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Kappa-opioid receptors mediate the antidepressant-like activity of hesperidin in the mouse forced swimming test

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ABSTRACT

The opioid system has been implicated as a contributing factor for major depression and is thought to play a role in the mechanism of action of antidepressants. This study investigated the involvement of the opioid system in the antidepressant-like effect of hesperidin in the mouse forced swimming test. Our results demonstrate that hesperidin (0.1, 0.3 and 1 mg/kg; intraperitoneal) decreased the immobility time in the forced swimming test without affecting locomotor activity in the open field test. The antidepressant-like effect of hesperidin (0.3 mg/kg) in the forced swimming test was prevented by pretreating mice with naloxone (1 mg/kg, a nonselective opioid receptor antagonist) and 2-(3,4-dichlorophenyl)-*N*methyl-*N*-[(1*S*)-1-(3-isothiocyanatophenyl)-2-(1-pyrrolidinyl)ethyl] acetamide (DIPPA (1 mg/kg), a selective κ-opioid receptor antagonist), but not with naloxone methiodide (1 mg/kg, a peripherally acting opioid receptor antagonist), naltrindole (3 mg/kg, a selective δ-opioid receptor antagonist), clocinnamox (1 mg/kg, a selective μ-opioid receptor antagonist) or caffeine (3 mg/kg, a nonselective adenosine receptor antagonist). In addition, a sub-effective dose of hesperidin (0.01 mg/kg) produced a synergistic antidepressant-like effect in the forced swimming test when combined with a sub-effective dose of morphine (1 mg/kg). The antidepressant-like effect of hesperidin in the forced swimming test on mice was dependent on its interaction with the κ-opioid receptor, but not with the δ-opioid, μ-opioid or adenosinergic receptors. Taken together, these results suggest that hesperidin possesses antidepressant-like properties and may be of interest as a therapeutic agent for the treatment of depressive disorders.

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1. Introduction

The monoamine deficiency hypothesis has been proven to be a typical model of the pathophysiology of depression. Currently, antidepressants are designed based on this hypothesis, as most of them inhibit the reuptake of monoamines (Nutt, 2008). Thus, intense research is underway to identify novel targets for antidepressant therapy. It has been well established that opioids are associated with mood regulation and, consequently, with depressive disorders (Berrocoso et al., 2009). Clinical findings indicate that depressed patients displayed a deficiency of endogenous opioid activity, while manic patients display excess opioid activity (Rahman, 2010). Other evidence also implies a role for the opioid system in the etiopathogenesis of depression, as traditional depressant compounds seem to indirectly modulate opioid neurotransmission (Berrocoso and Mico, 2009).

The biological effects of endogenous opioid peptides are mediated through three classes of naloxone-sensitive opioid receptors: mu (μ), kappa (κ), and delta (δ) (Law et al., 2000). μ-Opioid receptor agonists, with morphine [(5α,6α)-7,8-didehydro-4,5epoxy-17-methylmorphinan-3,6-diol] as the prototype, provide a gold standard for the treatment of pain (Gutstein and Akil, 2006). δ-Opioid receptor agonists can also produce analgesia, but they have been known to cause convulsions as a side effect, which has limited their use in the pharmacological industry. The endogenous κ-opioid receptor system is involved in a number of physiological processes, rendering it opportunistic for drug development (Aldrich and McLaughlin, 2009; Wang et al., 2010). Some studies have also indicated antidepressant-like effects in the forced swimming test following the administration of mianserin, mirtazapine, PF-04455242 and Trp isomers of CJ-15,208, which are known to modulate κ-opioid receptor activity (Carr et al., 2010; Grimwood et al., 2011; Olianas et al., 2012; Ross et al., 2012).

Adenosine is an important neuroactive nucleoside that exerts two parallel effects on the central nervous system, serving as a homeostatic modulator and as a modulator at the synaptic level (Cunha, 2001). Adenosine modifies the release of neurotransmitters,

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post-synaptic responsiveness and the action of a number of other neurotransmitter systems (Ribeiro et al., 2003). In addition, adenosine also modulates cognitive states and is associated with affective and mood disorders, such as anxiety and depression (Mendonça et al., 2000; Ribeiro et al., 2003; Kaster et al., 2004, 2005).

Hesperidin (4′-methoxy-7-*O*-rutinosyl-3′,5-dihydroxyflavanone), a naturally occurring flavanone glycoside, is predominantly found in citrus fruits (Yang et al., 2012). Hesperidin has also been described to have other therapeutically useful properties, such as antioxidant (Ahmad et al., 2012; Yang et al., 2012), neuroprotective (Hwang and Yen, 2008) and anticancer (Cho, 2006; Lee et al., 2010) activities. The mechanisms responsible for the sedative and antinociceptive effects of hesperidin in mice act through the opioid system (Loscalzo et al., 2008, 2011).

Considering the need for novel compounds that could improve conventional therapies, the primary aim of the present study was to investigate the antidepressant-like effect of hesperidin as a potential treatment for associated disorders in the forced swimming test in mice. The second objective of this study was to investigate the involvement of opioid and adenosinergic receptors in the antidepressant-like activity of hesperidin in the forced swimming test in mice.

