treatment, and decreased elevated hippocampal AChE, MAO-A and MAO-B activity caused by CXM. Therefore, the present results further confirmed that puerarin normalized the monoaminergic (such as increased NE and DA levels and MAO-B activity) and acetylcholinergic systems (such as the decreased AChE activity, especial in the hippocam-

pus), to ameliorate CXM-induced impairment of memory consolidation. Although Liu *et al*. [22] indicated that puerarin prevented lead-induced neurotoxicity partially via reversing altered neurotransmitter-enzyme activity (such as increasing AChE and MAO activity), we posit that this difference was due to the different pathologic mechanisms between the two neurotoxic models. We also found that puerarin alone (50 mg/kg, i.p.) increased DA levels in the hippocampus of the Sham rats, and that it inhibited AChE and MAO-B activity in the hippocampus and prefrontal cortex of the Sham rats. So, puerarin (50 mg/kg, i.p.) decreased the DA turnover rate in the prefrontal cortex and hippocampus of Sham rats. Hence, we further confirmed that puerarin (50 mg/kg, i.p.) elevated central cholinergic and dopaminergic activities via the inhibition of AChE and MAO-B activity in the brain, especially the hippocampus. In addition, puerarin belongs to a common isoflavonoid and is a known antioxidant [51]. Many reports have indicated that puerarin can protect against oxidative stress or neurotoxins such as H_2O_2, MPP^+ and Aβ via its antioxidant and free radical scavenging activities [52–55]. The present results showed that puerarin (50 mg/kg, i.p.) restored GSH-recycle-system activity, SOD activity and catalase activity, and decreased elevated MDA levels in the hippocampus and prefrontal cortex that were caused by CXM treatment. These results also confirmed that puerarin exerts antioxidant properties and has protective effects against CXM-induced central neurotransmitter dysfunction and against impairment of memory consolidation. We suggest that the attenuating effects of puerarin on CXM-induced impairment of memory consolidation may be related to decreased oxidative damage and normalized central neural function (especially the cholinergic and dopaminergic systems) via its radical scavenging potency and restoriation of the activities of antioxidant defense systems in the hippocampal-prefrontal cortex circuit.

5. Conclusions

The findings from this study confirm that treatment with the antifungal antibiotic CXM impairs memory consolidation and causes retrograde amnesia, as indicated by a passive avoidance test. The impairment of memory consolidation caused by CXM treatment may be related to the inhibition of protein synthesis and the decrease in antioxidant-defense-system activation, and it may cause neurotransmitter dysfunction. Puerarin attenuates the toxicity of the antifungal antibiotic CXM, protecting the process for memory consolidation. Puerarin restored CXM-induced impairment of memory consolidation and the consequent retrograde amnesia. The protective mechanism of puerarin against CXM-induced impairment of memory consolidation may be related to decreased oxidative damage and normalization of neurotransmitter function in the prefrontal cortex-hippocampal circuit (Fig. 8).

Abbreviations

5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine; 5,7-DHT, 5,7-dihydroxytryptamine; 6-OHDA, 6-hydroxydopamine; AChE, acetylcholinesterase; ActCH, acetylthiocholine iodide; AD, Alzheimer's diseae; ChAT, choline acetyltransferase; CXM, cycloheximide; DA, dopamine; DOI, 1-(2,5-dimethoxy-4-iodophenyl)-2 aminopropane; DOPAC, 3,4-dihydroxyphenyl acetic acid; DPAT, 8-hydroxy-2-(di-n-propylamino) tetralin hydrobromide; DTNB, 5,5′-dithiobis (2-nitrobenzoic acid); ECD, electrochemical detector; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; HPLC, high performance liquid chromatography; HVA, homovanillic acid; MAO, monoamine oxidase; MDA, malondialdehyde; MECA, mecamylamine; MHPG, 3-methoxy-4-hydroxyphenyl glycol; MK-801, dizocilpine; NE, norepinephrine; PCA, *p*-chloroamphetamine; PHEN, phenoxybenzamine; PROP, propranolol; PUR, puerarin; SCOP, scopolamine; SEM, standard errors; SOD, superoxidase dismutase; STL, step-through latency; TBA, thiobarbituric acid; TBARS, thiobarbituric acid reactive substances.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

CRW designed the research study. JCL and KJW performed the research. JCL and KJW analyzed the data. CRW wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

All rats handling, manipulation and treatment were conducted according to the Guiding Principles for the Care and Use of Laboratory Animals, and approved by The Institutional Animal Care and Use Committee of China Medical University (CMUIACUC-2018-243).

