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Dynamic regulation of cerebral DNA repair genes by psychological stress

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ABSTRACT

Neuronal genotoxic insults from oxidative stress constitute a putative molecular link between stress and depression on the one hand, and cognitive dysfunction and dementia risk on the other. Oxidative modifications to DNA are repaired by specific enzymes; a process that plays a critical role for maintaining genomic integrity. The aim of the present study was to characterize the pattern of cerebral DNA repair enzyme regulation after stress through the quantification of a targeted range of gene products involved in different types of DNA repair. 72 male Sprague–Dawley rats were subjected to either restraint stress (6 h/day) or daily handling (controls), and sacrificed after 1, 7 or 21 stress sessions. The mRNA expression of seven genes (*Ogg1, Ape1, Ung1, Neil1, Xrcc1, Ercc1, Nudt1*) involved in the repair of oxidatively damaged DNA was determined by quantitative real time polymerase chain reaction in the prefrontal cortex (PFC) and hippocampus (HC). DNA repair gene expression in PFC exhibited a general trend towards an induction after acute stress and a decrease after subchronic exposure compared to control animals. After chronic stress, a normalization towards control levels was observed. A similar pattern was seen in HC, but with overall smaller effects and without the induction after acute stress. Nuclear DNA damage from oxidation as measured by the comet assay was unaffected by stress in both regions. We conclude that psychological stress have a dynamic influence on brain DNA repair gene expression; however, since we were unable to identify concurrent changes in DNA damage from oxidation, the down-stream consequences of this regulation, if any, remains unclear.

1. Introduction

According to the World Health Organization, major depression is the leading cause of disability worldwide and a major contributor to the global burden of disease [1]. A very harmful aspect of recurrent depression, both from an individual and a socioeconomic perspective, is that the cumulative exposure to depression is associated with the development of cognitive dysfunction in the euthymic state [2,3], and an increased risk for out-right dementia [4,5]. The cognitive deterioration occuring with each depressive episode may render the individual more susceptible to new episodes [6,7].

There is compelling evidence that depression and neurocognitive dysfunction are interconnected through the neurohormonal

Abbreviations: HPA-axis, Hypothalamic-pituitary-adrenal axis; ROS, reactive oxygen species; BER, base excision repair; NER, nucleotide excision repair; SSBR, single strand break repair; Ogg1, 8-oxoguanine glycosylase 1; Neil1, endonuclease VIII-like 1; Ung1, uracil DNA N-glycosylase 1(Ung1); Ape1, apurinic/apyrimidinic endonuclease 1; Xrcc1, X-ray repair cross-complementing protein 1; Ercc1, excision repair cross-complementing rodent repair deficiency, complementation group 1; Nudix, (nucleoside diphosphate linked moiety X)-type motif 1; PFC, prefrontal cortex; HC, hippocampus; RT-PC, Rreverse transcriptase polymerase chain reaction.

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stress response, in particular a prolonged activation of the HPA-axis, which results in the systemic release of glucocorticoids [7]. In rodents, both experimental stress and glucocorticoid administration cause a reduced dendritic branching in the frontal cortex and the hippocampus, with corresponding impairments in cognitive function [8–10]. In humans, analogous reductions of hippocampal volume, cognitive deficiencies and increased dementia risk occur after prolonged stress exposure and in association to elevated glucocorticoid levels [11,12].

DNA damage from oxidation is considered to be a key event in aging per se [13,14], as well as an early pathogenic event in many neurodegenerative disorders, including Alzheimer's disease [15,16]. Previous studies have found increased levels of oxidatively generated DNA damage in the brain after acute and subchronic stress [17,18]. Correspondingly, both in vitro and in vivo studies in rodents and humans have linked increased glucocorticoid levels to increased levels of DNA damage [19–21]. DNA damage activates signaling cascades which are known to regulate neuronal survival and synaptic plasticity, including apoptosis [22], and thereby have down-stream effects that plausibly connect depression with neuronal degeneration and dysfunction. In line with this, a recent post-mortem study of gene expression profiles in the frontal cortex of depressed individuals found evidence of inflammation as well as oxidative stress and apoptosis [23]. Thus, neuronal genotoxic insults from oxidative stress is a plausible molecular link between stress and depression on the one hand, and cognitive dysfunction and dementia on the other.

Oxidatively damaged DNA is repaired by specific enzymes, and therefore, the steady-state DNA lesions reflect a balance between the ROS-induced damage and repair activity. Consequently, the cellular toxicity of genomic stress from oxidation is not solely determined by the load of ROS-induced insults, but also by the ability to repair such insults. Specific phenotypes of accelerated aging and neurodegeneration have been described in rodents and humans deficient in enzymatic DNA repair [24], and reduction in cerebral DNA repair capacity has been established in the early phases of Alzheimer's disease [25,26]. In neurons, the predominating repair pathway for oxidized DNA lesions is BER, which detects and repairs single-base damage. However, other repair pathways with different substrate specificities, such as NER or SSBR, are also active in neurons [27].

