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Neurochemical factors associated with the antidepressant-like effect of flavonoid chrysin in chronically stressed mice

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ABSTRACT

Chrysin is a flavonoid which is found in bee propolis, honey and various plants. Antidepressant-like effect of chrysin in chronically stressed mice was previously demonstrated by our group. Conversely, neurochemical factors associated with this effect require further investigations. Thus, we investigated the possible involvement of pro-inflammatory cytokines, kynurenine pathway (KP), 5-hydroxytryptamine (5-HT) metabolism and caspases activities in the effect of chrysin in mice exposed to unpredictable chronic stress (UCS). UCS applied for 28 days induced a depressive-like behavior, characterized by decrease in the time of grooming in the splash test and by increase in the immobility time in the tail suspension test. Oral treatment with chrysin (5 or 20 mg/kg, 28 days), similarly to fluoxetine (10 mg/kg, positive control), culminated in the prevention of these alterations. UCS elevated plasma levels of corticotropin-releasing hormone and adrenocorticotropic hormone, as well the tumor necrosis factor-α, interleukin-1β, interleukin-6 and kynurenine levels in the prefrontal cortex (PFC) and hippocampus (HP). UCS induced the decrease in the 5-HT levels in the HP and the increase in the indoleamine-2,3-dioxygenase, caspase 3 and 9 activities in the PFC and HP. Treatment with chrysin, similarly to fluoxetine, promoted the attenuation of these alterations occasioned by UCS. These results corroborated with the antidepressant potential of chrysin in the treatment of psychiatric diseases. Furthermore, this work indicated the association of pro-inflammatory cytokines synthesis, KP, 5-HT metabolism and caspases activities with the action exercised by chrysin in mice exposed to UCS.

1. Introduction

Initially developed as a model to screen antidepressant drugs, unpredictable chronic stress (UCS) is increasingly used as a means to investigate behavioral, endocrine and neurochemical changes underlying depression (Willner et al., 1987; Willner, 1997). Depression is a debilitating, commonly occurring, and life-threatening psychiatric disorder, with a worldwide prevalence of approximately 17% (Liu et al., 2013). Depression is the main type of affective disorders which are due to the biological, psychological, social, and other factors. In this way, the syndrome is characterized by significant and lasting low mood (Lu et al., 2015). Depression is a major cause of disability, and imposes a substantial health threat to the modern society.

Although the pathophysiology of depression is not yet fully clarified, it is known that depression is generally associated with reductions in the central monoamines levels (mainly 5-hydroxytryptamine, 5-HT), although not all depressed subjects having serotonin reductions. Furthermore, also is observed excessive stimulation of hypothalamic-pituitary-adrenal (HPA) axis, characterized by hypersecretion of corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH) and glucocorticoids, mainly cortisol (corticosterone in rodents) (Lee et al., 2010). Excessive stimulation of HPA has been associated with the production of pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6),

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that have been found to reduce the production of 5-HT by interference of cytokines in the kynurenine (KYN) pathway (KP), by activation of the tryptophan (TRP)-metabolizing enzyme indoleamine-2,3-dioxygenase (IDO) (Maes et al., 2011). In addition, it has been shown that the excessive activation of HPA axis produces neurotoxic effects in several brain regions related to depression, such as prefrontal cortex (PFC) and hippocampus (HP) (Anacker et al., 2011; Liu et al., 2014), and that stressed animals exhibit the increase of caspases activities in cerebral structures (Bachis et al., 2008), suggesting the occurrence of apoptosis in animals that present excessive stimulation of HPA axis.

Chrysin (5,7-dihydroxyflavone, Fig. 1) is a flavonoid which is found in bee propolis, honey and various plants (Barbaric et al., 2011). As a result of its effect on inhibition of the aromatase enzyme, which converts testosterone to estradiol, chrysin is commercially available as a dietary supplement (500 mg per capsule), aiming the elevation of testosterone levels. Research has shown that chrysin has multiple other biological activities, such as anti-inflammatory, antineoplastic, hipolipdemic and antioxidant (Borges Filho et al., 2013; Cho et al., 2004; Lapidot et al., 2002; Zarzecki et al., 2014). A recent study of our group demonstrated the antidepressant potential of crhysin when administered for 28 days in chronically stressed mice (Borges Filho et al., 2015). However, this study needs to be expanded for the evaluation of other neurochemical parameters strongly associated with depression.

Thus, our study investigated the possible involvement of the pro-inflammatory cytokines and 5-HT levels, KP and caspases activities in the antidepressant-like effect of chrysin treatment in female mice exposed to UCS. Our working hypothesis is that pro-inflammatory cytokines and 5-HT levels, KP, and caspases activities may be associated with the antidepressant-like effect of chrysin in mice subjected to UCS.

