Psychoneuroendocrinology 90 (2018) 92–101

Contents lists available at ScienceDirect

Psychoneuroendocrinology

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

Corticotrophin releasing factor receptor 1 antagonists prevent chronic stress-induced behavioral changes and synapse loss in aged rats

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ARTICLE INFO

Keywords:
Stress
Aging
Corticotrophin releasing factor receptor 1 antagonist
Memory
Neurodegeneration
Rats

ABSTRACT

Mounting evidence suggests that chronic stress can alter brain structure and function and promote the development of neuropsychiatric disorders, such as depression and Alzheimer's disease. Although the results of several studies have indicated that aged brains are more vulnerable to chronic stress, it remains unknown whether antagonists of a key stress regulator, the corticotrophin releasing factor receptor 1 (CRF1), can prevent stress-induced anxiety and memory deficits in animal models. In this study, we evaluated the potential benefits of two CRF1 antagonists, R121919 and antalarmin, for preventing stress-induced anxiety-related behavioral and memory deficits and neurodegeneration in aged rats. We stressed rats using isolation-restraint for 3 months starting from the 18 months of age. Subsets of animals were administrated either R121919 or antalarmin through food chow for 3 months, followed by a series of behavioral, biochemical and morphological analyses. We found that stressed aged rats displayed body weight losses and increased corticosterone levels, as well as anxiety-related behaviors and memory deficits. Additionally, chronic stress induced a loss of cortical dendritic spines and synapses. However, R121919 and antalarmin both prevented stress-induced behavioral changes including anxiety-related behaviors and memory deficits and prevented synapse loss, perhaps through reversing HPA axis dysfunction. These results suggest that CRF1 antagonists may hold promise as a potential therapy for preventing stress-induced anxiety and memory deficits in aged individuals.

1. Introduction

There is widespread evidence that chronic stress can affect both the structure and function of the brain (Oliveira et al., 2016; Prenderville et al., 2015) with important consequences for learning, memory, decision-making and emotional responses (Scott et al., 2015). Moreover, stress has been linked to the development of neuropsychiatric disorders, such as depression and Alzheimer's disease (AD) (Carroll et al., 2011; Csernansky et al., 2006; Daulatzai, 2014; Dong et al., 2004; Greenberg et al., 2014; Kang et al., 2007). Moreover, the impact of stress on the brain may be dependent on the stage of brain development (Barnum et al., 2012; Kingsbury et al., 2016; Lesse et al., 2016; Lin et al., 2016; Naninck et al., 2015). For instance, neonatal exposure to stress has been observed to affect brain structure and function later in life, and increase the risk of developing age-related neuropsychiatric disorders (Barnum et al., 2012; Kingsbury et al., 2016; Lesse et al., 2016; Sousa et al., 2014). Surprisingly, the behavioral consequences of chronic stress in aged animals has not been studied in similar detail. The aged brain may show decreased resiliency in response to environmental stressors (Prenderville et al., 2015). Moreover, aging may be seen as a stressor itself, since the effects of aging can mimic the effects of chronic stress in regard to hyperactivation of the hypothalamic–pituitary–adrenal (HPA) axis and increases in basal glucocorticoid release, impaired HPA negative feedback and reduced central glucocorticoid receptor expression (Gaffey et al., 2016; Holmes et al., 2010).

Corticotrophin releasing factor (CRF) and its receptor 1 (CRF1) are critical components of a signaling cascade that regulates the HPA axis in response to stressors that vary depending on changes in the internal and external environment (Bao et al., 2008; Johnson et al., 1992). In addition to its central role in regulation of the HPA axis, CRF/CRF1 signaling pathway has a second, interrelated function in the brain (Birnbaum et al., 2004; Blank et al., 2002; Grammatopoulos et al., 2001; Hains et al., 2009). CRF1 is distributed extensively throughout the cortex, hippocampus and amygdala, where it modulates neural

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https://doi.org/10.1016/j.psyneuen.2018.02.013
Received 7 June 2017; Received in revised form 25 January 2018; Accepted 14 February 2018

activity related to stress-sensitive cognitive processes, such as learning and memory (De Souza and Battaglia, 1988; Gallagher et al., 2008; Orozco-Cabal et al., 2006).

Some stressors, such as physical illness and psychosocial loss, may be unavoidable during aging. Therefore, decreasing the effects of such stressors through the manipulation of stress-related signaling pathways may help to prevent or reduce stress-related disorders. To date, there has been relatively little study on the effects of chronic stress on CRF1 activation in aged animals and age-related declines in brain structure and function (Romeo et al., 2007). In this study, we evaluated the effects of isolation-restraint stress in aged rats by measuring body weight and plasma corticosterone changes, as well as dendritic spine and synapse density and anxiety-related behaviors and memory deficits. Then, we examined the ability of CRF1 antagonists to block the effects of stress-related changes in these biological and behavioral measures.

2. Material and methods

2.1. Animals

A total of 48 rats (Sprague Dawley, 24 males and 24 females) at 18 months (n = 8 per group) of age from the Charles River Laboratories (Bar Harbor, Maine) were used for this study. Animals were housed on a 12-h light/dark cycle and given food and water ad libitum. All procedures were performed according to NIH guidelines for the treatment of animals and the Current Guide for the Care and Use of Laboratory Animals (2011, 8th edition) under a protocol approved by the Northwestern University Animal Care and Use Committee.