2. Materials and methods

2.1. Animals

The behavioral experiments were conducted using male adult Swiss mice (25–35 g) that were maintained at 22–25 °C with free access to water and food, under a 12 h light/dark cycle, with lights on at 6:00 a.m. All manipulations were conducted between 08.00 a.m. and 04.00 p.m. All experiments were performed on separate groups of animals, and each animal was used only once in each test. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, the Federal University of Santa Maria, Brazil. All possible efforts were made to minimize suffering and reduce the number of animals used in the experiments.

2.2. Chemicals

Hesperidin (Sigma–Aldrich Chemical Co, USA) was dissolved by the sequential addition of dimethylsulfoxide (final concentration of 5%), a water solution of 0.25% Tween 80 (final concentration of 20%) and saline. Hesperidin doses were chosen based on our previously published data (Fernández et al., 2005) and on preliminary experiments.

The following drugs were used in this study: naloxone, naloxone methiodide, naltrindole, clocinnamox, 2-(3,4-dichlorophenyl)-*N*methyl-*N*-[(1*S*)-1-(3-isothiocyanatophenyl)-2-(1-pyrrolidinyl)ethyl] acetamide (DIPPA), caffeine and morphine (Sigma–Aldrich Chemical Co, USA).

All drugs were dissolved in saline, except clocinnamox and DIPPA which were diluted in saline with 10% dimethylsulfoxide. Groups were also simultaneously treated with the appropriate vehicle. The drugs or vehicle were administered by intraperitoneal (i.p.) injection in a constant volume of 10 ml/kg, except for morphine and naloxone methiodide, which were administered by subcutaneously (s.c.).

2.3. Antidepressant-like activity evaluation

To assess the antidepressant-like effect of hesperidin, the compound was administered (dose range: 0.01–1 mg/kg, i.p.) 30 min before the forced swimming test or the open-field test. Fluoxetine (32 mg/kg, i.p.) and imipramine (15 mg/kg, i.p.) were used as positive controls (Kaster et al., 2007), and they were administered 30 min prior to the forced swimming test. To evaluate locomotor and exploratory activities, mice were treated with hesperidin (0.01–1 mg/kg, i.p.) 30 min before the open-field test (Loscalzo et al., 2011).

Previously published studies were used to determine the doses of antagonists, which demonstrated effects in pharmacological and biochemical studies, and the doses of antidepressants, which did not modify the basal response in the forced swimming test or locomotor activity in the open-field test. Several authors have confirmed the selectivity and efficacy of these antagonists and antidepressants at the chosen doses (Berrocoso et al., 2004; Brocardo et al., 2009).

To test the hypothesis that the antidepressant-like effect of hesperidin is mediated through an interaction with the opioid system, animals were pretreated with the nonselective opioid receptor antagonist, naloxone (1 mg/kg, i.p., a dose that produces no effect in the forced swimming test), naloxone methiodide, a nonselective opioid receptor antagonist that does not cross the blood–brain barrier (1 mg/kg, s.c., a dose that produces no effect in the forced swimming test) or vehicle. Thirty minutes after receiving the indicated opioid receptor antagonists or vehicle, the mice received hesperidin (0.3 mg/kg, i.p.) and were subjected to the forced swimming test and open-field test (Berrocoso et al., 2004; Kaster et al., 2007).

In another series of experiments, we investigated the involvement of μ-, δ-, and κ-opioid receptor subtypes in the antidepressant-like effect of hesperidin (0.3 mg/kg, i.p.) in the forced swimming test. To this end, mice were pretreated with the selective δ-opioid receptor antagonist, naltrindole (3 mg/kg, i.p., a dose that produces no effect in the forced swimming test; Kaster et al., 2007), a selective μ-opioid receptor antagonist, clocinnamox (1 mg/kg, i.p., a dose that produces no effect in forced swimming test; Kaster et al., 2007), or the selective κ-opioid receptor antagonist DIPPA (1 mg/kg, i.p., a dose that produces no effect in the forced swimming test; Kaster et al., 2007). Thirty minutes after the administration of the indicated opioid receptor antagonist, the mice received hesperidin (0.3 mg/kg, i.p.) and were subjected 30 min later to the forced swimming test and open-field test.

To assess the possible involvement of the adenosinergic system in the antidepressant-like effect of hesperidin in the forced swimming test, independent groups of animals were pretreated with caffeine (3 mg/kg, i.p., a nonselective adenosine receptor antagonist) or vehicle (Lobato et al., 2008). Thirty minutes after receiving caffeine or vehicle, the mice received hesperidin (0.3 mg/kg, i.p.) or vehicle and were subjected to the forced swimming test and open-field test.

In another set of experiments, a sub-effective dose of morphine (1 mg/kg, s.c.) was administered 20 min before the administration of hesperidin (0.01 mg/kg, i.p.). Fifteen minutes later, the forced swimming test or open-field test was conducted. The doses of both morphine and hesperidin were shown to be sub-effective based on dose–response curves of these compounds in the forced swimming test (Brocardo et al., 2009).

2.3.1. Forced swimming test

The test was conducted using the method previously described by Porsolt et al. (1977). Briefly, individual mice were 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). Each mouse was recorded as being immobile when floating motionless or making only those movements necessary to keep its head above water.