Antidepressant-Like Effect of *Cordyceps sinensis* in the Mouse Tail Suspension Test

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Cordyceps sinensis **(CS) has been known as a component of traditional medicines that elicit various biological effects such as anti-fatigue, immunomodulatory, and hypoglycemic actions. Since it has been well-established that fatigue is closely related to depression, we used the tail suspension test (TST) in mice to examine the antidepressant-like effects of hot water extract (HWCS) and supercritical fluid extract (SCCS) of CS. Immobility time in the TST was reduced by administration of SCCS (2.5—10 ml/kg, *p.o.*) dose-dependently though it was not reduced by treatment with HWCS (500—2000 mg/kg, *p.o.*). Neither HWCS nor SCCS altered locomotor activity in the open field test, excluding the possibility that the effect of SCCS is due to activation of locomotion. Pretreatment with prazosin (an adrenoreceptor antagonist) or sulpiride (a dopamine D2 receptor antagonist) reduced the effect of SCCS on the immobility time. In contrast, pretreatment with *p*-chlorophenylalanine (*p*-CPA, a serotonin synthesis inhibitor) did not alter the anti-immobility effect of SCCS. The last finding is consistent with an additional observation that SCCS had no effect on head twitch response induced by 5-hydroxy-L-tryptophan in mice. Taken altogether, these results suggest that SCCS may elicit an antidepressant-like effect by affecting the adrenergic and dopaminergic systems, but not by affecting the serotonergic system.**

Key words *Cordyceps sinensis*; depression; tail suspension test; noradrenaline; dopamine

Cordyceps sinensis (CS) is a fungus parasitic to *Hepialidae* larvae and has been known as a traditional medicine in China. Many studies have shown that CS possesses anti-fatigue,[1] antitumor,[2,3] antioxidant,[4,5] immunomodulatory,[6—8] hypoglycemic,[9,10] and vasorelaxant activities.[11] Since a relationship between depression and fatigue has been well established,[12—14] we determined to evaluate its antidepressive effect using the tail suspension test (TST)[15,16] that, in parallel with the forced swimming test,[17] has been proposed as a primary screening test for antidepressant drugs.

The most prevalent theory for the pathogenesis of depression is the "monoamine hypothesis" in which monoamines (*e.g.*, norepinephrine, dopamine, and serotonin) are thought to play important roles in the development of symptoms. Many antidepressant drugs work by modulating these neurotransmission systems.[18,19] Recently, behavioral tests have been used to study mechanisms underlying some antidepressant-like substances with inhibitors of neurotransmitters such as alpha-1 adrenoreceptor antagonists, selective dopamine D2 receptor antagonists, and serotonin synthesis inhibitors.[20—23] In addition to these screening behavioral tests of antidepressants, counting the numbers of head twitches induced by 5-hydroxytryptophan in mice is an effective method to evaluate serotoninerigic effects of drugs *in vivo*.[24] It has been generally accepted that numbers of head twitches represent the level of 5-hydroxytryptamine (5-HT) in the synapses.

In the present study, we have evaluated the antidepressant-like effects of hot water extract (HWCS) and supercritical fluid extract (SCCS), obtained from CS, using the TST in mice. We have also dissected the mechanism underlying the antidepressant actions of SCCS by examining the effects of pretreatment with an alpha-1 adrenoreceptor antagonist, a selective dopamine D2 receptor antagonist, and an inhibitor of serotonin synthesis. Furthermore, to determine whether the antidepressive effect of SCCS is mediated by the serotoninergic system, we have evaluated the effect of SCCS on 5-HTP-induced head twitch response in mice.

MATERIALS AND METHODS

Animals Five-week-old male C57 BL/6 mice were purchased from Japan SLC Inc. (Hamamatsu). They were housed in clear plastic cages (15×22×13 cm high) in groups of 5 mice per cage with free access to food and water. The temperature of the room in which the animals were housed, treated, and observed was maintained at 23±1 °C with constant humidity (55±7%). The room was illuminated from 8:00 a.m. to 8:00 p.m. The animals were acclimatized for at least 5 d before behavioral experiments. All experiments were performed according to guidelines for the care and use of laboratory animals of the Keio University School of Medicine.

