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Serotonergic and noradrenergic systems are implicated in the antidepressant-like effect of ursolic acid in mice

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ABSTRACT

Ursolic acid (UA) is a natural pentacyclic triterpenoid carboxylic acid that exerts antidepressant-like effects in the tail suspension test (TST) and in the forced swimming test, and this effect was reported to be mediated by the dopaminergic system. Many studies show that currently available antidepressant agents have effects on multiple neurotransmitter systems which account for their efficacy. Therefore, this study was aimed at investigating the possible involvement of the serotonergic, noradrenergic, glutamatergic and opioid systems in the antidepressant-like effect of UA. To this end, several pharmacological agents were administered to verify their ability to influence the antidepressant-like responses elicited by UA in the TST in mice. The open-field test was used to assess the locomotor activity. The results show that the pre-treatment of mice with ρ-chlorophenylalanine (100 mg/kg, i.p., 4 days) or α-methyl-ρ-tyrosine (100 mg/kg, i.p.) but not with N-methyl-D-aspartate (0.1 pmol/mouse, i.c.v.) or naloxone (1 mg/kg, i.p.), was able to prevent the antidepressant-like effect of UA (0.1 mg/kg, p.o.). Sub-effective doses of fluoxetine (5 mg/kg, p.o.) or reboxetine (2 mg/kg, p.o.), but not ketamine (0.1 mg/kg, i.p.) or MK-801 (0.001 mg/kg, p.o.), was capable of potentiating the effect of a sub-effective dose of UA (0.001 mg/kg, p.o.) in the TST. None of the treatments affected locomotor activity. Altogether, the results show an involvement of the serotonergic and noradrenergic systems, but not the glutamatergic or opioid systems, in the antidepressant-like effect of UA.

1. Introduction

Major depression is one of the most commonly diagnosed neuropsychiatric disorders, with a worldwide lifetime prevalence of approximately 17% (Greenberg et al., 2003). By the year 2030, it is estimated that depression will account for the second greatest increase in morbidity. Therefore, it is a chronic, recurring and potentially life threatening disorder that represents a significant socioeconomic burden (Branchi et al., 2013; Mathers and Loncar, 2006).

Many studies reveal important roles for monoaminergic systems in the pathophysiology and treatment of depression. Particularly monoaminergic neurotransmitters, serotonin, norepinephrine and dopamine, exert major influence on brain circuits implicated in the regulation of mood (Dell'Osso et al., 2011; Elhwuegi, 2004; Hamon and Blier, 2013; Schildkraut, 1965). Indeed, the antidepressant pharmacotherapy is still based almost exclusively on the drugs that enhance monoamine transmitter function as selective serotonin reuptake inhibitors, selective noradrenaline reuptake inhibitors, tricyclics and monoamine oxidase inhibitors (Hamon and Blier, 2013; Nemeroff and Owens, 2002).

Besides the monoaminergic system, the glutamatergic and opioid systems have been proposed to be implicated in the etiology of depressive disorder and efforts have been done for searching novel antidepressant agents that modulate these systems (Jutkiewicz and Roques, 2012; Lang and Borgwardt, 2013). In line with this, studies have reported the antidepressant-like effect of different pharmacological agents that act as N-methyl-D-aspartate (NMDA) receptor antagonists (Machado-Vieira et al., 2012; Salvadore and Singh, 2013). Interestingly, ketamine, a non-competitive NMDA receptor antagonist, has been reported to exhibit a rapid antidepressant action in preclinical and clinical studies (Salvadore and Singh, 2013; Zarate et al., 2013). Furthermore, evidence also supports the notion that the activation of the opioid system is implicated in the mechanisms underlying the effect of antidepressant compounds (Brocardo et al., 2009; Schreiber et al., 2002).

Similar to antidepressants, a natural pentacyclic triterpenoid carboxylic acid, ursolic acid (UA), elicits an antidepressant-like effect in the tail suspension test (TST) and forced swimming test, two different behavioral tests usually performed to screen antidepressant effects of compounds. This effect was reported to be mediated, at least in part, by the dopaminergic system through the activation of dopamine D_1 and D_2 receptors (Machado et al., 2012b). Moreover,

Abbreviations: UA, ursolic acid; TST, tail suspension test; PCPA, ρ-chlorophenylalanine; NMDA, N-methyl-D-aspartate; AMPT, α-methyl-ρ-tyrosine.

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UA produces antinociceptive effect involving serotonergic system (Verano et al., 2013) as well as anti-inflammatory and antitumor activities (Liu, 1995; Lu et al., 2010; Zang et al., 2014). However, the exact mechanism and neurotransmitter systems involved in the antidepressant-like effect of UA have not been investigated.

Taking into account that many studies have shown that currently available antidepressant agents have effects on multiple neurotransmitter systems that account for their antidepressant efficacy (Hamon and Blier, 2013), the aim of this study was to explore the putative involvement of the serotonergic, noradrenergic, glutamatergic and opioid systems in the antidepressant-like effect of UA in the TST in mice.

2. Methods

2.1. Animals

Swiss mice (35–45 g, 55–60 days old) of either sex (homogeneously distributed among groups) obtained from the Central Biothery of Universidade Federal de Santa Catarina (UFSC) were used. They were housed in groups of fourteen animals per plastic cage under controlled conditions of light (lights on at 07:00 h) and temperature (20–22 °C) with free access to water and food. Mice were allowed to acclimatize to the holding room for 24 h before the behavioral procedure. All manipulations were conducted in the light phase ($n = 8$ animals per group). All procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Ethics Committee of the Institution.

