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Chronic restraint stress in rats causes sustained increase in urinary corticosterone excretion without affecting cerebral or systemic oxidatively generated DNA/RNA damage

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ABSTRACT

Increased oxidatively generated damage to nucleic acids (DNA/RNA) may be a common mechanism underlying accelerated aging in psychological stress states and mental disorders. In the present study, we measured the urinary excretion of corticosterone and markers of systemic oxidative stress on nucleic acids, 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) and 8-oxo-7,8-dihydroguanosine (8-oxoGuo), respectively, in rats subjected to chronic restraint stress. To reliably collect 24 h urine samples, the full 3-week restraint stress paradigm was performed in metabolism cages. We further determined frontal cortex and hippocampal levels of oxidatively generated nuclear DNA damage, as measured by oxoguanine DNA glycosylase and formamidopyrimidine DNA glycosylase sensitive sites detected by the comet assay, as well as the expression of genes involved in DNA repair (*Ogg1* and *Nudt1*) and inflammation (*Ccl2* and *Tnf*). The metabolism cage housing in itself did not significantly influence a range of biological stress markers. In the restraint stress group, there was a sustained 2.5 fold increase in 24 h corticosterone excretion from day 2 after stress initiation. However, neither whole-body nor cerebral measures of nucleic acid damage from oxidation were affected by stress. In contrast, cerebral DNA repair enzymes exhibited a general trend towards an induction, which was significant for hippocampal *Nudt1*. The results and their implications for stress sensitivity and resilience are discussed.

1. Introduction

Accumulating evidence has substantiated that psychological stress states are detrimental to health and accelerate aging. Stress is associated with an increased risk of various age-related medical disorders, in particular cardiovascular disease (Brotman et al., 2007). In the brain, experimental chronic stress leads to neuronal remodeling and dysfunctions of the prefrontal cortex and the hippocampus (Goldwater et al., 2009; Watanabe et al., 1992). Correspondingly, epidemiological evidence suggests that stress and mental disorders such as depression and schizophrenia increase the risk of cognitive dysfunction or out-right dementia (Jeste et al., 2011; Johansson et al., 2010; Saczynski et al., 2010).

Attempts have been made to identify common mechanisms underlying a stress-induced syndrome of accelerated aging. One important finding was that telomere length – a biochemical correlate of aging – is reduced in psychological stress and mental disease (Epel et al., 2004; Kao et al., 2008; Simon et al., 2006). A related candidate phenomenon is oxidative stress, in which the generation of reactive oxygen species (ROS) exceeds the antioxidant potential of the cell, thereby causing damage to proteins, lipids or nucleic acids. The continuous ROS attacks on DNA are considered to be a key mediator of aging (Chen et al., 2007; Finkel and Holbrook, 2000). Specifically,

Abbreviations: 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; 8-oxoGuo, 8-oxo-7,8-dihydroguanosine; ROS, reactive oxygen species; CORT, corticosterone; FCM, fecal corticosterone metabolites; OGG1, 8-oxoguanine DNA glycosylase; NUDT1, Nudix (nucleoside diphosphate linked moiety X)-type motif 1; TNF, tumor necrosis factor; CCL2, C–C motif ligand 2.

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genotoxic stress from oxidation accelerates telomere shortening and activates DNA damage signaling pathways, thereby increasing the risk of cellular senescence or apoptosis, which are known to be key events in tissue aging processes (Sahin and DePinho, 2010). Indeed, a recent meta-analysis showed that an age-dependent accumulation of oxidatively damaged DNA occurs in various organs of rodents (Moller et al., 2010). Finally, oxidative stress has been implicated as a pathogenic event in age-related medical disorders such as cardiovascular disease and dementia (Bossy-Wetzel et al., 2004; Harrison et al., 2003).

Various measures of cerebral oxidative stress, *e.g.* protein or lipid oxidation markers, have been found to be increased in animal models of psychological stress (Fontella et al., 2005; Madrigal et al., 2003). In an early study, DNA oxidation levels were increased in the cerebral cortex of rats after acute restraint stress (Liu et al., 1996). In clinical studies peripheral markers of oxidative stress have been found to be associated with occupational stress (Irie et al., 2001), caregiver stress (Epel et al., 2004) and depression (Forlenza and Miller, 2006). Several post-mortem studies have found signs of mitochondrial dysfunction and oxidative stress in the brains of depressed (Gawryluk et al., 2011), bipolar disorder (Andreazza et al., 2010), and schizophrenia patients (Prabakaran et al., 2004). However, a recent post-mortem study found no activation of several oxidative stress-response genes in depressed subjects (Teyssier et al., 2011). Hence, questions regarding the specificity of oxidative stress to various psychiatric disorders, the temporal relationship between stress exposure and oxidative stress induction, and the relationship between peripheral biomarkers and cerebral oxidative stress levels, remain largely unanswered.

