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Research report

Evaluation of the antidepressant- and anxiolytic-like activity of α-spinasterol, a plant derivative with TRPV1 antagonistic effects, in mice

Katarzyna Socała*, Piotr Wlaź

Department of Animal Physiology, Institute of Biology and Biochemistry, Maria Curie-Skłodowska University, Lublin, Poland

HIGHLIGHTS

- α-Spinasterol exerted antidepressant-like effect in mice.
- α-Spinasterol was devoid of anxiolytic-like effect in mice.
- No hyperthermia after α-spinasterol administration was observed.
- TRPV1 receptors may represent a new molecular target for treatment of depression.

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ABSTRACT

The transient receptor potential vanilloid 1 (TRPV1) receptor has recently gained attention as a new molecular target in the treatment of mental disorders such as depression and anxiety. α-Spinasterol is a plant steroid that acts as a TRPV1 antagonist. The present study was undertaken to evaluate the antidepressant- and anxiolytic-like properties of α-spinasterol in mice. The obtained results showed that α-spinasterol (at doses of 1 and 2 mg/kg) exerted anti-immobility effect in mice subjected to the forced swim test. Furthermore, co-administration of an ineffective dose of α-spinasterol (0.5 mg/kg) with an ineffective dose of another TRPV1 antagonist – capsazepine (50 μg/mouse) produced a synergistic effect in the forced swim test. This compound was, however, devoid of anxiolytic-like effects in the elevated plus maze (at doses of 0.5–2 mg/kg) and the light/dark box test (at a dose of 2 mg/kg) in mice. Of note, α-spinasterol did not produce significant changes in body temperature and did not alter spontaneous locomotor activity in mice. The present study adds further support to the thesis that antagonism of the TRPV1 receptors may produce antidepressant effects. α-Spinasterol may represent a new therapeutic approach towards the development of novel antidepressant therapy. However, further detailed studies on the antidepressant potential of α-spinasterol are warranted.

1. Introduction

Depression and anxiety are highly prevalent mental disorders that contribute significantly to the global burden of disease [1]. Depression, with an approximately 20% lifetime incidence, is characterized by enduring sadness, loss of interest, low self-esteem, hopelessness, feeling of guilt, insomnia, decreased appetite and libido, recurrent thoughts of death and many others symptoms [2]. Anxiety may occur as a separate disorder or it co-occurs with depression. Lifetime prevalence for anxiety disorders ranges from 5.2% to 31%. Several different types of anxiety disorders can be distinguished such as generalized anxiety disorder, phobias, panic disorder, post-traumatic stress disorder or obsessive-compulsive disorder [1,3].

Both depression and anxiety represent a severe medical, social and economic problem. Although several classes of antidepressant and anxiolytic drugs are available, pharmacotherapy of depression and anxiety in many individuals is not satisfactory. For instance, up to 30% of the patients do not achieve adequate response following antidepressant treatment. Moreover, the usage of antidepressant and anxiolytic drugs is frequently associated with one or more side effects [3,4]. Therefore, there is a need for the development of new

Abbreviations: DMSO, dimethyl sulfoxide; i.c.v., intracerebroventricularly; i.p., intraperitoneally; NO, nitric oxide; SEM, standard error of the mean; TRPV1, transient receptor potential vanilloid 1 receptor.

* Corresponding author. Fax: +48 81 5375901.
E-mail address: ksocala@op.pl (K. Socała).

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medications against depression and anxiety that are devoid of the limitations in efficacy, have no or less adverse effects and better safety profile [3,5]. Herbal medicines are the most commonly used forms of alternative therapies and plants continue to be a promising source of new biologically active compounds including antidepressant and anxiolytic agents [6].

α-Spinasterol is a plant-derived sterol found in a variety of plants such as spinach leaves, alfalfa, cucumber, pumpkin seeds and other plants [7]. It exerts some pharmacological effects such as antinociceptive [8,9], anti-inflammatory [10–12], cytoprotective [11], antiulcerogenic [13] and anticonvulsant [14]. Trevisan et al. [9] showed that α-spinasterol displaces [^{3}H]resiniferatoxin binding to the transient receptor potential vanilloid 1 (TRPV1) receptors in spinal cord membranes and diminishes Ca^{2+} influx mediated by capsaicin. Based on observations made by Trevisan et al. [9], α-spinasterol emerged as a novel TRPV1 antagonist with low toxicity and ability to cross the blood–brain barrier after systemic administration. Noteworthy, it is devoid of hyperthermic effects, in contrast to commonly known TRPV1 antagonists [9,14].

The TRPV1 receptors are non-selective cation channels activated by thermal stimuli (>43 °C), voltage, low pH, capsaicin, piperine, resiniferatoxin and endovanilloids (e.g., anandamide). They are involved in the perception of noxious stimuli and have been extensively studied as a therapeutic target for the development of new analgesic drugs [15,16]. Although TRPV1 receptors are predominantly expressed in peripheral sensory neurons, they were also found in various brain regions, including prefrontal cortex, hypothalamus, thalamus, locus coeruleus, amygdala, hippocampus and cerebellum [15,17]. The widespread presence of TRPV1 receptors within the brain suggests that they may be implicated in the neurobiology of many mental illnesses, including depression and anxiety. Indeed, several lines of evidence implicates TRPV1 receptors in anxiety disorder but their role in depression remains unclear [18–20].