2.2. Drugs

The following drugs were used: ursolic acid, DL-ρ-chlorophenylalanine ethyl ester (PCPA), fluoxetine, α-methyl-ρ-tyrosine (AMPT), N-methyl-D-aspartate (NMDA), ketamine hydrochloride, naloxone hydrochloride (Sigma Chemical Co, St. Louis, MO, USA), MK-801 hydrogen maleate (RBI, Natick, MA, USA) and reboxetine mesylate (Pfizer, SP, Brazil). UA was dissolved in distilled water with 10% of Tween 80. Fluoxetine, reboxetine and MK-801 were dissolved in distilled water, AMPT was dissolved in saline with 10% Tween 80, whereas all the other drugs were dissolved in isotonic saline solution (NaCl 0.9%).

2.3. Tail suspension test (TST)

Mice were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. The total duration of immobility was recorded during a 6 min period and a decreased immobility time is considered an antidepressant-like effect according to the method previously described (Steru et al., 1985). Mice were considered immobile only when they hang passively or stay completely motionless. The immobility time was recorded in real time by an investigator present in the behavioral room that was blind to the experimental groups.

2.4. Open-field test

In order to rule out the possibility that the alteration in the immobility time in the TST was due to interference of the locomotor activity, mice were individually submitted to the open-field test paradigm, a wooden box (40 × 60 × 50 cm) with the floor divided into 12 equal squares. The number of crossings in the squares with the four paws was registered during a period of 6 min. The floor of the open-field test apparatus was cleaned with 10% ethanol between tests (Rodrigues et al., 2002). The number of crossings was recorded in real time by an investigator present in the behavioral room that was blind to the experimental groups.

2.5. Pharmacological treatments

To assess if the antidepressant-like effect of UA in the TST involves the serotonergic system, mice were administered with PCPA (100 mg/kg, intraperitoneal injection, i.p., inhibitor of tryptophan hydroxylase, involved in the serotonin synthesis) or vehicle once daily for 4 consecutive days. Thirty minutes after the last PCPA administration, mice received vehicle or UA (0.1 mg/kg, oral administration, p.o.) and, after 60 min, were submitted to the TST or open-field test. In another set of experiments, the effect of the administration of a sub-effective dose of UA (0.001 mg/kg, p.o.), followed by the immediate administration of a sub-effective dose of fluoxetine (5 mg/kg, p.o., a selective serotonin reuptake inhibitor), was investigated 60 min later in the TST or open-field test. The sub-effective dose of fluoxetine was selected based on a dose–response curve in which mice were administered with fluoxetine (5 and 10 mg/kg, p.o.) and, after 60 min, submitted to the TST or open-field test.

In the experiments designed to study a possible role of the noradrenergic system in the antidepressant-like effect of UA, mice were pretreated with AMPT (100 mg/kg, i.p., an inhibitor of tyrosine hydroxylase, a critical enzyme for the synthesis of noradrenaline and dopamine), or vehicle 4 h before UA administration (0.1 mg/kg, p.o.). After 60 min, the behavioral tests were carried out. In another set of experiments, the effect of the administration of a sub-effective dose of UA (0.001 mg/kg, p.o.), followed by the administration of a sub-effective dose of reboxetine (2 mg/kg, p.o., a selective noradrenaline reuptake inhibitor), was investigated after 60 min in the behavioral tests. The sub-effective dose of reboxetine was selected based on a dose–response curve in which mice were administered with reboxetine (2 and 20 mg/kg, p.o.) and, after 60 min, submitted to the TST or open-field test.

To evaluate whether an inhibition of NMDA receptors is implicated in the antidepressant-like effect of UA in the TST, mice were treated with UA (0.1 mg/kg, p.o.) or vehicle and after 45 min, NMDA (0.1 pmol/mouse, i.c.v., a selective agonist of NMDA receptors) or vehicle was administered. Fifteen minutes later, the behavioral tests were carried out. In another set of experiments the effect of the combined administration of UA with ketamine was investigated. Mice were treated with a sub-effective dose of UA (0.001 mg/kg, p.o.) 30 min before the treatment with a sub-effective dose of ketamine (0.1 mg/kg, i.p., a non-competitive NMDA receptor antagonist) and the behavioral tests were performed after 30 min. We also investigated the effect of the combined administration of a sub-effective dose of UA (0.001 mg/kg, p.o.) with a sub-effective dose of MK-801 (0.001 mg/kg, p.o., a non-competitive NMDA receptor antagonist). In this protocol, MK-801 or vehicle was administered immediately after UA or vehicle and 60 min later the behavioral tests were carried out.

To investigate a possible involvement of the opioid system in the antidepressant-like effect of UA, mice were pretreated with naloxone (1 mg/kg, i.p., a nonselective opioid receptor antagonist), or vehicle, 15 min before the administration of UA (0.1 mg/kg, p.o.). Sixty minutes later the behavioral tests were performed.

All drugs were administered in a constant volume of 10 ml/kg body weight and the control animals received appropriate vehicle, except NMDA which was administered by i.c.v. route (5 μl/mouse). The dose of NMDA was chosen based on a dose–response curve carried out by our group in which NMDA caused neither overt signs of toxicity nor alteration in the locomotor activity. The administration schedule and the doses of the drugs used, including the sub-effective and effective dose of UA, were chosen based on experiments previously performed in our laboratory and literature data confirm the efficacy of the abovementioned protocols (Berrocoso et al., 2013; Bettio et al., 2012; Cunha et al., 2013a,b; Machado et al., 2008, 2012b).

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