Pharmacology, Biochemistry and Behavior xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Quercetin alleviates predator stress-induced anxiety-like and brain oxidative signs in pregnant rats and immune count disturbance in their offspring

Mohamed Lamine Toumi [a,*], Sameha Merzoug [a,b], Bruno Baudin [c], Abdelkrim Tahraoui [a]

[a] Laboratoire de Neuro-endocrinologie Appliquée, Département de Biologie, Université Badji Mokhtar, BP 12, 23000, Annaba, Algeria
[b] Département de Biologie, Institut des sciences de la nature et de la vie, Faculté des Sciences, Université d'El-Tarf, BP 73, 36000, El-Tarf, Algeria
[c] Service de Biochimie A, Hôpital Saint-Antoine, 184 rue de Faubourg Saint-Antoine, Paris 75571 Cedex 12, France

ARTICLE INFO

Article history:
Received 2 November 2012
Received in revised form 28 February 2013
Accepted 16 March 2013
Available online xxxx

Keywords:
Prenatal stress
Anxiety-like behavior
Oxidative stress
Immune cell count
Sex differences
Quercetin

ABSTRACT

This study was performed in rats to investigate the effect of a psychogenic stress during late gestation on the immediate behavior and brain oxidative status in dams as well as on the immune cell counts in their offspring up to weaning. Besides, the ability of quercetin (a natural flavonoid) to prevent stress effects was evaluated. Quercetin was orally administered for 6 consecutive days before the pregnant rats were acutely exposed to predator stress on gestational day 19. Post-stress corticosterone level, brain oxidative stress parameters and anxiety-like behavior were assessed in dams, whereas immune cell counts were postnatally determined in both male and female pups. Predator stress caused an oxidative stress in the brain and elicited an elevation in plasma corticosterone with concomitant behavioral impairment in dams. Prenatally-stressed pups mainly showed a decrease in total leukocytes and lymphocytes along with monocytosis and granulocytosis, but these changes were sex-dependent throughout the postnatal period studied. Quercetin pretreatment blocked the stress-induced corticosterone release and alleviated the brain oxidative stress with the maternal anxiety measures being slightly attenuated, whereas its effects on the offspring immune cell counts were mostly revealed at birth. Our findings suggest that late gestational exposure to traumatic events may cause anxiety symptoms in dams, in which corticosterone and brain oxidative stress play a certain role, and trigger negative immune changes in the early postnatal life of progeny. Notably, quercetin intake before such adverse events seems to be beneficial against negative outcomes in both dams and offspring.

1. Introduction

Psychogenic stressors during gestation are thought to evoke physiological alterations in both maternal and offspring organisms (Koenig et al., 2005; Patin et al., 2005). Pregnant women who experienced severe stressful events have been reported to manifest anxiety symptoms and several obstetrical complications, such as preeclampsia, fetal distress and preterm birth, with their offspring's development being compromised (Chang et al., 2002; Engel et al., 2005; Talge et al., 2007). In rats, exposure to a natural predator creates a species-relevant life-threatening experience, which is considered to be ecologically valid in mimicking an intense stressful situation in human beings (Adamec et al., 1998). Predator stress provokes an acute sense of fear, agitation and helplessness with intense activation of the hypothalamo–pituitary–adrenocortical (HPA) axis in experimental animals (Sullivan and Gratton, 1998; Zoladz et al., 2008). With regard to the stress-induced neurobehavioral disorders, a close link between anxiety and oxidative stress has been recently determined (Rammal et al., 2008). Oxidative stress is an overload of oxidants caused by elevated free radicals production and/or declined antioxidant defense mechanisms (Ates et al., 2006). In fact, free radicals are highly reactive oxygen-derived moieties generally referred to as reactive oxygen species (ROS), which play an essential role in maintaining homeostasis but their overproduction in response to stressful stimuli could lead to behavioral impairment. The brain is particularly vulnerable to ROS-induced damage because of its low mitotic rate, high oxygen request, abundant content of oxidizable polyunsaturated fatty acids, considerable amount of iron, and low concentration of antioxidants (Julka and Gill, 1996; Peker et al., 2010). The ROS-scavenging system of brain cells is particularly based on the reduced glutathione (GSH), an endogenous thiol-containing molecule (Raghavendra and Kulkarni, 2001). GSH depletion may lead to lipid peroxidation, generation of cytotoxic lipid peroxides such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE) in the brain tissue, and ultimately a series of neurobehavioral alterations (Chakraborti et al., 2008). GSH effectively contributes to the enzymatic antioxidant mechanisms that include glutathione peroxidase (GPx) and glutathione-S-transferase (GST). It was reported that the GST activity is much higher than that of the GPx in the brain, representing a relevant indicator of neuronal oxidative status (Carmagnol et al., 1983). GST catalyzes the conjugation of GSH to lipid peroxides and exogenous

