Neuroscience Letters 639 (2017) 173–178

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

# Sarsasapogenin reverses depressive-like behaviors and nicotinic acetylcholine receptors induced by olfactory bulbectomy

Bo Feng, Xiao-Yang Zhao, Yi-Zhou Song, Wen-Na Liang, Ji-Liang Liu*

*The Third People's Hospital of Liaocheng, Department of Neurology, Shandong, China*

## HIGHLIGHTS

- Sarsasapogenin reverses depressive-like behaviors in OB rat model.
- Sarsasapogenin modulating α4-nAChR and α7-nAChR expression in OB rat model.
- Sarsasapogenin reversed AChE activity in OB rat model.

## ARTICLE INFO

*Article history:*
Received 26 May 2016
Received in revised form 31 October 2016
Accepted 13 December 2016
Available online 14 December 2016

*Keywords:*
α4-nAChR
α7-nAChR
Acetylcholinesterase
Olfactory bulbectomy
Depression
Sarsasapogenin

## ABSTRACT

Cholinergic signalling in the hippocampus may contribute to the aetiology of mood regulation. Antidepressants can reverse the increase in acetylcholinesterase (AChE) activity induced by olfactory bulbectomy. The activation of nicotinic acetylcholine receptors (nAChRs) also alleviates the symptoms of depression. This study advances the development of sarsasapogenin, which interacts with cholinergic signalling and has a favourable antidepressant profile in olfactory bulbectomised (OB) rats. We examined OB-induced changes in cholinergic signalling, as well as AChE, α4-nAChR, and α7-nAChR expression in the hippocampus. The results indicate that abnormal cholinergic signalling in the hippocampus contributes to the development of depression in the OB rat model. This depression may be alleviated following treatment with sarsasapogenin.



## 1. Introduction

Depression and stress-related mental disorders result in significant personal, economic, and social burdens. The mechanisms underlying depression remain unclear. However, interactions between molecular and cellular abnormalities and genetic and environmental factors may be responsible for the onset of depression [1]. Several studies have shown that symptoms of depression can be induced by blocking acetylcholinesterase (AChE) activity. However, another line of evidence indicates that AChE inhibitors may improve the efficacy and decrease the adverse effects of tricyclic antidepressants. Moreover, antidepressant-like effects can be enhanced through the activation of nicotinic acetylcholine receptors (nAChRs) [2–4].

It has been shown that fluoxetine and rivastigmine can reverse the depressive symptoms and AChE activity induced by olfactory bulbectomy (OB). While this line of evidence focuses on AChE, recent evidence also suggests that nAChRs may also be involved in the aetiology of depression [5]. In particular, findings suggesting that nicotine may improve mood in individuals with depression have prompted investigations of the relationship between nAChRs and depression [6,7]. nAChRs function as excitatory cation channels and comprise α-subunits (α2-α7) and β-subunits (β2-β4). The principal subunits of nAChRs expressed in the brain are the α4 and α7 subunits. α4-nAChR is expressed throughout the nervous system, while α7-nAChR is mainly expressed in the hippocampus, cerebral cortex, and several brainstem nuclei [8,9]. Rodent studies indicate that either nicotine or agonists of α7- and α4-nAChRs may enhance the positive effects of antidepressants [10–12].

OB is a classical model used to investigate pathophysiological mechanisms underlying depression, and is considered to have high predictive validity for the exploration of neurobiological mechanisms and the detection of antidepressant effects [13]. Surprisingly, the relationships between the expression of α4- and α7-nAChR subunits and OB-induced depression have not been thoroughly investigated.

* Corresponding author.
*E-mail address:* jiliangliu2016@163.com (J.-L. Liu).

http://dx.doi.org/10.1016/j.neulet.2016.12.025
0304-3940/

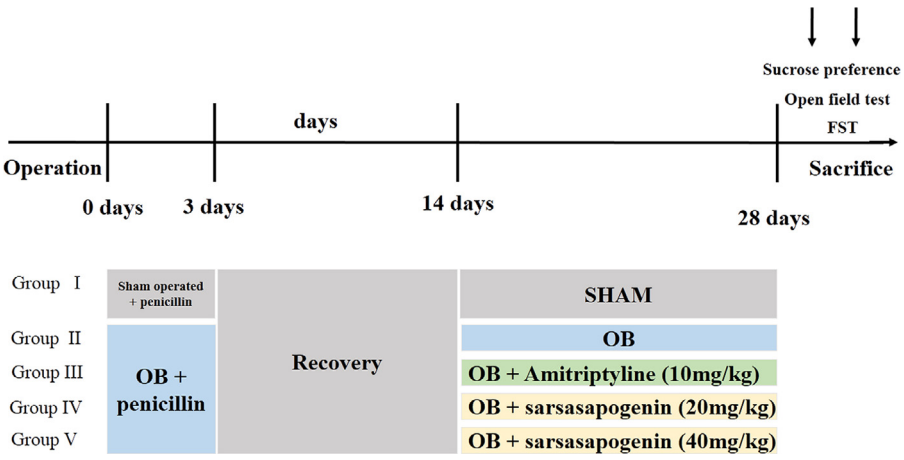


**Fig. 1.** Schematic representation of the experimental procedure for OB and treatments in rat. Abbreviations: FST, the forced swimming tests.

