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Effects of ginseng stem-leaves saponins on one-way avoidance behavior in rats

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ABSTRACT Using a multiple-trial, training-to-criterion procedure, the effects of repeated administrations of ginseng stem-leaves saponins (GSLS) on learning and memory of one-way avoidance in rats were studied in shuttle-box. In acquisition, GSLS 10, 30, and 60 mg · kg^{-1} ip shortened the latency of avoidence in a bell-shaped manner from 1.0 ± 0.2 s in saline rats to 0.8 ± 0.5, 0.5 ± 0.1, 0.8 ± 0.2 s (d 3 learning) and from 0.9 ± 0.2 s to 0.8 ± 0.2, 0.7 ± 0.1, and 0.8 ± 0.2 s (10 × 24 h memory), and the best dose was 30 mg · kg^{-1}. GSLS 30 mg · kg^{-1} ip shortened the latency of avoidance prolonged by scopolamine 0.8 mg · kg^{-1} sc from 5.2 ± 1.3 s to 3.9 ± 0.8 s (d 1 learning acquisition) and from 2.2 ± 0.6 s to 0.8 ± 0.3 s (3 × 24 h memory acquisition). In retention, GSLS 30 mg · kg^{-1} ip shortened the latency prolonged by cycloheximide 2.5 and 5 mg · kg^{-1} ip from 3.4 ± 1.0 s to 1.4 ± 0.5 s (4 h memory) and from 1.6 ± 0.3 s to 0.9 ± 0.2 s (24 h memory), and increased the avoidance number decreased by cycloheximide 5 mg · kg^{-1} from 38.1 ± 8.8% to 72.4 ± 10.8% (4 h memory). The results indicate that GSLS facilitated the acquisition of learning and memory in rats, and improved the scopolamine amnesia and cycloheximide amnesia.

KEY WORDS saponins; avoidance learning; memory; scopolamine; cycloheximide; acquisition; retention (psychology); ginseng

The root of *Panax ginseng* CA Meyer has been used as restorative drug, sedative, psychic energizer and an agent to counter senile changes and to prolong vital force[1]. Pharmacological studies have shown that GSLS have the similar actions to the ginseng roots[2].

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Some investigators studied ginseng in many behavioral models[3]. The reports of ginseng on active avoidance having not been found, we explored the effects of GSLS on one-way avoidance behavior.

MATERIALS

Rats Wistar rats, ♂, were obtained from the Laboratory Animal Center in Chinese Medical University. The rats were housed 5 or 6 per cage on a natural light-dark cycle. They had free access to food and water.

Drugs Scopolamine (Scop, Fluka AG), cycloheximide (CXM, Nacalai Tesque Inc Kyoto), GSLS (Xinbin County 2nd Pharmaceutical Factory, Liaoning Province) with a 90.3% content of ginsenosides were dissolved in normal saline (NS) before use. These drugs and NS were injected ip in a volume of 5 ml · kg^{-1} except Scop (sc, 1 ml · kg^{-1}).

Behavioral apparatus The apparatus was made of a wooden box 61 cm long divided into 2 equal compartments (30 cm × 24 cm × 41 cm) by a vertical wall with a 9 cm diameter hole[4]. One compartment was used as shock side illuminated by a 100 W lamp, and the other as the safe side. Each compartment contained separate grid floor consisting of 0.5 cm diameter brass rods 1 cm apart. The shock side grid could be electrified by a voltage device.

METHODS AND RESULTS

Behavioral procedure There were 3 phases to the procedure: adaptation, training, and testing. Rats were habituated to both compartments for 90 s before first training trial. The rats were trained to cross the

hole from the shock side into the safe side within the duration of a conditioned stimulus (CS, flash light), and if they failed to do so, they were punished with a footshock, an unconditioned stimulus (US, AC 30 V). Each rat received 30 trials daily. One trial consisted of 15 s intertrial interval followed by 15 s CS. The last 5 s of CS overlapped with US. Testing procedure was the same as training procedure.

Parameters and statistics In this study, we counted the number and / or latency of avoidance to evaluate the learning or memory performance of the daily training or testing. Group comparisons were performed by t test.

Effects of GSLS on the acquisition of learning and memory Four groups of 6 rats each, weighing 206 ± 20 g, received either NS, GSLS 10, 30 or 60 mg · kg^{-1} bid (at 800 and 1600) 2 d prior to d 1 training and once 30 min prior to d 1–3 training. Memory acquisition 10× 24 h test was performed after d 3 training.

GSLS 10, 30, 60 mg · kg^{-1} shortened the latency of avoidance in a bell-shaped manner except in d 1 training, and the optimal dose was 30 mg · kg^{-1}. These results indicated that GSLS facilitated the acquisition of learning and memory (Tab 1).