Drug Treatment The following drugs were used: prazosin hydrochloride, (±)-sulpiride, DL-*p*-chlorophenylalanine (*p*-CPA), 5-hydroxy-L-tryptophan (5-HTP), fluoxetine hydrochloride, and polyoxyethylene sorbitan monooleate (Wako Pure Chemical Indusries, Ltd., Osaka); clorgyline hydrochloride (MP Biomedicals, Inc., Solon, OH, U.S.A.); desipramine hydrochloride (Sigma-Aldrich, St. Louis, MO, U.S.A.); bupropion hydrochloride (Toronto Research Chemicals Inc., North York, Canada). Prazosin hydrochloride, clorgyline hydrochloride, desipramine hydrochloride, bupropion hydrochloride and fluoxetine hydrochloride were dissolved in saline (0.9% NaCl solution). (±)-Sulpiride and *p*-CPA were suspended in 1% polyoxyethylene sorbitan monooleate-saline. All drugs were injected in a volume of 10 ml/kg. The same amounts of vehicles were administered for control

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mice.

Preparation of HWCS A hot water extract of CS (HWCS) was prepared by autoclaving 20 g of CS in 400 g of water at approximately 120 °C for 20 min, followed by filtration and freeze-drying (yield: approximately 30%). HWCS was dissolved in distilled water and administered in a volume of 10 ml/kg.

Preparation of SCCS A supercritical fluid extract (SCCS, 4.82 kg) was obtained from 55.2 kg of CS by CO_2 extraction conducted at a pressure of 25 MPa, 40 °C using a supercritical fluid extraction system (batch method, 300L, UHDE GmbH, Germany).

Measurement of the Total Duration of Immobility in the TST Immobility time during tail suspension was measured according to the method described previously.[15] The tail suspension apparatus consisted of a gray polyethylene box (35×35×40 cm high) with a hook in the center of the ceiling. Each mouse was individually suspended by the tail from the hook with an adhesive tape. Total duration of immobility during the 6-min test was calculated as immobility time. To evaluate the effects of HWCS and SCCS, mice were treated with each extract (HWCS: 500, 1000, 2000 mg/kg, *p.o.*, SCCS: 2.5, 5, 10 ml/kg, *p.o.*) or with water for 5 consecutive days. The last administration was conducted 1 h before the test.

To dissect the mechanism underlying the antidepressant-like effect of SCCS in the TST, we assessed the effects of pretreatment with prazosin (an alpha 1 adrenoceptor antagonist, 1 mg/kg, i.p., administered 45 min before the test), sulpiride (a selective dopamine D2 receptor antagonist, 20 mg/kg, i.p., administered 45 min before the test), or *p*-CPA (a serotonin synthesis inhibitor, 300 mg/kg, i.p., administered 72, 48, and 24 h before the test), on the antidepressive action of SCCS at the most effective dose (10 ml/kg, *p.o.* for 5 d). As a positive control, desipramine (30 mg/kg, i.p.), bupropion (10 mg/kg, i.p.), or fluoxetine (20 mg/kg, i.p.) was administered for 5 consecutive days. The last administration was conducted 30 min before the test. Prazosin, sulpiride, or *p*-CPA was administered according to the same schedule as above.

Measurement of Locomotor Activity in the Open Field Test (OFT) The open field apparatus was made of a gray polyethylene box (90×70×40 cm high). The floor of the apparatus was divided into 63 squares of equal area (10×10 cm) by black lines. Each mouse was moved from its home cage to the center square of the open field. Crossings of the black lines were counted for 10 min. Mice were treated with each extract (HWCS: 500, 1000, 2000 mg/kg, *p.o.*, SCCS: 2.5, 5, 10 ml/kg, *p.o.*) or with water for 6 consecutive days. The last administration was conducted 1 h before the test.