The aim of the present study was to characterize the pattern of DNA repair enzyme regulation in the brain after experimental psychological stress (restraint stress) through the quantification of a targeted range of gene products involved in different types of DNA repair. The gene expression levels were assessed in brain regions relevant to the cognitive domains affected by clinical depression (e.g., attention and memory) [2,28] and abundant in glucocorticoid receptors [9], namely PFC and HC. The genes selected for mRNA quantification *Ogg1*, *Neil1*, *Ung1*, *Ape1*, and *X-ray repair cross-complementing protein 1* (all involved in various steps of BER and SSBR); *Ercc1* (involved in NER); and *Nudt1* (involved in the removal of oxidatively damaged nucleotides from to nucleotide pool) [27,29]. All genes have previously been implicated in neuroprotection and/or found to be inducible by stress paradigms [30–37]. In parallel to the gene expression measurements, we determined the nuclear levels of oxidatively damaged DNA in the same brain regions. Both parameters were determined after acute stress (AS), subchronic stress (SCS) and chronic stress (CS).

Based on findings from a previous study [37], and the hypothesis that genomic protection is part of the brain's adaptation to stress, we hypothesized that experimentally induced psychological stress would increase the expression of genes involved in DNA and nucleotide pool repair. To our knowledge, the regulation of cerebral DNA repair as an allostatic adaptation to stress has not previously been specifically addressed.

2. Materials and methods

2.1. Animal model

Male Sprague–Dawley rats (Charles River, $n = 72$, initial weight: 220–230 g) were pair housed (Scanbur type III high Techniplast) and acclimatized 10 days prior to the experiment. Room temperature was $21 \pm 1\,^{\circ}C$, 12:12 light/dark cycle between 7AM and 7PM. The animals were fed Altromin 1319 (Brogaarden, Gentofte, Denmark) and had access to acidified water ad libitum except during restraint sessions. All animals were weighed every third day and immediately prior to sacrificing.

The animals were matched by weight and randomly distributed into 6 groups ($n = 12$ per group), treated either as control (daily handling) or with restraint stress for 6 h/day during 1, 7 or 21 consecutive days, representing AS, SCS, and CS, respectively. Chronic restraint stress is a widely validated animal model of depression, which reliably induces depressive behavior, which in turn is reversed by antidepressant treatments [10,38,39]. It further induces dendritic retractions in the hippocampus and prefrontal cortex [8,10,40]. The stress groups were restrained in their home cage daily from 9AM–3PM, using a fitted wire mesh restrainer, designed to allow natural breathing and to avoid problems of overheating, as previously described [10,37]. To avoid sonic and ultrasonic influences between the groups, stress and control groups were housed in separate rooms.

Animals were sacrificed by decapitation immediately after the first restraint stress session (AS group) or in the morning following the last session (SCS and CS groups). This procedure was applied to avoid recording acute effects of the last session in the SCS and CS groups. Animals from the stress and control groups were sacrificed in random order, and decapitation took place within <30 seconds after removal from the home cage to avoid induction of a corticosterone response. A sample of trunk blood was collected in 10 ml EDTA tubes (BD Vacutainer, K2E 18,0 mg. Plymouth. PL6 7BP. UK), followed by manual bilateral dissection and weighing of the adrenal glands. Trunk blood was stored at 4 °C until centrifugation at 3000 rcf for 15 min at 4 °C. Plasma was removed and stored at −80 °C until assayed for corticosterone, by a commercially available ELISA kit as previously described [41]. Immediately upon blood sampling, the PFC and HC were dissected mannually on an ice-cold metalic plate. Both regions were instantly frozen in liquid nitrogen for storing at −80 °C. The left brain-area was used for comet assays and the right for RT-PCR-analysis.

2.2. Gene expression analysis

The relative change in mRNA expression of specific target genes was determined by RT-PCR as described previously [42], using primers from Life Technology; Ogg1(Rn00578409_m1), Ung1 (Rn01499386_m1), Neil1(Rn01422336_g1), Ape1(Rn00821186_g1), Xrcc1(Rn01457689_m1), Nudt1(Rn00589097_m1) and Ercc1(Rn01498472_m1). Endogenous control gene was eukaryotic 18 S rRNA (P/N 4352930E) [43]. Isolation of total RNA was performed with TRIzol reagent (Invitrogen, Carlsbad, CA, USA). After dilution of samples in RNase-free water, 1:10, purity of RNA was assesed and concentration of sample-RNA was calculated with a spectrophotometer (Eppendorf BioPhotometer, 8.5 mm light center hight). The purity (A_{260}/A_{280}) of the mRNA was 1.60 ± 0.12 and 1.73 ± 0.07 in the PCF and HC samples, respectively. To avoid DNA contamination, DNase treatment of the purified RNA samples was performed using the RQ1 RNase-Free DNase kit (Promega, Madison, WI, USA). Thereafter the samples were stored at −20 °C. RT-PCR reactions were run with PFC, HC and a Standard (St) in 3 different sets, with a minus RT-sample as control each time, using the High-Capacity cDNA transcription Kit (Applied Biosystems,

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