2.2. Isolation-restraint stress

Isolation-restraint stress was performed by individually housing rat at 18 months of age in a cage one-third smaller ($27 \times 14 \times 11\ cm^3$) than standard-sized rat cages ($43.2 \times 34.0 \times 19.8\ cm^3$), in which individual rats (body weight > 500 g) could still freely move and reach food and water. The isolation-restraint rat cages were placed in a separate area (a satellite facility next to the main laboratory) from other animals to prevent visual contact. Control animals were group-housed with three animals per standard cage for the same period of time. The body weight and food consumption of each rat was measured weekly.

2.3. CRF1 antagonist administration

CRF1 antagonists, R121919 and antalarmin (20 mg/kg, from Dr. Rice's laboratory at NIDA and NIAAA), were administrated by mixing them in food chow (LabDiet, St Louis, MO 64144) to stressed and non-stressed rats for 3 months; i.e., from 18 to 21 months of age. We selected R121919 and antalarmin because they were commonly used for preclinical studies (Kehne and Cain, 2010). Further, R121919 has been used in patients for clinical trials up to phase II (Saunders and Williams, 2001; Zorrilla and Koob, 2010). Both drugs pass through the blood brain barrier (BBB) reasonably well (Marinelli et al., 2007; Sabino et al., 2013). We selected a daily doses of 20 mg/kg based on our previous work (Dong et al., 2014) and dosages used in previous animal studies from other laboratories (Marinelli et al., 2007; Sabino et al., 2013). Using measure of average food intake/day (FI, 129.75 ± 10.32 g/7), average body weight at 18 months of age (BW, 606.82 ± 69.16 g), dosage = mg of compound per kg of body weight (CK, 20 mg/kg), we were able to calculate the concentration of CRF1 antagonist to be mixed in the food chow based on the formula (((CK × BW)/FI)/10,000 = concentration of the drug in the diet). In our study, our concentration in the food chow was 0.06%.

Food consumption was measured weekly. Given that the drug concentration in food chow was calculated using average food consumption, and each rat's daily consumption of CRF1 antagonists was different, we calculated daily drug administration for each individual rat using the formula (((weekly food chow consumption/7) × 0.06%)/body weight).

To determine whether the administration of CRF1 antagonists through food chow was comparable to our previous study (Dong et al., 2014), the concentration of R121919 and antalarmin in the plasma was measured by liquid–liquid extraction with methyl *tert*-butyl ether (MTBE), using a previously published method (Dong et al., 2014). Plasma concentrations of R121919 were 32.4 ± 5.34 ng/ml and antalarmin were 37.825 ± 6.56 ng/ml, which are comparable to plasma concentrations achieved by delivering the drugs through drinking water and 12 h following IP injection (Dong et al., 2014).

After 3 months of CRF antagonist administration, tests of anxiety- and memory-related behaviors were performed. During behavioral testing, animals were still exposed to isolation-restraint stress and/or CRF1 administration in their food chow. After behaviors testing was complete, blood from each animal was collected, and brain tissues including the cortex and hippocampus were prepared for measurements of dendritic spine and synapse density.

2.4. Corticosterone levels

Blood was collected by rapid retro-orbital phlebotomy in the early morning (7:00am) after CRF1 antagonist treatment and after behavioral testing was finished. Plasma corticosterone levels were measured using an EIA kit (Cayman Chemical Company, Ann Arbor, MI) according to the instruction manual. The final corticosterone concentration was calculated in nanograms per milliliter (ng/mL) according to the manufacturer's instructions.

2.5. Open field

General locomotion and anxiety-related behavior were measured using the Open Field Test. An automated tracking system (Any-Maze, Stoelting Co. Wood Dale, IL 60191) tracked the locomotion of each subject in an open box (72 cm × 72 cm × 36 cm) for 5 min. Anxiety-related behaviors were determined by comparing the number of times the animals went to the center zone, the amount of time the animals spent in the center zone, and the amount of time the animals stayed in the periphery zone (wall-hugging) of the open field.

2.6. Novel object recognition (NOR)

NOR test is commonly used to assess memory-related behavior in rodents. The test was modified from previously published methods (Horiguchi et al., 2012, 2011). The apparatus for this test consists of an evenly illuminated Plexiglas box (72 cm × 72 cm × 36 cm). The procedure included three phases: habituation, acquisition and retention. During habituation (Days 1–3), each individual mouse was allowed to freely explore the box absent of objects for 10 min. During the acquisition trial, two identical objects were placed in the box and positioned 6 cm away from the walls in opposite corners. The rats were allowed to explore the identical objects for 10 min and then returned to their home cages. During the retention trial, one of the two familiar objects was replaced with a novel object. Following a one-hour gap, rats were returned to the box and allowed to explore for 10 min. All objects used in this study were different in shape and color but identical in size. To minimize olfactory cues, the box and objects were cleaned with 70% ethanol after each trial. Object exploration was defined as sniffing, licking or touching the objects using the forepaw, but not leaning against, turning around, standing, or sitting on the objects. Behavioral data were video-recorded for further analysis by an experimenter blind to the treatments. The exploration time (in seconds) was recorded manually using 2 stopwatches. Total exploration time of both objects in the acquisition and retention trials and total exploratory activity was calculated. *Discrimination index (DI)* was used as the memory-related measure, and was calculated as the proportion of the total time spent