Measurement of Head Twitch Response (HTR) Induced by 5-HTP Plus Clorgyline Clorgyline (1 mg/kg, i.p.), a monoamine oxidase inhibitor, was given as pretreatment 1 h prior to injection of 5-HTP (150 mg/kg, i.p.). After the administration of 5-HTP, each mouse was placed in a clear plastic cage (15×22×13 cm high). The frequencies of head twitches were counted for 2 min at 10 min intervals from 10 to 50 min after injection of 5-HTP as head twitch response (HTR). SCCS (10 ml/kg, *p.o.*) was administered 1 h before the injection of 5-HTP while fluoxetine (10 mg/kg, i.p.) was administered as a positive control 30 min before the 5-HTP injection.

Statistics Data are shown as the mean±S.E.M. The data indicating the dose dependency of HWCS and SCCS in the TST and the OFT were analyzed by the Dunnett's test after the Bartlett test and one-way analysis of variance (ANOVA). The data indicating the effects of SCCS pretreated with the inhibitors of neurotransmitters in the TST were evaluated using the Newman–Keuls test after the Bartlett test and the one-way ANOVA. The data for the effects of SCCS on the HTR were analyzed by the Student *t*-test or the Aspin–Welch *t*-test after dispersal analysis using the *F*-test. *p* values less than 0.05 were considered to indicate statistical significance.

RESULTS

The Effects of HWCS and SCCS on Immobility Time in the TST and on Normal Behaviour in the OFT in Mice Administration of HWCS (500—2000 mg/kg, *p.o.* for 5 d) showed no effects on immobility time in the TST (Fig. 1A). In contrast, administration of SCCS (2.5—10 ml/kg, *p.o.* for 5 d) decreased the immobility time in a dose-dependent manner (Fig. 1B). Meanwhile, neither HWCS (500—2000 mg/kg, *p.o.* for 6 d) nor SCCS (2.5—10 ml/kg, *p.o.* for 6 d) affected the number of crossings in the OFT (Figs. 2A, B), indicating that these extracts did not affect normal behavior of mice. There were also no effects of HWCS and SCCS on the frequencies of rearing, glooming, defecating and urinating (data not shown).

The Effect of Pretreatment with Prazosin on the Antidepressive Action of SCCS and Desipramine in Mice Pretreatment with prazosin (1 mg/kg, i.p.), an alpha-1 adrenoceptor antagonist, significantly blocked the decrease in immobility time elicited by SCCS in the TST (Fig. 3A). De-

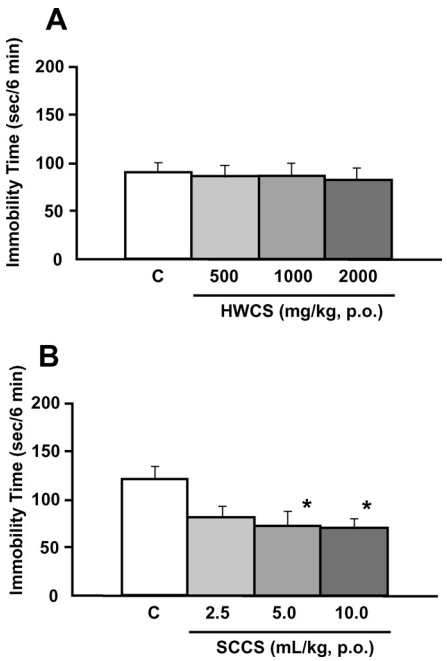

Fig. 1. The Effects of HWCS (500—2000 mg/kg, *p.o.* for 5 d, Panel A [ANOVA: $F_{(3,64)}=0.104$; $p=0.96$]) and SCCS (2.5—10 ml/kg, *p.o.* for 5 d, Panel B [ANOVA: $F_{(3,64)}=3.49$; $p=0.021$]) on Immobility Time in the Mouse TST

The last administration of HWCS or SCCS was conducted 1 h before the test. Values are shown as mean±S.E.M. ($n=17$). $*p<0.05$ *vs.* vehicle-treated control group (C).

