Antidepressant-Like Effects of Sarsasapogenin from *Anemarrhena asphodeloides* BUNGE (Liliaceae)

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The aim of this study was to investigate the effects of sarsasapogenin from *Anemarrhena asphodeloides* BUNGE (Liliaceae) on the forced swimming test, and the central noradrenergic, dopaminergic and serotonergic activities in mice. Our results showed that sarsasapogenin treatment at 12.5, 25 and 50 mg/kg (p.o.) for 14 d significantly reduced the duration of immobility in the forced swimming test. These doses that affected the immobile response did not affect locomotor activity. In addition, the neurochemical assays showed that sarsasapogenin produced a marked increase of noradrenaline and serotonin levels at 50 mg/kg in both the hypothalamus and the hippocampus. Moreover, sarsasapogenin showed a monoamine oxidase inhibitory activity in the mouse brain. These findings suggest that the antidepressant activity of sarsasapogenin may involve the central monoaminergic neurotransmitter systems.

Key words: *Anemarrhena asphodeloides*; sarsasapogenin; antidepressant activity; forced swimming; monoamine; monoamine oxidase

Depression is a common illness associated with high rates of chronicity, relapse, and recurrence; psychosocial and physical impairment; and a high suicide rate. Nonetheless, many currently available antidepressants have low rates of response and remission. Moreover, contemporary antidepressants can produce many unwanted side effects. Therefore, research for new antidepressants with greater effectiveness without any (or with lower) adverse effects is still desirable.

In Oriental society, herbs and herbal preparations have been widely used as medicines by consumers for many centuries. Some of the herbal medicines may be effective alternatives in the treatment of neuropsychiatric diseases such as depression. *Anemarrhena asphodeloides* BUNGE (Liliaceae) is commonly found in traditional Chinese herbal medicines, and has been shown to have antidepressant effects in mouse models of behavioral despair tests. Sarsasapogenin, 5β,20α,22α,25S-spirostan-3β-ol, is a major active component of *Anemarrhena asphodeloides*, which exhibits a variety of pharmacological effects such as the promotion of neurogenesis activity, antioxidative action and improving cognitive impairment; however, no information is available about its antidepressant activity. In the present study, we assessed the potential antidepressant effects of sarsasapogenin from *Anemarrhena asphodeloides* by means of behavioral, pharmacological and neurochemical procedures.

MATERIALS AND METHODS

Materials Sarsasapogenin with a purity of 98% was obtained as described in a previous study. In short, the method involved acid hydrolysis, extraction with organic solvent and repeated recrystallization.

Animals Male Swiss mice, weighing 20—24 g, were used throughout the study. The animals were supplied by the Experimental Animal Centre of Shenyang Pharmaceutical University. Mice were maintained under standard housing conditions in a 12L:12D light/dark cycle (light on 6:30) with free access to water and standard food.

Dose and Route of Administration All drugs were suspended in 0.5% CMC–Na. Sarsasapogenin in doses of 12.5, 25 or 50 mg/kg and fluoxetine hydrochloride in a dose of 20 mg/kg were administered orally once daily for 14 d. A control group of animals received 0.5% CMC–Na only. The behavioral tests and the neurochemical assay were performed 1 h after the last administration on the 14th day.

Forced Swimming Test The forced swimming test (FST) was similar to that described by Porsolt et al. The animals were individually placed in a glass-poly carbonate cylinder (height: 25 cm; diameter: 10 cm) filled to a depth of 10 cm with water maintained at 24 °C and were allowed to swim for 6 min, and the durations of immobility were recorded during the last 4 min of the test.

Open-Field Test The open-field apparatus was a field, 80 cm in diameter, which was demarcated into 36 approximately equal areas. The animals were placed individually in the center of the arena and allowed to explore freely. The number of times the animal crossed squares was recorded for 3 min.

Determination of Monoamines and Metabolites Mice were sacrificed by decapitation. The hypothalamus and hippocampus were rapidly isolated from the cerebrum on an ice-cold plate and immediately frozen in liquid nitrogen until homogenization. The contents of 5-HT, noradrenaline, dopamine, 5-HIAA and DOPAC were measured using HPLC with electrochemical detection. Each frozen tissue sample was weighed and homogenized in 0.1 mol/l perchloric acid. The mixture was centrifuged at 10000 r/min (4 °C) for 20 min, and then the pellet was discarded. The resultant supernatant was filtered, and 20 μl was directly injected into an EP-10 liquid chromatography system equipped with a reversed-phase C18 column (5 μm, 150×4.6 mm, Eicom, Japan). The measurements were done at electrode potentials of a glassy carbon electrode +700 mV vs. Ag/AgCl reference electrode with Eicom ECD-100 electrochemical detector. The mobile phase consisted of 0.1 mol/l citric acid–sodium citrate (pH 3.7), 0.2 mmol/l EDTA, 1 mmol/l sodium octanesulfonate and 10% methanol. The flow rate was 1.0 ml/min. Tissue levels were determined by means of the internal standard 3,4-dihydroxybenzylamine and expressed in terms of nanograms per gram of tissue.