Good safety profile and bioavailability of α-spinasterol makes it a useful tool for studying the role of TRPV1 receptors in the pathophysiology of mental disorders. Therefore, the aim of the present study was to evaluate the acute antidepressant-like effect of α-spinasterol in the forced swim test and the anxiolytic-like effects in the elevated plus maze test as well as in the light/dark test in mice. Furthermore, a synergistic effect of α-spinasterol and capsazepine (a well-known TRPV1 antagonist) in the forced swim test was evaluated. All of the tests employed in the present study are the most widely used tools for the primary screen of antidepressant and anxiolytic agents.

2. Materials and methods

2.1. Animals

The experiments were carried out on 266 naive male Albino Swiss mice weighing 23–25 g. The animals were purchased from a licensed breeder (Laboratory Animals Breeding, Ilkowice, Poland) and housed in groups of 8–10 in Makrolon cages (37 cm × 21 cm × 15 cm) at a controlled temperature (22–23 °C) and relative humidity (45–55%). They were maintained on a 12 h light–dark cycle (lights on at 6 a.m.). Tap water and food pellets (Agropol S.J., Motycz, Poland) were available ad libitum. Animals were used in the study after at least one week of acclimatization. All experiments were performed between 8 a.m. and 2 p.m, after a minimum 30-min acclimatization to the experimental room. The animals were randomly assigned to the experimental groups. Each animal was used only once.

The study was performed under experimental protocols approved by the Ethical Committee of the Medical University in Lublin. Housing and experimental procedures were conducted in accordance with the European Union Directive of 22 September 2010 (2010/63/EU) and Polish legislation concerning animal experimentation. All efforts were made to minimize animal suffering as well as the number of animals used in the study.

2.2. Treatment

α-Spinasterol (5α-Stigmasta-7,22-dien-3β-ol; also known as bessisterol, hitodesterol or α-spinasterin) purchased from TRC (Toronto Research Chemicals Inc., Canada) was used in the present study. The compound was dissolved in a 1% solution of dimethyl sulfoxide (DMSO, ICN Biomedicals, Inc., Aurora, OH, USA) in normal saline and administrated intraperitoneally (i.p.) in a constant volume of 10 ml per kg of body weight. The route of administration and the treatment time for α-spinasterol (30 min) was based on other reports [8,10,14]. In the time-course study in the light/dark test, α-spinasterol was administered 15, 30, 60 or 120 min before the test.

Capsazepine (Sigma–Aldrich) was dissolved in a 20% solution of DMSO in normal saline and administered intracerebroventricularly (i.c.v.) in a constant volume of 5 μl/mouse, at a dose of 50 μg/mouse. The injection was performed 30 min before the test according to a modified method described by Lipman and Spencer [21] with the usage of a 10 μl glass Hamilton microsyringe (type 701) with the 26 gauge needle. The needle was shortened to a length of 7 mm, sharpened and polished to a fine tip. Rigid PVC tubing was put on the needle to limit its penetration to 3 mm. The injection site was approximately 2 mm posterior to an imaginary line intersecting the posterior extent of the orbits of the eyes and 1 mm lateral to the midline. The accuracy of the i.c.v. injections by using this method was previously confirmed in separate animals by injection of Evans Blue dye and observing the distribution of the injected dye in the ventricular space. The route of administration and the treatment time for capsazepine was based on the report of Miyanohara et al. [22].

Control animals received i.p. injection of 1% DMSO or i.p. injection of 1% DMSO and i.c.v. injection of 20% DMSO, depending on the tested group. All solutions were prepared freshly before use.

2.3. Forced swim test

The test procedure was performed according to the method described by Porsolt et al. [23]. Mice were placed individually into glass cylinders (height 25 cm, diameter 10 cm) containing 11 cm of water maintained at temperature of 23–25 °C. Animals were allowed to swim for 6 min. After the initial 2 min of vigorous activity, the total duration of immobility was recorded during the last 4 min of the test. Mice were considered immobile when they stopped struggling, remained floating passively, made no attempts to escape and showed only slow limb movements necessary to keep its head above the water. Water in the beakers was regularly changed between subjects. The immobility time was recorded by a trained observer with the help of cumulative stopwatches. Data obtained in groups of 11–12 mice were expressed as means (in s) ± the standard error of the mean (SEM).

2.4. Elevated plus maze test

The elevated plus maze apparatus was made of matte black material and consisted of four arms (30 cm long and 5 cm wide) elevated 50 cm above the floor, with two arms enclosed by 15 cm high walls. The arms extended from a central platform (5 × 5 cm). The maze was illuminated by red light. Animals were placed individually on the central platform of the apparatus, facing an enclosed arm and they were allowed to explore freely. Two trained observers