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Hesperidin reverses cognitive and depressive disturbances induced by olfactory bulbectomy in mice by modulating hippocampal neurotrophins and cytokine levels and acetylcholinesterase activity

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ABSTRACT

Depression is a serious mental disorder that is becoming more common. To better treat patients suffering from this illness, elucidation of the underlying psychopathological and neurobiological mechanisms of depression is needed. Based on the evidence, we sought to investigate the effects of hesperidin in a model of depression induced by olfactory bulbectomy (OB). C57BL/6 mice were treated with hesperidin (50 mg/kg) and imipramine (10 mg/kg, positive control) after OB induction. The brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), interleukin 1β (IL-1β) and interleukin 6 (IL-6) levels and acetylcholinesterase activity were analyzed in the hippocampus of the mice. The behavioral parameters were also verified in the model of depression induced by OB. This study demonstrated that OB increased the pro-inflammatory cytokines levels and acetylcholinesterase activity in the hippocampus, exploratory activity in the open field test and immobility in the forced swimming test in mice. In addition, OB decreased the BDNF and NGF levels in the hippocampus, grooming time in the splash test and memory consolidation in the Morris water maze task. Treatment with hesperidin, similar to imipramine, was effective in preventing these behavioral and neurochemical alterations. We suggest that the main targets of hesperidin are pro-inflammatory cytokine modulation, helping to maintain brain plasticity and acetylcholinesterase activity regulation, which are closely linked with antidepressant-like action, as shown by behavior tests. This study demonstrated that there is a pharmacological effect of hesperidin in alterations induced by OB in mice, indicating that hesperidin could be useful as a treatment for depression.

1. Introduction

Depression is a serious mental disorder that is becoming more common. In 2005, 17% of the population around the globe was affected by this condition (Kessler et al., 2005), and it is the fourth major cause of morbidity worldwide at present and will become the second by 2020 according to the World Health Organization (Kessler et al., 2003, 2011). Depressive disorders are clinically characterized by a prevalent and persistent low mood, accompanied by inappropriate guilt, low self-esteem, hopelessness and thoughts of death or suicide (De Bodinat et al., 2010). Despite the increasing number of available antidepressants drugs, over 30% of patients do not respond to pharmacotherapy, and full remission was only able to be achieved in only half of patients (Mihaljević-Peleš et al., 2011); there is still a clear need for drugs that have improved efficacy and fewer side effects. Based on the evidence, we examined the antidepressant-like activity of the bioflavonoid hesperidin, a specific flavonoid glycoside that is predominant in citrus fruits (Yang et al., 2012). Hesperidin has been reported to possess significant anti-inflammatory, antiviral, anticancer (Gaur and Kumar, 2010), and antidepressant-like properties in mice (Antunes et al., 2014; Donato et al., 2014).

In the current study, olfactory bulbectomy (OB) has been adopted as animal model to evaluate the effects of hesperidin in mice. OB causes numerous behavioral changes, such as hyperactivity after being exposed to a novel environment, signs of anhedonia, and cognitive deficits (Harkin et al., 2003; Hendriksen

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et al., 2015). Moreover, OB could cause an increase of inflammatory reactions in several brain regions (Myint et al., 2007; Rinwa and Kumar, 2013). These changes are similar to the clinical symptoms of human depression and could be improved by repeated antidepressant treatment; therefore, this model is often used to study the pathophysiology of depression and to screen antidepressants (Eisenstein et al., 2010; Oral et al., 2013). Our present study investigated the effects of hesperidin on the brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), interleukin 1β (IL-1β) and interleukin 6 (IL-6) levels and acetylcholinesterase (AChE) activity in the hippocampus of mice. Moreover, we analyzed behavior in the open field test (OFT), forced swimming test (FST), splash test, Morris water maze task (MWMT) and object recognition test (ORT) in the model of depression induced by OB in mice.

2. Materials and methods

2.1. Animals

The experiments were conducted using male C57BL/6 mice (25–35 g, 4–6 months old). The animals were maintained at constant room temperature (21 ± 1 °C) with free access to water and food under a 12:12 h light: dark cycle (lights on at 07:00 h). The manipulations were carried out during the light portion of the cycle. All efforts were made to minimize animal suffering and to reduce the number of animals used. The procedures of this study were conducted according to the guidelines of the Committee on Care and Use of Experimental Animals Resources and with the approval of Ethical Committee for Animal Use (CEUA protocol #002/2013) of the Federal University of Pampa, Brazil.

2.2. Reagents

All reagents used in this study were purchased from Sigma Chemical Co. (St. Louis, MO, USA) with a degree of purity of 99%.

