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Guanosine prevents behavioral alterations in the forced swimming test and hippocampal oxidative damage induced by acute restraint stress

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ABSTRACT

Guanosine is a guanine-based purine that modulates glutamate uptake and exerts neurotrophic and neuroprotective effects. In a previous study, our group demonstrated that this endogenous nucleoside displays antidepressant-like properties in a predictive animal model. Based on the role of oxidative stress in modulating depressive disorders as well as on the association between the neuroprotective and antioxidant properties of guanosine, here we investigated if its antidepressant-like effect is accompanied by a modulation of hippocampal oxidant/antioxidant parameters. Adult Swiss mice were submitted to an acute restraint stress protocol, which is known to cause behavioral changes that are associated with neuronal oxidative damage. Animals submitted to ARS exhibited an increased immobility time in the forced swimming test (FST) and the administration of guanosine (5 mg/kg, p.o.) or fluoxetine (10 mg/kg, p.o., positive control) before the exposure to stressor prevented this alteration. Moreover, the significantly increased levels of hippocampal malondialdehyde (MDA; an indicator of lipid peroxidation), induced by ARS were not observed in stressed mice treated with guanosine. Although no changes were found in the hippocampal levels of reduced glutathione (GSH), the group submitted to ARS procedure presented enhanced glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD) activities and reduced catalase (CAT) activity in the hippocampus. Guanosine was able to prevent the alterations in GPx, GR, CAT activities, and in SOD/CAT activity ratio, but potentiated the increase in SOD activity elicited by ARS. Altogether, the present findings indicate that the observed antidepressant-like effects of guanosine might be related, at least in part, to its capability of modulating antioxidant defenses and mitigating hippocampal oxidative damage induced by ARS.

1. Introduction

Stressful life experiences are recognized as a major risk factor for cardiovascular, metabolic and neuropsychiatric diseases (Musazzi et al., 2010). Among the stress related psychiatry disorders, a great amount of evidence indicates the involvement of stressful events with major depression, a debilitating disease with high prevalence worldwide (Krishnan and Nestler, 2008; Paykel, 2003). This observation can be explained by the occurrence of morphological remodeling as well as molecular, neurochemical and electrophysiological changes associated with stress that can lead to the cognitive deficits observed in depressive patients (Herman and Cullinan, 1997). In this scenario, the hippocampus, an encephalic area that plays a key role in learning and memory, is also deeply involved in the pathophysiology of depression and in the action of antidepressant drugs (Kim et al., 2012; McEwen, 1999, 2000). Due to its high levels of glucocorticoid receptors (the main intracellular mediators of stress response), this brain structure is particularly sensitive to stressful events (McEwen, 2005). Moreover, the hippocampus is interconnected and exerts synaptic influence in the prefrontal cortex and amygdala, which are also key structures implicated in depression (Degenetais et al., 2003; Ishikawa and Nakamura, 2003; Price and Drevets, 2010). This integrated circuitry suggests that the hippocampus is functionally related with these structures. Therefore, an impairment in its function caused by stress may cause neurochemical dysfunctions in neuroplasticity pathways in distinct corticolimbic regions (Godsil et al., 2013).

The acute exposition to a stressful event and the consequent increase in circulating levels of glucocorticoids induce a rapid depolarization-evoked glutamate release in cortical and limbic regions (Lowy et al.,

Abbreviations: ANOVA, analysis of variance; ARS, acute restraint stress; CAT, catalase; CNS, central nervous system; FST, forced swimming test; GSSG, glutathione disulfide; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; H_2O_2, hydrogen peroxide; OH•, hydroxyl radical; HO-1, heme-oxygenase-1; MDA, malondialdehyde; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NPSH, non-protein thiols; Nrf-2, nuclear factor (erythroid-derived-2)-like 2; $O_2^{\bullet-}$, superoxide anion radical; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances.

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1995; Musazzi et al., 2010). This neurotransmitter displays an important role in neuronal plasticity through the activation of its synaptic receptors (Mattson, 2008). However, excessive levels of glutamate can cause an overstimulation of extrasynaptic receptors, leading to mitochondrial dysfunction, impairment in the cellular calcium homeostasis and generation of reactive oxygen species (ROS) (Hardingham et al., 2002; Reynolds and Hastings, 1995).

The precursor of most ROS is superoxide anion ($O_2^{\bullet-}$) a product of the reduction of oxygen by one electron. Its dismutation produces hydrogen peroxide (H_2O_2), which in turn may be fully reduced to water or partially reduced to hydroxyl radical (OH•), one of the strongest oxidants in nature (Turrens, 2003). Although ROS participate in normal cellular processes, they may cause damage to cell components including proteins, nucleic acids, carbohydrates and lipids when associated with an imbalance in antioxidant capacity (Hovatta et al., 2010). Human defenses against excessive ROS generation include the enzyme superoxide dismutase (SOD), which accelerates the dismutation of $O_2^{\bullet-}$ into H_2O_2 and molecular oxygen. Thereafter, H_2O_2 is decomposed by glutathione peroxidase (GPx) or by catalase (CAT) (Turrens, 2003). Besides, many different agents are involved in the nonenzymatic detoxification of ROS including glutathione (GSH), the main nonprotein thiol of the mammalian cell. This tripeptide protects cells by serving as a substrate in the cytosolic redox cycle or directly inactivating ROS such as $O_2^{\bullet-}$ or OH• (Knapen et al., 1999).