Acknowledgment

We would like to express our gratitude to all peer reviewers for their opinions and suggestions.

Funding

This research was funded by the Ministry of Science and Technology, grant number 103-2320-B-039-028, 104-2320-B-039-027-MY2, 104-2622-B-039-005-CC2 and 105-2622-B-039-003-CC2.

Conflict of Interest

The authors declare no conflict of interest. Chi-Rei Wu is serving as one of the Editorial Board members of this journal. We declare that Chi-Rei Wu had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Gernot Riedel.

References

[1] Roesler R. Molecular mechanisms controlling protein synthesis in memory reconsolidation. Neurobiology of Learning and Memory. 2017; 142: 30–40.

[2] Barraco RA, Stettner LJ. Antibiotics and memory. Psychological Bulletin. 1976; 83: 242–302.

[3] Davis HP, Squire LR. Protein synthesis and memory: a review. Psychological Bulletin. 1984; 96: 518–559.

[4] Jodar L, Kaneto H. Synaptic plasticity: stairway to memory. Japanese Journal of Pharmacology. 1995; 68: 359–387.

[5] Rosenberg T, Gal-Ben-Ari S, Dieterich DC, Kreutz MR, Ziv NE, Gundelfinger ED, *et al*. The roles of protein expression in synaptic plasticity and memory consolidation. Frontiers in Molecular Neuroscience. 2014; 7: 86.

[6] Randt CT, Barnett BM, McEwen BS, Quartermain D. Amnesic effects of cycloheximide on two strains of mice with different memory characteristics. Experimental Neurology. 1971; 30: 467–474.

[7] Nabeshima T, Noda Y, Itoh K, Kameyama T. Role of cholinergic and GABAergic neuronal systems in cycloheximide-induced amnesia in mice. Pharmacology, Biochemistry, and Behavior. 1988; 31: 405–409.

[8] Nabeshima T, Itoh K, Kawashima K, Kameyama T. Effects of 5-HT2 receptor antagonist on cycloheximide-induced amnesia in mice. Pharmacology, Biochemistry, and Behavior. 1989; 32: 787–790.

[9] Geller A, Robustelli F, Barondes SH, Cohen HD, Jarvik ME. Impaired performance by post-trial injections of cycloheximide in a passive avoidance task. Psychopharmacologia. 1969; 14: 371–376.

[10] Squire LR, Smith GA, Barondes SH. Cycloheximide affects memory within minutes after the onset of training. Nature. 1973; 242: 201–202.

[11] Quinton EE, Kramarcy NR. Memory impairment correlates closely with cycloheximide dose and degree of inhibition of protein synthesis. Brain Research. 1977; 131: 184–190.

[12] Yu CC, Du YJ, Li J, Li Y, Wang L, Kong LH, *et al*. Neuroprotective Mechanisms of Puerarin in Central Nervous System Diseases: Update. Aging and Disease. 2022; 13: 1092–1105.

[13] Wang H, Hsu T. Effects of Pueraria lobata and Cindium officinale on cognitive function in rodents. Journal of China-Japan Friendship Hospital. 1995; 4: 191–194.

[14] Yu CL, Chang KC, Chao HC. Effects of total flavonoids isolated from Pueraria lobata on memory behavior in mice. Journal of China Pharmaceutical University. 1997; 28: 350–353. (In Chinese)

[15] Xu XH, Zhao TQ. Effects of puerarin on D-galactose-induced memory deficits in mice. Acta Pharmacologica Sinica. 2002; 23: 587–590.

[16] Tao J, Cui Y, Duan Y, Zhang N, Wang C, Zhang F. Puerarin attenuates locomotor and cognitive deficits as well as hippocampal neuronal injury through the PI3K/Akt1/GSK-3β signaling pathway in an *in vivo* model of cerebral ischemia. Oncotarget. 2017; 8: 106283–106295.

[17] Zhao SS, Yang WN, Jin H, Ma KG, Feng GF. Puerarin attenuates learning and memory impairments and inhibits oxidative stress in STZ-induced SAD mice. Neurotoxicology. 2015; 51: 166–171.

[18] Liu X, Mo Y, Gong J, Li Z, Peng H, Chen J, *et al*. Puerarin ameliorates cognitive deficits in streptozotocin-induced diabetic rats. Metabolic Brain Disease. 2016; 31: 417–423.

[19] Wu L, Tong T, Wan S, Yan T, Ren F, Bi K, *et al*. Protective Effects of Puerarin against Aβ 1-42-Induced Learning and Memory Impairments in Mice. Planta Medica. 2017; 83: 224–231.