2.3. Experimental design

After undergoing an OFT, all animals were submitted to surgery to remove their olfactory bulbs or only to surgery (SHAM). The animals had 14 days of recovery and were then again subjected to an OFT after exposure to treatments. The 48 animals were divided in 6 groups. Imipramine (10 mg/kg of body weight, positive control) or hesperidin (50 mg/kg of body weight) were used as treatments. Imipramine was administered intraperitoneally (i.p.), and hesperidin was dissolved in distilled water and given by gavage (per oral, p.o.). Mice were treated with vehicle, imipramine or hesperidin once a day for two weeks. Solutions were freshly prepared each day. The controls received an identical volume of distilled water (vehicle). After finishing the treatments, the mice were subjected to behavior (we used different animal groups for each behavioral test) and biochemistry tests (Fig. 1).

2.4. Bilateral olfactory bulbectomy (OB) surgical procedure

After a 2-week acclimatization period, OB was performed according to the procedure described by Leonard and Tuite (1981). Briefly, mice were anesthetized with xylazin (20 mg/kg) in combination with ketamine (100 mg/kg) diluted in saline (0.9% NaCl) administered intraperitoneally (i.p., 10 ml/kg body weight). The skull covering the olfactory bulbs was exposed by skin incision, and two burr holes were drilled using a dentist drill. The olfactory bulbs were bilaterally aspirated using a blunt hypodermic needle (1.0–1.2 cm long and with a rounded tip of 0.80–1.2 mm in diameter) attached to a 10-ml syringe, taking care not to cause damage to the frontal cortex. Finally, the burr hole was filled with acrylic resin to avoid bleeding and contamination of the surgical site. SHAM-operations were performed in the same way, but the olfactory bulbs were left intact. After surgery, all animals were allowed to recover in a post-operative cage (maintained at 24 °C) for 3 h. After this time period, the mice were returned to their home cage. The technique was adapted (Leonard and Tuite, 1981; Van Riezen and Leonard, 1990). After behavioral testing, all animals were killed and the presence of lesions was verified. The bulbectomized animals that showed incomplete removal of olfactory bulbs or damage to other brain areas were excluded from the subsequent analysis following the criteria previously described (Jarosik et al., 2007 and Kelly et al., 1997).

2.5. Behavioral assessment

2.5.1. Open field test (OFT)

To verify the effects of imipramine and hesperidin administration on locomotor activity, animals were submitted individually to 5 min in the OFT (Insight model EP 154C) 24 h after the last treatment in the morning. The parameters observed included the distance traveled (unit: mm) (Prut and Belzung, 2003).

2.5.2. Forced swimming test (FST)

The test conducted using the method described by Porsolt et al. (1977). Briefly, the mice were individually forced to swim in open cylinders (25-cm height × 10-cm diameter) containing 19 cm of water at 25 ± 1 °C. The duration of immobility was scored during the 6 min test period as described previously (Rodrigues et al., 2002). The test occurred in the morning. Each mouse was recorded as immobile when floating motionless or making only those movements necessary to keep its head above the water.

2.5.3. Splash test

The splash test was adapted from Yalcin et al. (2005). This test evaluates grooming behavior, defined as cleaning of the fur by licking or scratching, after vaporization of a 10% sucrose solution onto the mouse's dorsal coat. The solution's viscosity prompts mice to initiate grooming behavior, with depressive symptoms characterized by an increased latency (idle time between spray and the initiation of grooming) and decreased time spent grooming (d'Audiffret et al., 2010). Latency and time spent grooming were recorded for 5 min.

2.5.4. Morris water maze task (MWMT)

The Morris water maze task (MWMT) was performed in a circular swimming pool similar to that described by Morris et al. (1982). The pool consisted of black painted fiberglass, 97 cm in diameter and 60 cm in height. For the tests, the tank was filled with water maintained at 23+2 °C. The target platform (10×10 cm^2) was made of transparent Plexiglas and was submerged 1–1.5 cm beneath the surface of the water. The starting points for the animals were marked on the outside of the pool as north (N), south (S), east (E) and west (W). Four distant visual cues (55×55 cm^2) were placed on the walls of the water maze room. The cues were positioned such that their the lower edges were situated 30 cm above the upper edge of the water tank, and in the standard setting, the position of each symbol marked the midpoint of the perimeter of a quadrant (circle¼NE quadrant, square¼SE quadrant, cross¼SW quadrant, and diamond¼NW quadrant). The apparatus was located in a room with indirect incandescent illumination. The mice were submitted to a spatial reference memory version of the water maze using a previously described protocol by Prediger et al. (2007). The training session consisted of ten consecutive trials during which the animals were left in the tank facing the wall and then allowed to swim freely to the submerged