* Corresponding author. Tel.: +213 663 429 282.
E-mail addresses: ml.toumi_annaba@yahoo.fr (M.L. Toumi), merzougs@yahoo.fr (S. Merzoug).

0091-3057/$ – see front matter © 2013 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.pbb.2013.03.009

electrophilic compounds through a detoxification process that implies a continual recycling of the oxidized GSH (GSSG) by glutathione reductase (Backos et al., 2012). In this respect, whether stress-related anxiety aspects occurring during gestation are associated with brain ROS excess is still unclear.

The impact of gestational stress on the offspring in utero is referred to as prenatal stress. Growing evidence in recent years suggests that prenatal stress impairs the immune system function in the developing progeny. Maternal glucocorticoids, neuromediators and cytokines, released in response to stressful stimuli, are potentially able to cross the placental barrier and affect the fetal immune milieu (Merlot et al., 2008). Previous experimental data indicated that prenatal stress generally inhibits the offspring's immune processes by reducing blood lymphocytes number (Götz and Stefanski, 2007), lymphocytes proliferation (Klein and Rager, 1995), cytotoxic activity of natural killer cells (Kay et al., 1998) and the immunization-induced antibody response (Gorczynski, 1992; Sobrian et al., 1997). In rodents, the perinatal stage is crucial for acquiring an appropriate immune functioning in later life because the immune system is relatively immature (Huling et al., 1992). Therefore, maternal stress experience at this period may perturb the fetal environment and adversely affect the course of postnatal immune development. In fact, studies dealing with developmental facets of both innate and acquired immunity have pointed out that prenatal psychological stress may alter the developing immunocompetence and predispose the offspring to a wide range of bacterial and viral infections (Gotz et al., 2007). Nevertheless, whether a psychotraumatic stress occurring at late gestational stage would postnatally have the same effects on the offspring immune capacities is not yet investigated.

Recently, a substantial attention was paid to flavonoids as antioxidant and/or anxiolytic agents having a prominent pharmacological effectiveness (Zhang, 2004). Among these plant molecules, quercetin is being increasingly used in experimental studies (Pu et al., 2007). Quercetin (3,5,7,3′,4′-pentahydroxyflavone) is a polyphenolic flavonol molecule that occurs in many fruits and vegetables, such as onions, apples, berries, peanuts, soybeans, potatoes, broccoli, grapes, citrus fruits and tea (Herrmann, 1976; Scalbert and Williamson, 2000). Since it is largely present in the human diet, up to 1 g/day average intake of quercetin has been reported (Manach et al., 2004), representing 60 to 75% of the overall polyphenols ingestion (Goldberg et al., 1995; Sampson et al., 2002). Quercetin efficiently scavenges free radicals, inhibits ROS-generating enzymes and prevents oxidative stress-induced neuronal injuries (Ansari et al., 2009; Heo and Lee, 2004). Along with this potent antioxidant property, quercetin has been found to exert an anxiolytic-like effect (Aguirre-Hernandez et al., 2010). The relationship between the antioxidant and anxiolytic abilities of quercetin is strengthened by several lines of evidence showing that dietary antioxidants can improve cognitive functions and prevent stress-induced neurobehavioral disorders (Chakraborti et al., 2007). These findings prompted us to hypothesize that quercetin may alleviate, even prevent, gestational stress adversity in dams and their offspring.