Due to its high rate of oxygen consumption, the central nervous system (CNS) is especially vulnerable to excessive generation of free radicals (Lee et al., 2012) and oxidative damage to neurons has been implicated in the pathogenesis of depression (Zhang and Yao, 2013). Therefore, the involvement of ROS-mediated pathways and lowered levels of antioxidants in the pathogenesis of depression have been target of several investigations, some of which have pointed to neuroinflammation and altered neurogenesis/neuroplasticity as pivotal phenomena (Maes et al., 2011a). Depressive patients have significantly elevated plasma levels of peroxide in comparison with normal controls (Maes et al., 2010) as well as higher levels of malondialdehyde (MDA), an indicative of the occurrence of oxidative stress (Ozcan et al., 2004; Sarandol et al., 2007). As mentioned above, this excessive ROS production can be neutralized by different antioxidant enzymes like superoxide dismutase (SOD), glutathione peroxidase (GPx) or catalase (CAT), which have been reported to be altered in depressive patients (Galecki et al., 2009; Maes et al., 2011b; Ozcan et al., 2004; Sarandol et al., 2007).

Supporting this hypothesis, there is increasing evidence demonstrating that current antidepressants used in clinical practice may exert their therapeutic effect by regulating oxidative stress (Herken et al., 2007; Khanzode et al., 2003; Kotan et al., 2011). Taking this into account, it is not surprising that novel antidepressant targets are being considered for investigation based on their antioxidant properties (Lee et al., 2012), since conventional antidepressants present side effects and may afford incomplete remission (Katalinic et al., 2013; Niciu et al., 2014).

In this context, guanosine, a guanine-based nucleoside that exhibits protective effects against glutamate excitotoxicity (Frizzo et al., 2002; Vinade et al., 2005) and oxidative stress (Dal-Cim et al., 2012; Petronilho et al., 2012; Tarozzi et al., 2010), represents an interesting molecule for investigation. In fact, we recently demonstrated that the administration of this nucleoside produces an antidepressant-like effect in the tail suspension test (TST) and in the forced swimming test (FST) similar to the one produced by fluoxetine (Bettio et al., 2012); however, the involvement of this effect with hippocampal anti-/pro-oxidative events has not yet been investigated. Considering this background, the present study investigated the protective effect of guanosine against behavioral alterations in the forced swimming test and hippocampal antioxidant imbalance induced by the acute restraint stress (ARS) protocol (Kumar et al., 2010; Moretti et al., 2013; Freitas et al., 2014).

2. Materials and methods

2.1. Animals

Female Swiss mice (35–45 g), maintained at 20–22 °C with free access to water and food and under a 12:12 h light/dark cycle (lights on at 7:00 a.m.) were used. The animals were caged in groups of 15 in a 41 × 34 × 16 cm cage. All behavioral tests were carried out between 9:00 a.m. and 5:00 p.m. Mice were used according to the NIH Guide for the Care and Use of Laboratory Animals and the experiments were performed after approval of the protocol by the Ethics Committee of the Institution. All efforts were made to minimize animal suffering and to reduce the number of animals used in the experiments.

2.2. Drugs and treatment

Guanosine and fluoxetine (obtained from Sigma Chemical Co., St. Louis, USA) were dissolved in distilled water and administered orally (p.o.) at a dose of 5 and 10 mg/kg, respectively, 1 h before the ARS procedure. The doses used were chosen based on previous studies (Bettio et al., 2012; Moretti et al., 2013). Solutions were freshly prepared before administration and administered in a volume of 10 ml/kg. The nonstressed group (control) received distilled water by p.o. route (10 ml/kg). To develop this study, mice were divided into four groups as follows: (1) nonstressed + vehicle; (2) nonstressed + guanosine; (3) stressed + vehicle; and (4) stressed + guanosine. The number of mice per group was 7–9. Fluoxetine was added as a positive control in the forced swimming test (FST) and open field test in another set of experiments.

2.3. Acute restraint stress procedure

ARS protocol was performed as previously described (Freitas et al., 2014; Moretti et al., 2013) 1 h after the treatment with vehicle, guanosine or fluoxetine. The immobilization was applied for a period of 7 h using an individual rodent restraint device made of Plexiglas fenestrate, restraining all physical movement without causing pain. The animals were deprived of food and water during the entire period of exposure to stress. Non-stressed groups (controls) were treated with vehicle or guanosine and were kept without food and water for 7 h (the same time period of stress). After this time period, independent groups of mice were released from their enclosure and 40 min post-release were submitted to the FST, to the open field test and were sacrificed for the biochemical studies. The control group was submitted to the same protocol, except that it was not submitted to stress.

2.4. Behavioral tests

2.4.1. Forced swimming test

Briefly, mice were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), containing 19 cm of water (depth) at 25 ± 1 °C; the total duration of immobility was measured during a 6-min test period by observers blind to the treatment conditions. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water (Porsolt et al., 1977).

2.4.2. Open field test

To assess possible interferences on locomotor activity, mice were evaluated in the open field paradigm as previously described. Animals were individually placed in a wooden box (40 × 60 × 50 cm) with the floor divided into 12 equal squares and the number of crossings with all paws was manually counted in a 6-min session. The light was maintained at minimum to avoid anxiety behavior and the apparatus was cleaned with a solution of 10% ethanol between tests in order to hide animal clues (Budni et al., 2013; Moretti et al., 2013).