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Research article

Royal jelly decreases corticosterone levels and improves the brain antioxidant system in restraint and cold stressed rats

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HIGHLIGHTS

- Restraint and cold stress increases the plasmatic corticosterone levels.
- Restraint and cold stress worsens the antioxidant defense system in the brain tissues.
- Royal jelly decreases plasmatic corticosterone levels.
- Royal jelly improves the antioxidant defense system in the brain tissues.

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ABSTRACT

Restraint and cold stress induces the hypothalamic-pituitary-adrenal (HPA) axis to release corticosterone from the adrenal gland, which can worsen the antioxidant defense system in the central nervous system. Here, we investigated the corticosterone levels and the antioxidant defense system in the cerebellum and brain, as well as in its isolated regions, such as cerebral cortex, striatum and hippocampus of stressed rats supplemented with royal jelly (RJ). Wistar rats were supplemented with RJ for 14 days and the stress induction started on the 7th day. Stressed rats increased corticosterone levels, glycemia and lipid peroxidation in the brain and cerebellum, cerebral cortex and hippocampus besides reduced glutathione defense system in the brain and striatum. Rats supplemented with RJ decreased corticosterone, maintained glycemia and decreased lipid peroxidation in the brain, cerebellum, as well as striatum and hippocampus, besides improved glutathione defense system in cerebral cortex and striatum. This study suggests an anti-stress and neuroprotective effect of RJ under stress conditions.

1. Introduction

Stress activates the hypothalamic–pituitary–adrenal (HPA) axis, releasing glucocorticoids from the adrenal gland [42], which may induces an activation of the cellular reduction–oxidation (redox) system [39]. An increase in blood corticosterone levels has already been verified in models of stress induction, such as restraint and cold [34,44]. Moreover, a prolonged elevation of corticosterone levels can leads to oxidative stress [45], decreasing the levels of antioxidative enzymes and promoting damage to the central nervous system (CNS) [27].

When compared to other tissues, brain tissue is more vulnerable to oxidative stress due to a higher oxygen consumption rate, higher levels of peroxidizable lipids and low levels of antioxidants [1,16,46]. Oxidative stress in the CNS is related to the etiopathogenesis of several diseases, including neurodegeneratives and depression [9,24,47]. Oxidative stress is also involved in the increase of impairments in brain tissue related to cognitive performance and memory space [33].

Abbreviations: RJ, royal jelly; HPA, hypothalamicpituitary-adrenal; CNS, central nervous system; TBARS assay, thiobarbituric acid reactive substances; FRAP assay, ferric reducing antioxidant power; RIA, radioimmunoassay; ROS, reactive oxygen species; SOD, superoxide dismutase; CAT, catalase; GST, glutathione-S-transferase; G_R, glutathione reductase; GPX, glutathione peroxidase; GSH, reduced glutathione; GSSG, oxidized glutathione; G6PDH, glucose-6-phosphate dehydrogenase.

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Although stress-induced brain damage has already been observed in several stress models, oxidative damage triggered by restraint and cold stress in isolated brain regions has been less investigated. One of the few studies using immobilization stress showed an increase of lipid peroxidation in the cerebral cortex, cerebellum, hippocampus, and midbrain compared to the non-stressed controls [23]. Another study showed that chronic mild stress produces an imbalance between SOD and catalase (CAT) in different brain regions [24], indicating that the stress worsened the enzymatic antioxidant defense system. It is well documented that the brain has a low or moderate activity of SOD and CAT [7,14], whereas glutathione is the major antioxidant system of the brain. This system is involved in the disposal of peroxides by brain cells and in protection against reactive oxygen species (ROS) [8]. However, glutathione system has been less investigated, making it difficult to understand how stress changes this important antioxidant defense.

Owing to the fact that oxidative stress damages the brain, several antioxidant products have been used to improve the antioxidant system. Previous study showed that royal jelly (RJ), secreted by the hypopharyngeal and mandibular glands of young worker bees (*Apis mellifera*) [22], has antioxidant properties [30], hypocholesterolemic action [18] and also shows neurotrophic effects [12]. This secretion has in its composition proteins, free amino acids, sugars, vitamins (B1, B2, B6, folic acid, pantothenic acid, nicotinic acid and biotin) and lipids such as 10-hydroxy-2-decenoic acid (HDA-10) [22,36,41].

Thus, the aims of this study were: 1- to assess if restraint and cold stress worse the glutathione defense system in the cerebellum and brain, as well as in its isolated regions, such as cerebral cortex, striatum and hippocampus; 2- to evaluate if RJ supplementation reduces the corticosterone levels and improves the antioxidant defense system in those nervous tissues of stressed rats.