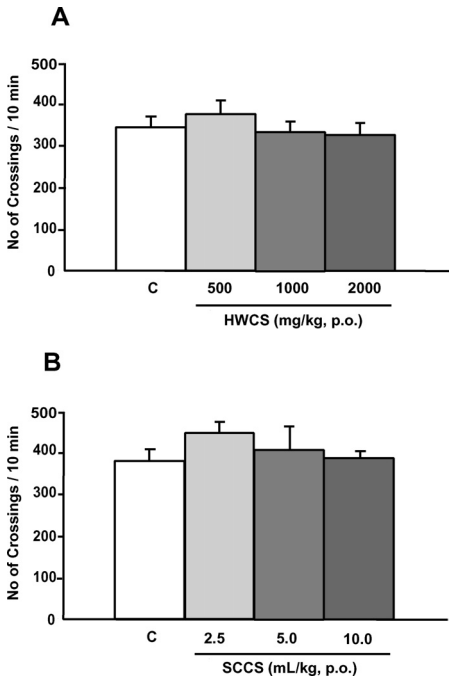

Fig. 2. The Effects of HWCS (500—2000 mg/kg, *p.o.* for 6 d, Panel A [ANOVA: $F_{(3,16)}=0.613$; $p=0.62$]) and SCCS (2.5—10 ml/kg, *p.o.* for 6 d, Panel B [ANOVA: $F_{(3,16)}=0.905$; $p=0.46$]) on Locomotor Activity in the Mouse OFT

The last administration of HWCS or SCCS was conducted 1 h before the test. Values are shown as mean±S.E.M. ($n=5$).

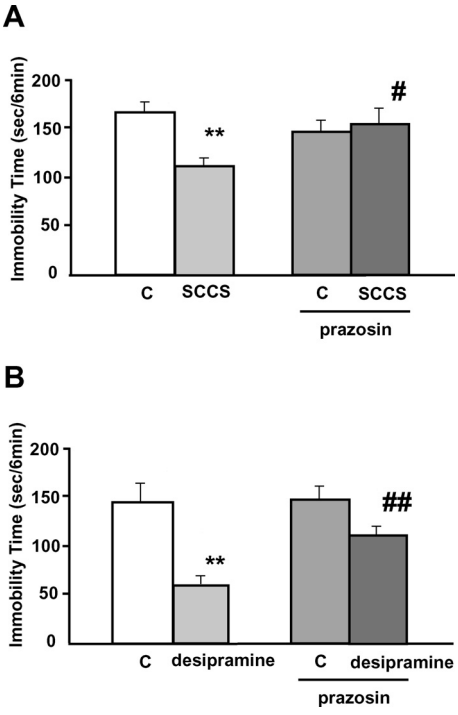

Fig. 3. The Effects of SCCS (10 ml/kg, *p.o.* for 5 d, $n=14$—16, Panel A [ANOVA: $F_{(3,56)}=4.40$; $p=0.0076$]) or Desipramine (30 mg/kg, i.p. for 5 d, $n=11$, Panel B [ANOVA: $F_{(3,40)}=10.4$; $p=0.000033$]) Alone and in Combination with the Alpha-1 Adrenoceptor Antagonist Prazosin in the Mouse TST

The last administration of SCCS or desipramine was conducted 1 h or 30 min before the test, respectively. Prazosin (1 mg/kg, i.p.) was administered 45 min before the test. Values are shown as mean±S.E.M. $^{**}p<0.01$ *vs.* vehicle-treated control group (C), $^{\#}p<0.05$, $^{\#\#}p<0.01$ *vs.* groups treated with SCCS or desipramine alone.

