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A chronic increase of corticosterone age-dependently reduces systemic DNA damage from oxidation in rats

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ABSTRACT

Stress and depression are associated with an acceleration of brain and bodily aging; effects which have been attributed to chronic elevations of glucocorticoids. We tested the hypothesis that a three week administration of stress-associated levels of corticosterone (CORT, the principal rodent glucocorticoid) would increase systemic and CNS DNA and RNA damage from oxidation; a phenomenon known to be centrally involved in the aging process. We also hypothesized that older individuals would be more sensitive to this effect and that the chronic CORT administration would exacerbate age-related memory decline. Young and old male Sprague-Dawley rats were non-invasively administered CORT by voluntary ingestion of nut paste containing either CORT (25 mg/kg) or vehicle for a total of 22 days. CORT increased the 24 h urinary excretion of the hormone to the levels previously observed after experimental psychological stress and caused a downregulation of the glucocorticoid receptor in the CA1 area of the hippocampus. Contrary to our hypothesis, 24 h excretion of 8-oxodG/8-oxoGuo (markers of DNA/RNA damage from oxidation) was reduced in CORT-treated young animals, whereas old animals showed no significant differences. In old animals, CORT caused a borderline significant reduction of RNA oxidation in CNS, which was paralleled by a normalization of performance in an object location memory test. To our knowledge, this is the first demonstration that chronic stress-associated levels of CORT can reduce nucleic acid damage from oxidation. These findings contradict the notion of elevated CORT as a mediator of the accelerated aging observed in stress and depression.

1. Introduction

An accumulating body of evidence suggests that prolonged psychological stress and stress-associated mental illnesses such as depression accelerate various aspects of aging. Both stress and depression are associated with an increased occurrence of age-related medical conditions, such as the metabolic syndrome, type 2 diabetes and cardiovascular disease [1–3]. Furthermore, stress and depression have been linked to accelerated cognitive decline [4], an increased risk of dementia [5,6], and age-related changes in the brain, including hippocampal atrophy and neuronal dendritic retractions [7,8]. Consistent with these observations, both perceived psychological stress, as well as suffering from depression or other mental disorders, have been shown to be associated with increased non-suicide mortality [9,10]. Biochemically, prolonged stress and depression have been associated with increased oxidative stress and telomere attrition; key mechanisms in the cellular aging process [11–13]. However, the biological mechanisms that mediate the connections between psycho-

List of abbreviations: CORT, Corticosterone; CNS, Central Nervous System; DNA, Deoxyribonucleic Acid; RNA, Ribonucleic Acid; HPA-axis, Hypothalamic-Pituitary-Adrenal axis; ROS, Reactive Oxygen Species; 8-oxodG, 8-oxo-7,8-dihydro-2-deoxyguanosine; 8-oxoGuo, 8-oxo-7,8-dihydroguanosine; Y-VHC, Young vehicle-treated rats; Y-CORT, Young CORT-treated rats; O-VHC, Old vehicle-treated rats; O-CORT, Old CORT-treated rats; CSF, Cerebrospinal Fluid; FCM, Fecal Corticosterone Metabolites; GluR, Glucorticoid Receptor; CA1, Cornu Ammonis of the hippocampus, area 1; CA3, Cornu Ammonis of the hippocampus, area 3; ELISA, Enzyme-linked Immonusorbent Assay; UPLC-MS/MS, Ultra-Performance Liquid Chromatography with Tandem Mass Spectrometry; ANOVA, Analysis of Variance

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logical states and accelerated aging remain largely unknown.

The hypothalamic-pituitary-adrenal (HPA) axis is activated upon psychological stress exposure, resulting in the release of glucocorticoids from the adrenal glands. Prolonged increase and/or dysregulation of glucocorticoid secretion are key components in the concept of allostatic load, in which prolonged stress leads to brain and bodily damage through metabolic dysregulation [14]. This has been suggested to be a central mediator of the age-advancing effects of stress and mental disorders [15,16]. Therefore, a critical question is to what extent circulating glucocorticoids at stress-associated physiological levels influence molecular aging mechanisms.

Oxidatively generated damage to DNA is an important mechanism in the aging process *per se*, as well as a putative early pathogenic event in age-related medical conditions such as the metabolic syndrome, type-2 diabetes, and dementia [17–19]. Reactive oxygen species (ROS) induced damage to DNA increases with age [20], and the inability to repair DNA damage from oxidation leads to a progeric phenotype in both animals and humans [19]. Furthermore, oxidative stress on telomeric DNA is an important negative regulator of telomere length [21]. Recently, an increased attention to the effects of RNA oxidation has emerged, with studies indicating that the oxidation of non-coding RNAs and mRNA negatively influences gene regulation and protein synthesis/folding [22,23], respectively; observations which could bear relevance to the pathogenesis of a range of medical disorders [24].

The main cellular source of ROS is the mitochondria, and mitochondrial dysfunctions may lead to increased ROS leakage [25]. High physiological levels of glucocorticoids have been shown to negatively influence a range of mitochondrial functions, e.g. levels of functional mitochondria and cytochrome *c* oxidase in muscle cells [26,27] and both *in vivo* and *in vitro* experiments have indicated that exposure to glucocorticoids increases oxidation of mitochondrial DNA in the liver [28] and causes DNA strand breaks [29].