2. Materials and methods

2.1. Animals

Experiments were realized with 42 female C57B/6J mice (20–25 g, 90 days old). Animals were maintained at 22–25 °C, with free access to water and food, under a 12:12-h light/dark cycle (except when the stressful activity involved continuous light during 24 h), with lights on at 7:00 a.m. The procedures of this study were conducted according to the guidelines of the Committee on the Care and Use of Experimental Animal Resources and with the approval of the Ethics Commission for Animal Use (CEUA protocol # 035/2013). It is important to clarify that this work was realized in female mice because women are more susceptible to development of depressive disorder than men (Parker and Brotchie, 2010; Posmontier, 2008).

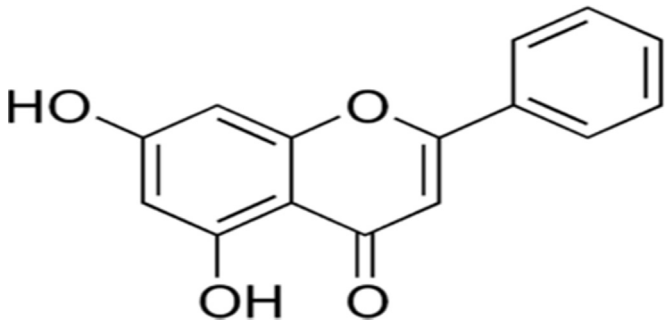

Fig. 1. Chemical structure of chrysin.

2.2. Drug solutions and administrations

Chrysin and fluoxetine were purchased from Sigma (St. Louis, MO, USA). All other chemicals used were obtained from standard commercial suppliers.

Chrysin was dissolved in a distilled water/propyleneglycol solution (80:20). Fluoxetine was dissolved in distilled water. Both drugs were administered per oral (p.o.) in the volume of 10 ml/kg. Mice were treated with chrysin at doses of 5 or 20 mg/kg, corresponding to a low dose and a high dose, respectively (Borges Filho et al., 2015), or fluoxetine at the dose of 10 mg/kg (Kumar et al., 2011). Both drugs were daily administered for 28 days, 30 min before the stressful activity (Borges Filho et al., 2015).

2.3. Experimental design and unpredictable chronic stress (UCS)

The mice were divided into eight groups ($n=5$–7): [1] Control (No stress+vehicle) {V},[2] Fluoxetine 10 mg/kg (No stress+-fluoxetine 10 mg/kg) {F10}, [3] Chrysin 5 mg/kg (No stress+chrysin 5 mg/kg) {C5}, [4] Chrysin 20 mg/kg (No stress+chrysin 20 mg/kg){C20}, [5] Stress (Stress+vehicle) {V}, [6] Stress+-fluoxetine 10 mg/kg {F10}, [7] Stress+Chrysin 5 mg/kg {C5} and [8] Stress+Chrysin 20 mg/kg {C20}. The UCS regimen was based on the procedures described by other researchers, with minor modifications (Borges Filho et al., 2015; Chen et al., 2012; Grippo et al., 2008; Liu et al., 2013, 2014; Peng et al., 2012; Zhang et al., 2012). The mice were housed in separate cages. Briefly, UCS-exposed mice were subjected to various stressors in a chronic and unpredictable way according to a random schedule for 28 days. The stressors were: damp bedding for 12 h; 45° cage tilting for 12–18 h; continuous light during 24 h; water and food deprivation for 12–18 h; strong level shaking for 5 min; electric shock foot (2 min; 0,5 mA, 3 s duration, average 1 shock/min) in an electrified grid; 2 min in the electrified grid, but without shock foot; 45 °C oven for 5 min; physically restraint for 2 h. Aleatory stressors were applied at random times in order to be completely unpredictable. All mice in the stress group were exposed to the same single stressor simultaneously in 1 day. None of stressful procedures was applied on two consecutive days. During the whole process of stress, each stressor was applied two to four times. On the 28th day, in the end of UCS, the animals were subjected to splash test (ST) and after 24 h were exposed to rota rod test and subsequently to tail suspension test (TST). After 24 h the animals were anesthetized and blood was collected by cardiac puncture and the mice were euthanized by decapitation and PFC and HP were dissected (Fig. 2).

2.4. Behavioral assessment

2.4.1. Splash test (ST)

This test was performed on the 28th day of stressful protocol, 24 h after the last stressing procedure, and consisted of squirting 200 μl of a 10% sucrose solution on the mouse's snout. Because of its viscosity, the sucrose solution dirties the mouse fur and animals initiate grooming behavior. After applying sucrose solution, the time spent for grooming was recorded in s for a period of 5 min. A decrease in grooming, which is a particular feature of mice submitted to stress was used as an index of self-care and motivational

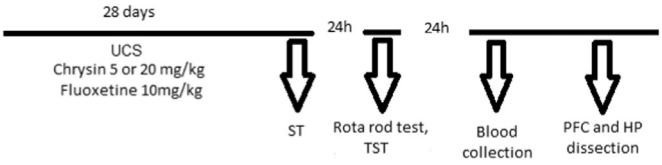

Fig. 2. Experimental design.