Tab 1. Effects of GSLS (ip) on acquisition of learning (d 1–3) and 10 × 24 h memory (d 13) of one-way avoidance in rats. $n = 6$, $\bar{x} \pm$ SD. $^{*}P > 0.05$, $^{**}P < 0.05$, *vs* NS group.

Drugs, mg · kg^{-1}		Avoidance latency (s) d 1	d 2	d 3	d 13
NS	–	2.4 ± 0.9	1.5 ± 0.7	1.0 ± 0.2	0.9 ± 0.2
GSLS	10	2.1 ± 1.0*	1.0 ± 0.4*	0.8 ± 0.5*	0.8 ± 0.2*
	30	1.8 ± 0.3*	0.8 ± 0.1**	0.5 ± 0.1**	0.7 ± 0.1**
	60	1.7 ± 0.4*	0.9 ± 0.2*	0.8 ± 0.2*	0.8 ± 0.2*

Effects of GSLS on Scop-induced amnesia Twenty three rats, weighing 176 ± 22 g, were divided into 3 groups: NS 7, Scop 7, and GSLS–Scop 8 rats. They received either NS, NS or GSLS 30 mg · kg^{-1} bid (at 800 and 1600) 2 d prior to d 1 training and received either NS–NS (sc), NS–Scop 0.8 mg · kg^{-1} or GSLS–Scop 30 min prior to d 1 training, and received either NS, NS or GSLS 30 min prior to d 2–5 training. Memory acquisition 3 × 24 h test was done after d 5 training. In this experiment, each rat was given 24 trials daily.

Scop prolonged the latency of avoidance throughout training and testing, and significantly in d 1 training, while GSLS significantly shortened the Scop-prolonged latency in the meantime. The results indicated that Scop impaired the acquisition of learning and memory and GSLS improved the Scop amnesia (Tab 2).

Tab 2. Avoidance latency (s) after GSLS 30 mg · kg^{-1} ip on acquisition impairment of learning (d 1–5) and 3 × 24 h memory (d 8) of one-way avoidance in rats induced by Scop 0.8 mg · kg^{-1} sc. $n = 7$–8, $\bar{x} \pm$ SD. $^{*}P > 0.05$, $^{***}P < 0.01$ *vs* NS group; $^{+}P > 0.05$, $^{++}P < 0.05$, $^{+++}P < 0.01$ *vs* Scop group.

	NS	Scop	GSLS+Scop
d 1	3.2 ± 0.7	5.2 ± 1.3***	3.9 ± 0.8^{++}
d 2	2.6 ± 0.7	3.4 ± 0.9*	1.9 ± 0.7^{++}
d 3	2.0 ± 0.4	2.1 ± 0.6*	1.4 ± 0.4^{++}
d 4	1.8 ± 0.4	2.0 ± 0.3*	1.0 ± 0.2^{++}
d 5	1.6 ± 0.5	2.1 ± 0.6*	0.8 ± 0.1^{+++}
d 8	1.7 ± 0.4	2.2 ± 0.6*	0.8 ± 0.3^{++}

Effects of GSLS on CXM-induced retention impairment of 4 h memory Twenty one rats, weighing 186 ± 22 g, were first adapted and trained as described above. The rats were divided into NS group, CXM group and GSLS–CXM group (7 per group) after they basically learned the behavioral task with more than 85% avoidance for each rat. They received daily either NS, NS or GSLS 30 mg · kg^{-1} 15 min prior to the following 3 d training and received either NS–NS,

NS–CXM 5 mg • kg^{-1} or GSLS–CXM immediately after the last day training, and then, 4 h memory retention was tested.

The latency and number of avoidance in the last day training showed no difference among 3 groups, and CXM (5 mg • kg^{-1}) significantly prolonged the latency and decreased the number in the retention testing of 4 h memory, and in the meantime, GSLS significantly shortened the CXM–prolonged latency and increased the CXM–decreased number. The results revealed that CXM impaired the retention of 4 h memory and GSLS improved the CXM amnesia (Tab 3).

Tab 3. Effects of GSLS 30 mg • kg^{-1} ip on retention impairment of 4 h and 24 h memory of one–way avoidance in rats induced by CXM 5 and 2.5 mg • kg^{-1} ip. AL: avoidance latency; AN: avoidance number. n=6–7, $\bar{x} \pm$ SD. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* NS group; $^{+}P>0.05$, $^{++}P<0.05$, $^{+++}P<0.01$ *vs* CXM group.