Sarsasapogenin is the major active component of Anemarrhena asphodeloides and has anti-oxidative properties. It also exhibits a variety of pharmacological effects and has been shown to enhance neurogenesis and ameliorate cognitive impairment [14]. Moreover, a study has confirmed that sarsasapogenin improves memory by increasing the density of acetylcholine receptors in the brain of a rat model of memory impairment [14]. Interestingly, sarsasapogenin also has antidepressant-like effects in mice, as assessed using the behavioural despair test [15]. Indeed, these findings suggest that cholinergic signalling may be a potential target for developing a sarsasapogenin-dependent treatment for depression. To our knowledge, few studies have investigated the effects of sarsasapogenin on depressive symptoms and the relationship between sarsasapogenin and cholinergic signalling in the OB rat model [16]. To investigate the antidepressant effect of sarsasapogenin and its relationship to cholinergic signalling, we designed a study to assess the relationship between OB-induced abnormal cholinergic signalling and the antidepressant action of sarsasapogenin.

## 2. Materials and methods

### 2.1. Drugs and reagents

Amitriptyline hydrochloride was obtained from Changzhou Siyao Pharmaceuticals Co., Ltd. (Changzhou, P.R. China). Sarsasapogenin (purity >90%) was obtained from Zelang Medical Technology Co., Ltd., (Nanjing, China). All other chemicals and reagents were of analytical grade.

### 2.2. Animals

Sixty male Sprague-Dawley rats weighing 180–220 g were purchased from the Experimental Animal Center in Shandong Province (Jinan, China) and all experiments were approved by the Animal Ethics Committee of China Pharmaceutical University. The animals were randomly housed in cages at room temperature ($25 \pm 2\,^{\circ}C$) under a 12-h light/dark cycle (lights on at 7:00 a.m.) with free access to food and water. The rats were allowed to adapt for 1 week before the beginning of the study. The animals were randomly divided into five groups (n = 12 per group), as follows: Group I (sham), Group II (OB model), Group III (OB + amitriptyline [10 mg/kg]), Group IV (OB + sarsasapogenin [20 mg/kg]), and Group V (OB + sarsasapogenin [40 mg/kg]) (Fig. 1). The doses of sarsasapogenin and amitriptyline were those used in previous studies [17]. These doses have been previously shown to have significant anti-depressive effects. All animal experiments were performed in accordance with the Provision and General Recommendation of Chinese Experimental Animals Administration Legislation. Every effort was made to minimise animal suffering.

### 2.3. Surgical procedure (olfactory bulb ablation)

The bilateral olfactory bulb ablation procedure was adapted from a previous study [18]. Briefly, the rats were anesthetised using chloral hydrate (10%, 3.3 mL/kg, intraperitoneal). Using sterile surgical equipment, the skull covering the olfactory bulbs was exposed using a midline incision and 2 holes (diameter = 2 mm) were drilled 8 mm anterior to the bregma and 2 mm lateral to the midline. Both olfactory bulbs were aspirated, the holes on the skull were filled with glass-ionomer cement, and the scalp was sutured. Sham-operated rats underwent the same surgical procedure but did not undergo aspiration of the bulbs. The rats received intramuscular (0.2 mL/300 g) penicillin ($8 \times 10^5$ U) once a day for 3 days post-surgery to prevent infection. They were subsequently housed individually in polypropylene cages. The experiments started 14 days after surgery. As depicted in Fig. 1, drug treatment began 14 days after surgery (1st–14th day, recovery period) and continued for a period of 14 days (15th–28th day, treatment period). After behavioural testing, all animals were sacrificed and the extent of the lesion was assessed. OB animals that had incomplete removal of olfactory bulbs or damage to other brain areas were excluded from subsequent analyses according to previously described criteria (i.e., less than 15% of tissue removed) [19].

### 2.4. Sucrose preference

All rats were provided with a 1% (w/v) sucrose solution for 24 h. After the initial 24-h period, rats had free access to both the sucrose solution and fresh water. After 48 h, all animals were food- and water-deprived for 23 h and were then provided with access to both water and a 1% sucrose solution for 1 h. Sucrose preference was calculated as follows: sucrose preference (%) = sucrose intake (g)/[sucrose intake (g) + water intake (g)] × 100.

### 2.5. Open field test

The open field test was performed as described by Kulkarni et al. [20]. The apparatus consisted of a four-sided 100 × 100 × 40 cm metallic cage, whose floor was divided into 16 small sections by red lines. Each rat was placed alone in the centre of the cage and allowed to explore the open field for 5 min. During this period, the