The aim of the present study was to investigate the urinary excretion of markers of oxidatively generated DNA/RNA damage in rats subjected to chronic restraint stress, and to relate these to post-stress brain levels of oxidatively generated nuclear DNA damage, as well as DNA repair enzyme and inflammation marker expression in the same animals. The restraint stress paradigm was chosen for its ability to reliably induce a range of the biological hallmarks of stress: increased glucocorticoid output and relative adrenal gland weight, reduced expression of the cerebral glucocorticoid receptor, as well as dendritic retractions in both the prefrontal cortex and the hippocampus (Goldwater et al., 2009; Hageman et al., 2008, 2009; Watanabe et al., 1992). Furthermore, the model is associated with depression-like behavioral changes (*e.g.* in the Forced Swim Test), thus mimicking the human depressive state (Hageman et al., 2009). Finally, the biological and behavioral consequences of restraint stress are reversible with antidepressive treatments such as electroconvulsive stimulations (Hageman et al., 2008, 2009; Maigaard et al., 2012).

We hypothesized an increased urinary excretion of the oxidation markers during stress, an increased level of DNA damage from oxidation in the brain of stressed animals, and an induction of the repair enzymes.

2. Methods

2.1. Experimental groups and general procedure

Male Sprague–Dawley rats (N = 36, weight 180–200 g, Charles-River, Germany) were randomly distributed in standard group cages (Macrolon type III cages (Tecniplast, Varese, Italy), 2 rats per cage), and left to acclimatize for one week. They were fed with food pellets (Altromin 1319; Brogaarden, Gentofte, Denmark) and acidified tap water provided *ad libitum*. Wooden chips (Tapvei Oy, Kortteinen, Finland) were used as bedding. Room temperature was maintained at 20 ± 2 °C, air humidity was 30–60% and the light regimen was a 12/12 h dark/artificial light cycle with lighting period starting at 7:00 AM.

We performed the full three-week restraint stress paradigm in metabolism cages (MC) (Tecniplast, Varese, Italy), which allowed for a continuous and non-invasive monitoring of urinary biomarkers. The day before the experiment began, animals were matched by weight and assigned to experimental groups. The three experimental groups (N = 12 per group) were 1) group cage control (GCC), 2) metabolism cage control (MCC) and 3) metabolism cage stress (MCS). The GCC group was included to allow for an evaluation of the stress effects of MC housing in itself. MCS and GCC/MCC were kept in separate rooms with similar conditions throughout the experiment to avoid sonic and ultrasonic influence between the groups.

One week after arrival at our facility, animals in the MCC and MCS groups were placed individually in the MCs for a further four days of acclimatization (day-3 through 0), while the GCC animals were left in the group cages throughout the experiment. The MCs were arranged in two racks with twelve cages each. The metabolism cages measured 26 cm in diameter, and had a grid floor to allow for the passage of excretions into a collection system. In the collection system, urine and feces are separated into two removable containers from which the samples were obtained. The MC animals had a plastic shelter (as did the GCCs) inside the cage, but no other forms of enrichment which could potentially interfere with the collection of urine and feces. A food trough and water dispenser were attached to the cage for *ad libitum* access (except during restraint). All animals received the same diet, which in the case of the MC rats was ground to avoid contamination of the collection system. Twenty-four hour urine and feces samples were collected daily at 9 AM, weighed and stored at −20 °C until analysis. The body weight of all animals was recorded daily.

2.2. Restraint stress

On day 1–21 MCS animals were subjected to restraint stress. At the initiation of restraint, the animals weighed on average 285 g, corresponding to an approximate age of postnatal day 64. The chronic restraint stress paradigm consists of 6 h of daily immobilization for 21 consecutive days. Every morning, the rats voluntarily entered wire mesh restrainers that were subsequently closed with wire clips. The restrainers were designed to tightly fit the animal without restricting breathing, interfering with thermoregulation, or causing pain. During restraint, animals remained in their metabolism cages to allow collection of the urine and feces produced while in the restrainer. The urine and feces samples from the previous 24 h were always collected before restraint stress began.

2.3. Isolation of brain tissue

On day 22 at 9 AM (*i.e.* the day after the last restraint stress session), rats from the two MC groups were sacrificed. To reduce diurnal variation in the time of death between the groups, GCC rats were sacrificed on day 23, also at 9 AM. The animals were deeply anesthetized by an intraperitoneal injection of pentobarbital (100–150 mg). Before blood circulation ceased, the animals were thoracotomized, the descending aorta was clamped, and the animals were perfused transcardially with cold 0.9% saline. Both adrenal glands were removed by manual dissection; adjacent fat and connective tissue were carefully removed, and the glands were weighed separately. The relative adrenal gland weight was defined as: (left + right adrenal gland weight (g) / total body weight (g)) × 1000‰.

The brains were removed and manually dissected on an ice-cooled metal plate. We isolated frontal cortex and hippocampal tissue for DNA oxidation and gene expression analysis. These regions where chosen for their established role in HPA-axis inhibition, sensitivity to glucocorticoids, and stress-associated plasticity (Ulrich-Lai and Herman, 2009). Frontal cortex was isolated by an incision in the coronal plane approximately 4 mm dorsal to the frontal pole, snap frozen on dry ice and stored at −80 °C. The remaining brain was snap frozen in isopentane, placed on dry ice and stored at −80 °C. To isolate hippocampal tissue for RT-PCR and comet analysis, the frozen brains were placed in an ice-cooled matrix, and consecutive 2 mm