In an aged human population, we previously observed a positive association between markers of oxidatively generated DNA and RNA damage (8-oxo-7,8-dihydro-2-deoxyguanosine (8-oxodG) and 8-oxo-7,8-dihydroguanosine (8-oxoGuo), respectively), and the urinary excretion of cortisol, the principal human glucocorticoid [15]. The purpose of the present study was to determine the effect of an experimental physiological increase in glucocorticoid exposure on markers of nucleic acid damage and on hippocampus-dependent memory in rats, and to study the influence of age on these effects. We hypothesized that elevated circulating levels of CORT would lead to greater oxidative damage of DNA/RNA, evident as increases in both systemic and CNS concentrations of 8-oxodG/8-oxoGuo, and attenuated performance in a spatial memory test. Furthermore, we hypothesized that these effects would be more pronounced in aged rats than in their younger conspecifics.

2. Material and methods

2.1. Animals and housing conditions

24 young (6 weeks old) and 24 old (46 weeks old; retired breeders) male Sprague-Dawley rats (Taconic, Ry, Denmark) were housed singly in individually ventilated standard cages (Tecniplast, Varese, Italy), and left to acclimatize for two weeks. They were provided pelleted feed (Altromin 1319; Brogaarden, Gentofte, Denmark) and acidified tap water *ad libitum*. Cages were lined with aspen chips (Tapvei Oy, Kortteinen, Finland) and enriched with nest building material (Lilico, Horley, UK), bite blocks (Tapvei Oy), plastic shelters (Lilico) and cardboard tubes (Lilico). Cage temperature was maintained at 22 ± 2 °C and 55% humidity with 63 h^{-1} airchanges. The light regimen was a 12/12 h dark/artificial light cycle with 30 min of “twilight” and the lighting period starting at 7:00 AM.

2.2. General experimental procedure

At the start of the study, the animals were randomized into four experimental groups: Young vehicle-treated rats (Y-VHC), young CORT-treated rats (Y-CORT), old vehicle-treated rats (O-VHC), and old CORT-treated rats (O-CORT) ($n = 12$ in each group). The animals were CORT treated for 22 days (days 1–22). On day 21, the animals were habituated to the behavioral testing apparatus and then returned to their home cages. On day 22, the animals were tested for their spatial memory, and, immediately following the behavioral test, transferred to metabolism cages for collection of urine and feces. On day 23, after 24 h of metabolism cage housing, cerebrospinal fluid (CSF) was collected, and the animals were euthanized while still in anesthesia.

2.3. Corticosterone dose titration

To match the 24 h CORT excretion to the levels previously observed in rats subjected to severe psychological stress [30], a dose titration study was conducted. Six animals (body weight 300–350 g) were administered corticosterone by voluntary ingestion of nut paste (Nutella®; Ferrero, Pino Torinese, Italy) dosed with corticosterone (Sigma-Aldrich, St. Louis, USA) dissolved in dimethyl sulfoxide (DMSO). The nut paste administration method has previously been validated for buprenorphine administration [31]. The animals were administered 0, 2, 4, 6, 8 or 10 mg/day for five consecutive days (one animal per dose), divided into two doses per day at 8 AM and 4 PM, respectively. Subsequently, 24 h urine and fecal samples were collected by placing the animals in metabolism cages, and the samples were assayed for CORT/corticosterone metabolite excretion (see below). We found that a dose of 10 mg/day, corresponding to a weight-adjusted dose of 25 mg/kg/day, resulted in 24 h urinary CORT and fecal corticosterone metabolites (FCM) levels observed in a previous study of rats subjected to severe psychological stress [30]. Accordingly, this dose was used in the subsequent experiments.

2.4. Corticosterone treatment

The animals were dosed for the entirety of the study, including during the metabolism cage housing on day 22. All animals were weighed daily. CORT (25 mg/kg/day) was administered as two voluntary ingestions – at 8 AM and 4 PM – of nut paste containing corticosterone (12.5 mg/kg) dissolved in sesame oil. The control groups received only nut paste with sesame oil. The amount of vehicle used per dose was 1 g of nut paste and 0.625 g/kg body weight of sesame oil. Throughout the experiment, the dose of CORT was continuously adjusted individually to the animal's weight. To avoid recording acute effects of the last dose, behavioral tests began at a minimum of 3 h after the 8 AM CORT administration.

2.5. Behavioral test

The animals were tested for spatial memory performance by an object location test [32], as previously described [33]. The test was chosen because of the dependence of spatial memory on hippocampal function [34], and because it does not involve training or reinforcement, which could act as a stressor in itself [32]. An open arena consisting of a black box measuring 77 (l) × 56 (w) × 41 cm (h) was used, allowing the animal spatial orientation using cues in the environment outside of the arena. The arena was constantly and evenly illuminated. Behavior was recorded by a video camera mounted vertically above the test arena and analyzed using the video tracking program EthoVision® (Noldus Information Technology, Wageningen, The Netherlands). In all experiments the computer automatically initiated registration of the rat's position (in four animals, the tracking was not successful). During testing, the room was sealed to minimize disturbing noise. Two arenas were used simultaneously. The arena was