	NS	CXM	GSLS+CXM
4 h memory			
Last day training			
AL, s	1.5±0.3	1.4±0.3*	1.3±0.3^{+*}
AN, %	100±0	100±0*	100±0^{+*}
Retention testing			
AL, s	1.4±0.4	3.4±1.0**	1.4±0.4^{+++}
AN, %	99.8±0.2	38.1±8.8**	72.4±10.8^{++}
24 h memory			
Last day training			
AL, s	1.6±0.1	1.5±0.1*	1.5±0.4^{+*}
AN, %	98.3±1.8	98.1±2.6*	97.1±2.3^{+*}
Retention testing			
AL, s	1.2±0.2	1.6±0.3**	0.9±0.2^{+++}
AN, %	97.2±2.5	95.2±6.9*	96.2±4.0^{+}

Effects of GSLS on CXM–induced retention impairment of 24 h memory Twenty rats, weighing 198± 20 g, were adapted, trained and tested as described above and in 4 h memory retention testing. The retention test of 24 h memory was performed, and the dose of CXM was 2.5 mg • kg^{-1}.

The latency and number in the last day training showed no difference among 3 groups, and CXM 2.5 mg • kg^{-1} significantly prolonged the latency and meanwhile, GSLS significantly shortened the CXM–prolonged latency in the retention testing. Both CXM and GSLS + CXM had no effect on the number in the memory testing. The results showed that CXM impaired the retention of 24 h memory and GSLS improved the CXM amnesia (Tab 3).

DISCUSSION

This study was to explore the mechanisms of GSLS facilitating learning and memory directly in respect of ethopharmacology. The experimental results indicated that GSLS 10, 30, 60 mg • kg^{-1} facilitated the acquisition of learning and memory in a bell–shaped manner and GSLS 30 mg • kg^{-1} improved the acquisition impairment induced by Scop. The former has been not yet reported in ginseng behavioral researches, and the latter may be one of the mechanisms of ginseng facilitating the learning and memory acquisition. We think GSLS may possess a central muscarine agonism and be used in the treatment of cognition deficits such as Alzheimer's disease resulting from cholinergic hypofunction.

Behavioral experiments have confirmed that cerebral protein synthesis is not necessary for learning or for memory for 3 h after training, but it is required for long–term memory[5]. In this study, CXM, a protein synthesis inhibitor, impaired the retention of 4 h and 24 h memory. These results demonstrated the above discoveries again. GSLS significantly improved the CXM amnesia, therefore, we think GSLS may increase the protein biosynthesis in rat brain, and this view was supported with the results of literature[6].

It should be mentioned that a high voltage, constant current scrambler has been

widely used in the study of conditioned avoidance responding. Our experimental conditions being limited, we used an ordinary voltage device in this study. In this case, the learning acquisition performance of control rats appeared too high to be convenient to the study of cognition-enhancing drugs, however, GSLS still showed the facilitation on learning and memory.

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人参茎叶皂甙对大鼠单路回避行为的影响

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提要 用穿梭箱法，研究多次 ip 人参茎叶皂甙(GSLS)对大鼠学习和记忆的影响. GSLS 10, 30, 60 mg·kg^{-1} 以钟形方式缩短回避潜伏期从而易化了学习和记忆获得. GSLS 30 mg·kg^{-1} 增加回避次数和/或缩短回避潜伏期从而改善了东莨菪碱(0.8 mg·kg^{-1} sc)遗忘和环己酰亚胺(2.5 和 5 mg·kg^{-1} ip)遗忘.

关键词 皂甙类；回避学习；记忆；东莨菪碱；环己酰亚胺；获得；保持 (心理学)；人参

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Inhibitory effect of triptolide on colony formation of breast and stomach cancer cell lines

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ABSTRACT Triptolide (Tri) is a diterpenoid triepoxide isolated from *Tripterygium wilfordii* Hook F. The effects of Tri on the colony formation of breast cancer cell lines MCF-7 and BT-20, stomach cancer cell lines MKN-45, MKN-7, and KATO-III, and promyelocytic leukemia cell line HL-60 were reported. Using Hamburger-Salmon's double layer agar technique with certain modifications, cancer cells were cultured in 0.3% agar in a highly humidified atmosphere of 5% CO_2 at 37℃ for 14-21 d. Colonies were counted on d 14 (occasionally d 21) with the colony analyzer system CA-7A. Of the 5 solid tumor cell lines tested, 4 showed duminished colony formation in soft agar by >70% of control value in Tri 10^{-8} mol·L^{-1} (continuous exposure). The magnitudes of the inhibitory effect of Tri on most breast and stomach cancer cell lines were similar to that on the leukemia cell line HL-60. IC_{50} were 0.504-1.22 μg·L^{-1}. The clinically achievable peak

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