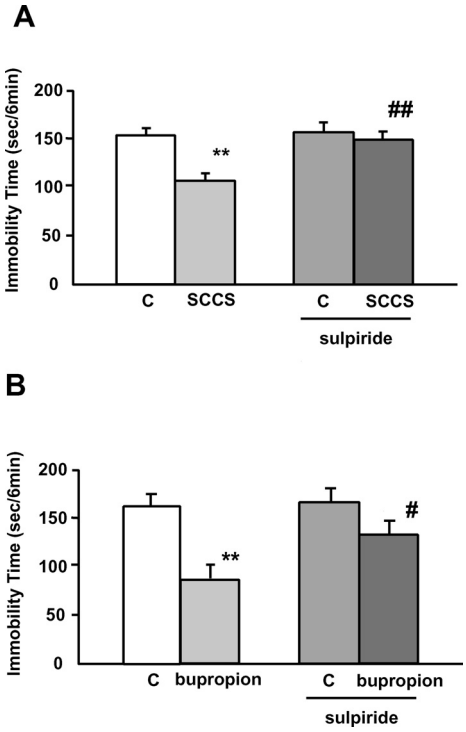

Fig. 4. The Effects of SCCS (10 ml/kg, *p.o.* for 5 d, $n=14$—16, Panel A [ANOVA: $F_{(3,56)}=7.44$; $p=0.00028$]) or Bupropion (10 mg/kg, i.p. for 5 d, $n=11$—12, Panel B [ANOVA: $F_{(3,42)}=6.92$; $p=0.00069$]) Alone and in Combination with the Selective Dopamine D2 Receptor Antagonist Sulpiride in the Mouse TST

The last administration of SCCS or bupropion was conducted 1 h or 30 min before the test, respectively. Sulpiride (20 mg/kg, i.p.) was administered 45 min before the test. Values are shown as mean±S.E.M. $^{**}p<0.01$ *vs.* vehicle-treated control group (C), $^{\#}p<0.05$, $^{\#\#}p<0.01$ *vs.* groups treated with SCCS or bupropion alone.

sipramine (30 mg/kg, i.p. for 5 d), a tricyclic antidepressant known as a noradrenaline reuptake inhibitor, also showed an anti-immobility effect. The anti-immobility effect of desipramine, as well as SCCS, was blocked by pretreatment with prazosin (Fig. 3B).

The Effect of Pretreatment with Sulpiride on the Antidepressive Action of SCCS and Bupuropion in Mice Pretreatment with sulpiride (20 mg/kg, i.p.), a selective dopamine D2 antagonist, significantly blocked SCCS action (Fig. 4A). Pretreatment with sulpiride also abolished the anti-immobility effect of bupropion (10 mg/kg, i.p.), a dopamine reuptake inhibitor (Fig. 4B).

The Effect of Pretreatment with *p*-CPA on the Antidepressive Action of SCCS and Fluoxetine in Mice Pretreatment with *p*-CPA (300 mg/kg, i.p. for 3 d) did not alter the action of SCCS in the TST (Fig. 5A). In contrast, the antidepressive effect of fluoxetine, a selective serotonin reuptake inhibitor, was significantly blocked by the pretreatment with *p*-CPA (Fig. 5B).

The Effects of SCCS or Fluoxetine on 5-HTP Plus Clorgyline-Induced HTR in Mice The effect of SCCS or fluoxetine on 5-HTP plus clorgyline-induced HTR in mice is demonstrated in Fig. 6A. SCCS treatment did not affect the HTR pattern. In contrast, fluoxetine treatment at a dose of 10 mg/kg, i.p. significantly increased the number of 5-HTP-induced HTR at 10—12 min and decreased the number of HTR at 30—32 min (Fig. 6B).