[20] Liu S, Cao XL, Liu GQ, Zhou T, Yang XL, Ma BX, . The in silico and in vivo evaluation of puerarin against Alzheimer's disease. Food & Function. 2019; 10: 799–813.

[21] Hsieh MT, Kuo LH, Tsai FH, Wang WH, Wu CR. Effects of puerarin on scopolamine-, mecamylamine-, p-chloroamphetamine- and dizocilpine-induced inhibitory avoidance performance impairment in rats. Planta Medica. 2002; 68: 901–905.

[22] Liu CM, Zheng GH, Ming QL, Sun JM, Cheng C. Protective effect of puerarin on lead-induced mouse cognitive impairment via altering activities of acetyl cholinesterase, monoamine oxidase and nitric oxide synthase. Environmental Toxicology and Pharmacology. 2013; 35: 502–510.

[23] Kong H, Wang X, Shi R, Zhao Y, Cheng J, Yan X, *et al*. Pharmacokinetics and Tissue Distribution Kinetics of Puerarin in Rats Using Indirect Competitive ELISA. Molecules. 2017; 22: 939.

[24] Li M, Long C, Yang L. Hippocampal-prefrontal circuit and disrupted functional connectivity in psychiatric and neurodegenerative disorders. BioMed Research International. 2015; 2015: 810548.

[25] Lieben CKJ, Steinbusch HWM, Blokland A. 5,7-DHT lesion of the dorsal raphe nuclei impairs object recognition but not affective behavior and corticosterone response to stressor in the rat. Behavioural Brain Research. 2006; 168: 197–207.

[26] Szot P, Franklin A, Miguelez C, Wang Y, Vidaurrazaga I, Ugedo L, *et al*. Depressive-like behavior observed with a minimal loss of locus coeruleus (LC) neurons following administration of 6-hydroxydopamine is associated with electrophysiological changes and reversed with precursors of norepinephrine. Neuropharmacology. 2016; 101: 76–86.

[27] Tsai FS, Peng WH, Wang WH, Wu CR, Hsieh CC, Lin YT, *et al*. Effects of luteolin on learning acquisition in rats: involvement of the central cholinergic system. Life Sciences. 2007; 80: 1692–1698.

[28] Hsieh MT, Wu CR, Hsieh CC. Ameliorating effect of p-hydroxybenzyl alcohol on cycloheximide-induced impairment of passive avoidance response in rats: interactions with compounds acting at 5-HT1A and 5-HT2 receptors. Pharmacology, Biochemistry, and Behavior. 1998; 60: 337–343.

[29] Paxinos G., Watson C. The Rat Brain in Stereotaxic Coordinates. Academic Press: San Diego, CA, USA. 2008.

[30] Shiao YJ, Su MH, Lin HC, Wu CR. Echinacoside ameliorates the memory impairment and cholinergic deficit induced by amyloid beta peptides via the inhibition of amyloid deposition and toxicology. Food & Function. 2017; 8: 2283–2294.

[31] Kuo HC, Chang HC, Lan WC, Tsai FH, Liao JC, Wu CR. Protective effects of Drynaria fortunei against 6-hydroxydopamine-induced oxidative damage in B35 cells via the PI3K/AKT pathway. Food & Function. 2014; 5: 1956–1965.

[32] Yi Y, Adrjan B, Li J, Hu B, Roszak S. NMR studies of daidzein and puerarin: active anti-oxidants in traditional Chinese medicine. Journal of Molecular Modeling. 2019; 25: 202.

[33] Zhou H, Li X, Shang Y, Chen K. Radical Scavenging Activity of Puerarin: A Theoretical Study. Antioxidants. 2019; 8: 590.

[34] Yamasaki M, Takeuchi T. Locus Coeruleus and Dopamine-Dependent Memory Consolidation. Neural Plasticity. 2017; 2017: 8602690.