2. Material and methods

2.1. Royal jelly

The sample of fresh RJ was supplied by a local distributor of apicultural products (Apiário Girassol, Uberlândia, MG, Brazil). This sample was transported on ice to the laboratory and stored in the freezer at −20 °C until the biological assay was performed. Fresh RJ samples was diluted with distilled water daily at a concentration of 200 mg/kg and given orally to animals with a gavage needle.

2.2. Animals

Male Wistar rats (207–250 g) were kept under standard conditions (22 ± 1 °C, humidity 60 ± 5%, 12h-light/12h-dark cycle) with free access to food and water. We followed the procedures for handling and use of the animals as proposed by the Brazilian Society of Laboratory Animal Science and by the Ethics Committee for Animal Research of the Federal University of Uberlandia, Brazil (CEUA/UFU-047/14).

2.3. Experimental design

Animals were allocated into three groups (n = 10 rats/group): No Stress (NS), Stress (S) and Stress supplemented with RJ (S + RJ). NS rats received vehicle by gavage for 14 days and were kept without any contact with restraint and cold stress. S rats received vehicle by gavage for 14 days and were submitted to restraint and cold stress after the 7th day of gavage. S + RJ rats were supplemented with RJ (200 mg/kg) by gavage for 14 days and were submitted to restraint and cold stress after the 7th day of supplementation. The groups submitted to restraint and cold stress received vehicle or RJ supplementation 45 min prior stress induction.

On the 14th day, S and S + RJ animals were subjected to the two stressors simultaneously for 2 h. Immediately after the stress session, all groups were anesthetized by an intraperitoneal injection of xylazine (20 mg/kg body weight) and ketamine (90 mg/kg body weight) [2]. Blood samples were collected and the rats were euthanized.

2.4. Stress procedure

Chronic stress protocol was carried out according to Paula-Freire et al. [34]. The chronic stress was induced by restraint for 2 h in the morning (9:00–10:00 a.m.) and cold for 2 h in late afternoon (4:00–6:00 p.m.). During restriction in acrylic restrainers (45 mm × 52 mm × 205 mm), the rats could slightly move forward and back in the restrainer but could not turn his head to tail, experiencing a tight position. During cold stress, the rats were allocated in individual clean cages without sawdust at 10 °C.

2.5. Glucose and corticosterone quantification

On the seventh day of stress induction, glucose levels were measured before and after the last session of stress by tail vein puncture, using reactive strips (n = 10rats/group) (Accu-Chek Performa; Roche Diagnostic Systems, Basel, Switzerland). For corticosterone evaluation, blood samples (n = 10rats/group) were centrifuged at 1500 × *g* at 4 °C for 10 min. Serum samples were used for corticosterone analysis by radioimmunoassay (RIA) [37]. The RIA used H3-corticosterone from NEN Life Science Products (Boston, USA) and a standard reference specific antibody from Sigma (St. Louis, MO, USA). Corticosterone was used to measure tritiated recovery. The intra-assay error was 4.5% and the minimum detectable dose was 0.08 ng/mL.

2.6. Tissue preparation

2.6.1. Brain dissection

The brain was removed after cutting the skull with an incision from the base up to the olfactory lobes. The brain was then washed out with deionized water to remove as much as possible of extraneous matter. Then, dissection of the brain regions was conducted on ice using established guidelines [10]. First the rhombencephalon was separated by a transverse section from the rest of the brain and the cerebellum was dissected. A sagittal section was made to separate the two lobes, then the hippocampus was dissected, as well as the striatum, which was dissected with the external walls of the lateral ventricles as internal limits and the corpus callosum as external limits. To obtain the cerebral cortex, the midbrain was gently separated and the remaining part was collected.

2.6.2. Homogenization tissues

Cerebellum, cerebral cortex, striatum and hippocampus (n = 4rats/group) were homogenized in 20 mM sodium phosphate buffer, containing 140 mM KCl, pH 7.4 (1:10 w/v). Homogenates were centrifuged at 800 × *g* for 10 min at 4 °C. Sediments were discarded and supernatants were collected for the quantification of oxidative stress parameters.

2.7. Thiobarbituric acid reactive substances (TBARS)

Lipid peroxidation was measured by the reaction between the malondialdehyde (MDA) in the sample and thiobarbituric acid (0.67% TBA). The organic-phase fluorescence was evaluated at 515 nm (excitation) and 553 nm (emission). An MDA standard