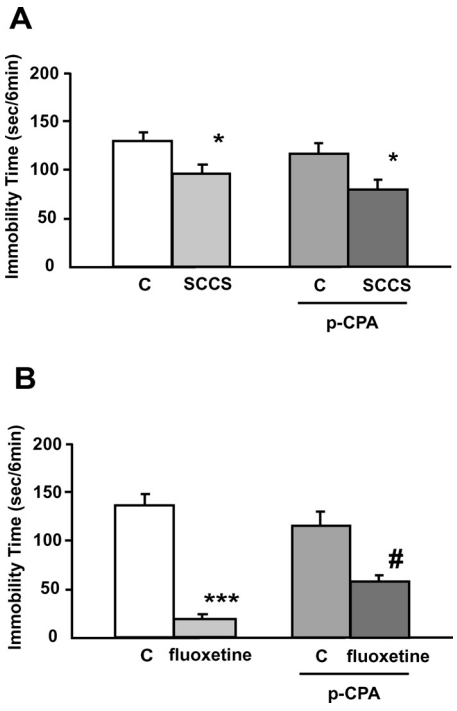

Fig. 5. The Effect of SCCS (10 ml/kg, *p.o.* for 5 d, $n=21$—23, Panel A [ANOVA: $F_{(3,84)}=5.05$; $p=0.0029$]) or Fluoxetine (20 mg/kg, i.p. for 5 d, $n=10$—11, Panel B [ANOVA: $F_{(3,42)}=27.5$; $p=0.00000000052$]) Alone and in Combination with the Serotonin Synthesis Inhibitor *p*-Chrolophenylalanine (*p*-CPA) in the Mouse TST

The last administration of SCCS or fluoxetine was conducted 1 hr or 30 min before the test, respectively. *p*-CPA (300 mg/kg, i.p.) was administered 72, 48, and 24 h before the test. Values are shown as mean±S.E.M. $*p<0.05$, $***p<0.001$ *vs.* vehicle-treated control group (C), $^{\#}p<0.05$ *vs.* the group treated with fluoxetine alone.

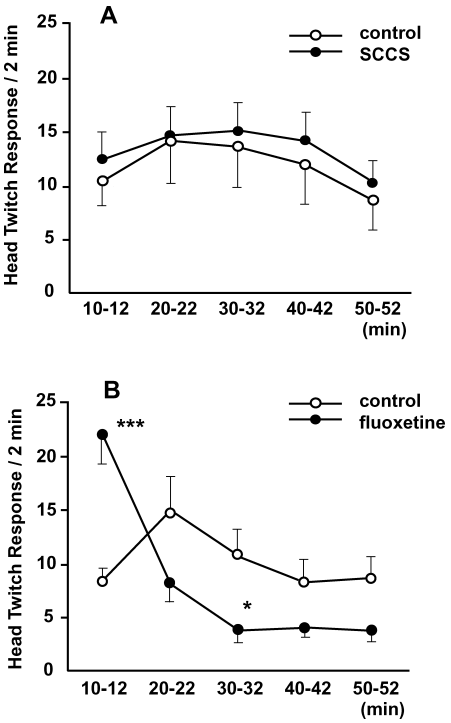

Fig. 6. The Effect of SCCS (10 ml/kg, *p.o.* for 5 d, $n=12$, Panel A) and Fluoxetine (10 mg/kg, i.p. for 5 d, $n=10$, Panel B) on 5HTP and Clorgyline-Induced Head Twitch Behavior in Mice

The last administration of SCCS or fluoxetine was conducted 1 h or 30 min before the injection of 5-HTP. After the injection of 5-HTP, the number of HTR was counted for 2 min at 10 min intervals from 10 to 50 min. Closed and open circles indicate treated and non-treated mice, respectively. $*p<0.05$, $***p<0.001$ *vs.* vehicle-treated control group (C).

DISCUSSION

In this study, based on the findings that administration of SCCS, but not that of HWCS, shortened immobility times in the mouse TST without affecting the locomotor activity in the mouse OFT, we have concluded that SCCS has significant antidepressant-like activity. This is the first study showing that the CS extract exerts an antidepressant-like effect.