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Measurements of Monoamine Oxidase Activity

Mouse brain mitochondrial fractions were prepared following the procedure of Schurr and Livne. Monoamine oxidase (MAO) activity was assessed spectrophotometrically as described previously. Briefly, the brain tissues were homogenized in 10 mmol/l cold sodium phosphate buffer (pH 7.4, containing 320 mmol/l sucrose). The mixture was centrifuged at 4000 r/min for 10 min; the supernatant was centrifuged at 15000 r/min for 20 min to deposit the protein, which was suspended in the same buffer. Protein concentration was estimated and adjusted to 1 g/l. The assay mixtures contained 4 mmol/l 5-HT or 2 mmol/l β-phenylethylamine (as specific substrates for MAO-A and MAO-B, respectively), 200 μl of the mitochondrial fraction, and 10 mmol/l sodium phosphate buffer (pH 7.4) up to a final volume of 1 ml. The reaction was allowed to proceed at 37°C for 20 min, and ceased by adding 200 μl of 1 mol/l hydrochloric acid. The reaction product was extracted with 4 ml of butyl-acetate (for MAO-A assay) or cyclohexane (for MAO-B assay), respectively. The organic phase was measured at a wavelength of 280 or 242 nm for MAO-A or MAO-B assay with a spectrophotometer. Blank samples were prepared by adding 1 mol/l HCl (200 μl) prior to reaction, and were subsequently treated in the same manner.

Statistical Analysis

All data were presented as mean±S.E.M. The effects of sarsasapogenin were analyzed by one-way analysis of variance (ANOVA). When significant variances (p<0.05) were found, post hoc comparisons were performed with Dunnett’s t-test.

RESULTS AND DISCUSSION

The antidepressant effect of treatment with sarsasapogenin was evaluated in the FST. Sarsasapogenin at the doses of 12.5, 25 and 50 mg/kg reduced, in a dose-dependent manner, the duration of immobility in the FST, resulting in a 26.6% (p<0.05), 32.7% (p<0.05) and 48.7% (p<0.01) immobility reduction compared with the control group, respectively (Fig. 1). The activity was comparable to the reference drug fluoxetine. The FST is the tool most widely used for assessing antidepressant activity preclinically. The widespread use of this model is largely a result of its ease of use, reliability across laboratories, and ability to detect a broad spectrum of antidepressant agents. Most clinically active antidepressants are effective in the FST, while neuroleptics and anxiolytics produce different effects. Antidepressants can also be distinguished from stimulants because stimulants cause marked motor stimulation, in contrast to antidepressants. In our study, the antidepressant effect of sarsasapogenin cannot be attributed to an increase in motor activity because it did not induce hyperlocomotion (p>0.05) in the open-field test (Fig. 2).

Basic neurobiological research as well as clinical studies have revealed that the monoamines have a crucial role in the development of the depression syndrome. It is known that an enhancement of neurotransmission of 5-HT, noradrenaline, or both, underlies the antidepressant response associated with most agents presently available to treat major depression. A recent review on the relationship between dopamine and depression suggested that the dopaminergic system is another appropriate target for antidepressant drugs. Therefore, we investigated the effects of subacute treatment with sarsasapogenin on 5-HT, noradrenaline, dopamine and their metabolites in mouse brain. In the present study, we focused our attention on two brain regions—the hypothalamus and the hippocampus. These brain regions are important because they are involved in important behavioral functions, such as emotion, motivation, learning and memory, all of which may be related to the expression of depression. As shown in Tables 1 and 2, sarsasapogenin (50 mg/kg) significantly elevated the concentration of 5-HT in these brain regions (p<0.05). The ratio of 5-HIAA/5-HT, a major index of 5-HT turnover, was decreased after subacute administration of sarsasapogenin in the hypothalamus (p<0.05). The present results suggest that the enhanced 5-HT level and the downward trend of 5-HT turnover produced by sarsasapogenin may be related, at least in part, to an effect on monoamine metabolism. The noradrenaline levels were also increased after treatment with 50 mg/kg sarsasapogenin in the hypothalamus (p<0.05), and with both 25 and 50 mg/kg sarsasapogenin in the hippocampus (p<0.05 and p<0.01, respectively), but not with fluoxetine (p>0.05). Moreover, sarsasapogenin (50 mg/kg) mildly elevated the dopamine levels in the hypothalamus and hippocampus (p>0.05). Fluoxetine, the reference control in this study, enhanced the concentration of 5-HT by inhibition of reuptake, and also enhanced dopamine in the hypothalamus (p<0.05),
which is consistent with previous findings. \(^{14}\) All these results allowed us to assume that the antidepressant property of sarsasapogenin was related to regulations of both 5-HT and the noradrenaline system.

In general, inhibitors of MAO cause an increase in the amount of monoamines stored and released from the nerve terminals, thus increasing monoaminergic activity. \(^{15}\) In order to clarify whether the changes of monoamines resulted from the inhibition of MAO activity, we assayed mouse brain MAO activity after sarsasapogenin administration. Monoamine oxidase is classified into types A and B. MAO-A preferentially metabolizes 5-HT and NE, whereas MAO-B has a higher affinity for phenylethylamine. Some MAO-A inhibitors are efficacious for treating depression, and the inhibitors of MAO-B appear to be effective in preventing and treating Parkinson’s disease. The present study showed that sarsasapogenin at a dose of 50 mg/kg significantly inhibited both the MAO-A activity (17%, \(p<0.05\)) and the MAO-B activity (15%, \(p<0.05\)) in mouse brain (Table 3). According to the present results, it is possible that the MAO inhibition contributes, at least in part, to the enhancement of 5-HT and noradrenaline levels in mouse brain.

In summary, the results of this study showed that sarsasapogenin possesses antidepressant properties in a behavioral despair test and that the effects may be related to the serotonergic and noradrenergic mechanisms. This report could be of interest in the study of potential therapeutic advantages of sarsasapogenin on depression treatment.

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REFERENCES