[35] Wilson MA, Fadel JR. Cholinergic regulation of fear learning and extinction. Journal of Neuroscience Research. 2017; 95: 836–852.
[36] Homberg JR. Serotonergic modulation of conditioned fear. Scientifica. 2012; 2012: 821549.
[37] Qiu ZK, Zhang GH, Zhong DS, He JL, Liu X, Chen JS, *et al*. Puerarin ameliorated the behavioral deficits induced by chronic stress in rats. Scientific Reports. 2017; 7: 6266.
[38] Ballinger EC, Ananth M, Talmage DA, Role LW. Basal Forebrain Cholinergic Circuits and Signaling in Cognition and Cognitive Decline. Neuron. 2016; 91: 1199–1218.
[39] Walsh TJ, Tilson HA, DeHaven DL, Mailman RB, Fisher A, Hanin I. AF64A, a cholinergic neurotoxin, selectively depletes acetylcholine in hippocampus and cortex, and produces long-term passive avoidance and radial-arm maze deficits in the rat. Brain Research. 1984; 321: 91–102.
[40] Zhou Y, Xie N, Li L, Zou Y, Zhang X, Dong M. Puerarin alleviates cognitive impairment and oxidative stress in APP/PS1 transgenic mice. The International Journal of Neuropsychopharmacology. 2014; 17: 635–644.
[41] Heo HJ, Suh YM, Kim MJ, Choi SJ, Mun NS, Kim HK, *et al*. Daidzein activates choline acetyltransferase from MC-IXC cells and improves drug-induced amnesia. Bioscience, Biotechnology, and Biochemistry. 2006; 70: 107–111.
[42] Wang Q, Shen ZN, Zhang SJ, Sun Y, Zheng FJ, Li YH. Protective effects and mechanism of puerarin targeting PI3K/Akt signal pathway on neurological diseases. Frontiers in Pharmacology. 2022; 13: 1022053.
[43] Schweri MM, Carr LA. The effects of Cycloheximide and Anisomycin on monoamine synthesis in a brain synaptosome preparation. Journal of Neural Transmission. 1982; 54: 41–50.
[44] Shiotani T, Tohyama K, Murase K, Ishihara S, Kameyama T, Yamasaki T, *et al*. Effects of nefiracetam, DM-9384 on amnesia and decrease in choline acetyltransferase activity induced by cycloheximide. Journal of Neural Transmission. General Section. 1992; 90: 103–111.
[45] Quartermain D, Botwinick CY. Role of the biogenic amines in the reversal of cycloheximide-induced amnesia. Journal of Comparative and Physiological Psychology. 1975; 88: 386–401.
[46] Robinson SW, Bourgognon JM, Spiers JG, Breda C, Campesan S, Butcher A, *et al*. Nitric oxide-mediated posttranslational modifications control neurotransmitter release by modulating complexin farnesylation and enhancing its clamping ability. PLoS Biology. 2018; 16: e2003611.
[47] Maltsev AV, Bal NV, Balaban PM. LTP suppression by protein synthesis inhibitors is NO-dependent. Neuropharmacology. 2019; 146: 276–288.
[48] Furukawa K, Estus S, Fu W, Mark RJ, Mattson MP. Neuroprotective action of cycloheximide involves induction of bcl-2 and antioxidant pathways. The Journal of Cell Biology. 1997; 136: 1137–1149.
[49] Zhang Y, Chen Y, Shan Y, Wang D, Zhu C, Xu Y. Effects of puerarin on cholinergic enzymes in the brain of ovariectomized guinea pigs. The International Journal of Neuroscience. 2013; 123: 783–791.
[50] Qiu ZK, Zhong DS, He JL, Liu X, Chen JS, Nie H. The anxiolytic-like effects of puerarin are associated with the changes of monoaminergic neurotransmitters and biosynthesis of allopregnanolone in the brain. Metabolic Brain Disease. 2018; 33: 167–175.
[51] Zhou YX, Zhang H, Peng C. Puerarin: a review of pharmacological effects. Phytotherapy Research. 2014; 28: 961–975.
[52] Wang K, Zhu X, Zhang K, Yao Y, Zhuang M, Tan C, *et al*. Puerarin inhibits amyloid β-induced NLRP3 inflammasome activation in retinal pigment epithelial cells via suppressing ROS-dependent oxidative and endoplasmic reticulum stresses. Experimental Cell Research. 2017; 357: 335–340.
[53] Zhang J, Guo W, Tian B, Sun M, Li H, Zhou L, *et al*. Puerarin attenuates cognitive dysfunction and oxidative stress in vascular dementia rats induced by chronic ischemia. International Journal of Clinical and Experimental Pathology. 2015; 8: 4695–4704.
[54] Zou Y, Hong B, Fan L, Zhou L, Liu Y, Wu Q, *et al*. Protective effect of puerarin against beta-amyloid-induced oxidative stress in neuronal cultures from rat hippocampus: involvement of the GSK-3β/Nrf2 signaling pathway. Free Radical Research. 2013; 47: 55–63.
[55] Bo J, Ming BY, Gang LZ, Lei C, Jia AL. Protection by puerarin against MPP+-induced neurotoxicity in PC12 cells mediated by inhibiting mitochondrial dysfunction and caspase-3-like activation. Neuroscience Research. 2005; 53: 183–188.