The main constituents of SCCS are fat-soluble components such as palmitic acid, oleic acid, triglyceride, ergosterol, and cholesterol while those of HWCS are carbohydrate and protein. Bioactivities of these main ingredients are hardly known. Furthermore, it has been demonstrated that minor components of CS such as cordycepin,[25] polysaccharides,[4,26] and cordyglucan[27] are responsible for some biological activities. Recently, Wang B. J. *et al.*[28] reported that the supercritical CO_2 fluid extractive fraction of CS had a strong scavenging ability and selectively inhibited the growth of cancer cells by promoting apoptosis. However, the constituents responsible for these activities, especially psychotropic activities, have not yet been determined. Further investigation is necessary to identify which constituents of SCCS have antidepressant-like activity.

TST is widely used as an animal behavior test to screen antidepressant drugs.[15,16] This test is quite sensitive and relatively specific to the antidepressing activities of tricyclics, serotonin-specific reuptake inhibitors, and monoamine oxidase inhibitors.

It has been generally accepted that antidepressants elicit their effects by modulating several neurotransmission systems, including the noradrenergic, dopaminergic, and serotoninergic systems. Therefore, the systems responsible for their antidepressant activities have been investigated by using specific inhibitors for these neurotransmission.[20,29—32] Poncelet M. *et al.*[29] reported that prazosin, an alpha-1 adrenoreceptor antagonist, reduced the effect of desipramine, a norepinephrine and serotonin reuptake inhibitor. Yamada J. *et al.*[30] showed that anti-immobility effect of bupropion, a dopamin reuptake inhibitors, was inhibited by sulpiride, a selective dopamine D2 receptor antagonist, in the forced swimming test.

With these neurotransmitter inhibitors, mechanisms underlying the antidepressant-like effect of some herbal extracts have been also investigated. Dhingra D. *et al.*[21] reported that the antidepressant-like effect of liquorice extract seems to be mediated by an increase of brain norepinephrine and dopamine, but not by an increase of serotonin. Rodrigues A. L. *et al.*[22] reported that the antidepressant-like effect of *Siphocampylus verticillatus* extract seems to involve an interaction with adrenergic, dopaminergic, glutamatergic, and serotonergic systems.

In the present study, we evaluated the effects of pretreatment with prazosin, sulpiride, and *p*-CPA on the antidepressant-like actions of SCCS in the mouse TST and found that these effects are mediated by both noradrenergic and dopaminergic neurotransmissions, but not by serotoninergic neurotransmission. We have confirmed the validity of these

inhibitors for these experiments by showing that they attenuated the anti-immobility effects of desipramine, bupropion, and fluoxetine.

Administration of large doses of 5-HTP, a precursor of 5-HT, induces head twitches that occur spontaneously and irregularly, probably *via* a central action of 5-HT. HTR, induced by 5-HTP in mice, provides a simple method of determining specific activities of potentiators and antagonists for 5-HT in the central nervous system.[24] In this study, the administration of SCCS did not affect 5-HTP-induced HTR in mice. This finding is consistent with another finding that *p*-CPA pretreatment did not attenuate the anti-immobility activity of SCCS in the TST (Fig. 5). In contrast, fluoxetine, a positive control compound, significantly increased the number of 5-HTP-induced HTR at 10—12 min and decreased it at 30—32 min. The potentiation of HTR by fluoxetine at 10—12 min may be due to the fluoxetine-mediated inhibition of the 5-HT reuptake and resulting increase of the content of 5-HT in synapses. Subsequent rapid attenuations of HTR by fluoxetine may be due to the exhaustion of 5-HT in synapses or the negative-feedback inactivation of the neurotransmission systems induced after high release of 5-HT into the synaptic clefts.

One possible mechanism underlying antidepressant-like activity of SCCS is that some constituents of SCCS might act as adrenoceptor and dopamine D2 receptor agonists or noradrenaline/dopamine reuptake inhibitors. This possibility needs to be systematically addressed in the future investigation.

In summary, this study has demonstrated that SCCS has an antidepressant-like activity and that its effect seems to originate from SCCS-mediated alterations in the noradrenergic and dopaminergic systems, but not in the serotonergic system.

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