The purpose of our study was to investigate the effect of predator stress, applied at late gestational stage in rats, on prepartum maternal behavior, brain oxidative status and plasma corticosterone levels as well as on the offspring immune aspects during the early postnatal life. We also examined the ability of quercetin pretreatment to prevent predator stress-induced changes in both maternal and offspring parameters.

2. Materials and methods

2.1. Animals and housing conditions

Three-month-old male and virgin female Wistar rats weighing 220 ± 10 g were purchased from Pasteur Institute (Algiers, Algeria) and randomly housed in large polyethylene cages (4 rats per cage for each sex) at standard facility conditions of temperature (25 ± 2 °C), humidity (65 ± 5%) and 12 h light/12 h dark regime (lights on at 07:30 h). Rats were supplied with commercial chow and tap water ad libitum. A six-month-old male domestic cat weighing approximately 1.6 kg was used as the predator stress. The cat was housed alone in a separate facility room under a natural light/dark cycle with constant temperature, humidity and food supply. The entire experimental procedures have been processed according to the revised policies of the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, 1996).

2.2. Mating

Two weeks later, sixty (60) female rats of the same weight (approximately 260 g) were individually housed in appropriate polyethylene cages and subjected to the first vaginal smears to determine estrus cycle phases based on a standard cytological analysis (Freeman, 1994). Each pro-estrus female was mated overnight with one sexually-experienced male. In the morning, the presence of sperm plugs in the second vaginal smears determined the first (1st) day of conception. Each pregnant female was then singly kept in its home cage for the entire experimental period.

2.3. Treatment groups and quercetin administration

The pregnant females were randomly assigned to the following treatment groups (15 rats per group): unstressed dams pretreated with vehicle (Control, C), unstressed dams pretreated with quercetin (Q), predator-stressed dams pretreated with vehicle (PS) and predator-stressed dams pretreated with quercetin (PS + Q). Pretreatment with quercetin was conducted in 6 consecutive days, from the fourteenth (14th) to the nineteenth (19th) day of gestation. Quercetin (quercetin dihydrate, 98% purity powder; Sigma Aldrich Co., Steinheim, Germany) was dissolved in warm saline (Brookes et al., 2002) and freshly administered by gavage in 50 mg/kg of body weight/day (in a volume of 1 ml/kg of body weight) at 09:00 h; C and PS groups received an equal volume of warm saline. The dose and duration of treatment were adopted from a previous report showing their effectiveness in male rats (Luangaram et al., 2007). Moreover, the dose used here is supposed to be safe, as quercetin oral administration in pregnant rats at a range of 20–200 mg/kg did not cause toxicological nor teratological effects (Wilhite, 1982).

2.4. Predator stress application

The acute predator stress procedure was applied at gestational day 19 (GD 19), at least two hours after the last quercetin or vehicle administration (between 11:00 h and 12:00 h). At GD 19 in rats, the fetal systems are well-differentiated and sensory capacities are grossly acquired (Patin et al., 2005). During the 15-min stress session, the pregnant female were placed in a small transparent Plexiglas box (28 × 9 × 14 cm) that was enclosed in a large Plexiglas chamber (57 × 57 × 76 cm) containing the cat and appropriate wall holes and slots were effected to elicit ventilation (Park et al., 2008). The apparatus permits the access of rats to the cat-associated visual, olfactory and acoustic stimuli while prohibiting any physical contact between animals. Throughout this procedure, the cat did not display any aggressive behavior or exaggerated attempts to catch the rat. The duration of 15-min predator stress was found to be very effective in male rats (Nanda et al., 2008). All stress sessions took place in an isolated testing room to which the animals were transported 20 min earlier. The unstressed pregnant rats of C and Q groups were placed into the small chamber of a similar apparatus as that used for the stressed rats, but the large chamber was empty (without a

Please cite this article as: Toumi ML, et al, Quercetin alleviates predator stress-induced anxiety-like and brain oxidative signs in pregnant rats and immune count disturbance ..., Pharmacol Biochem Behav (2013), http://dx.doi.org/10.1016/j.pbb.2013.03.009