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Acute restraint stress induces rapid changes in central redox status and protective antioxidant genes in rats

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ABSTRACT

The stress-induced imbalance in reduction/oxidation (redox) state has been proposed to play a major role in the etiology of neurological disorders. However, the relationship between psychological stress, central redox state, and potential protective mechanisms within specific neural regions has not been well characterized. In this study, we have used an acute psychological stress to demonstrate the dynamic changes that occur in the redox system of hippocampal and striatal tissue. Outbred male Wistar rats were subject to 0 (control), 60, 120, or 240 min of acute restraint stress and the hippocampus and striatum were cryodissected for redox assays and relative gene expression. Restraint stress significantly elevated oxidative status and lipid peroxidation, while decreasing glutathione ratios overall indicative of oxidative stress in both neural regions. These biochemical changes were prevented by prior administration of the glucocorticoid receptor antagonist, RU-486. The hippocampus also demonstrated increased glutathione peroxidase 1 and 4 antioxidant expression which was not observed in the striatum, while both regions displayed robust upregulation of the antioxidant, metallothionein 1a. This was observed with concurrent upregulation of 11β-hydroxysteroid dehydrogenase 1, a local reactivator of corticosterone, in addition to decreased expression of the cytosolic regulatory subunit of superoxide-producing enzyme, NADPH-oxidase. Together, this study demonstrates distinctive regional redox profiles following acute stress exposure, in addition to identifying differential capabilities in managing oxidative challenges via altered antioxidant gene expression in the hippocampus and striatum.

1. Introduction

Acute psychological stress is an adaptive response to a multitude of adverse stimuli perceived to be noxious or threatening. This response is governed by the respective tripartite activation of the sympatho-adrenal-medullary (SAM), hypothalamic-spinal-adrenal (HSA), and hypothalamic-pituitary-adrenal (HPA) axes (Ulrich-Lai and Herman, 2009). While the SAM and HSA axes promote immediate mobilization of stored adrenal hormones and simultaneously sensitize components of the HPA for release of glucocorticoids, the HPA axis subsequently generates the classical hormonal cascade resulting in glucocorticoid synthesis and release (Lowry, 2002). In the brain, the concentration of glucocorticoids is dependent on both adrenal secretion and intracellular regeneration of cortisol (or corticosterone) by the enzyme, 11β-hydroxysteroid dehydrogenase type 1 (11β-Hsd1) (Harris et al., 2001; Morgan et al., 2014). A primary function of glucocorticoids is the liberation of glucose from hepatic and adipose stores by promoting glycogenolysis, gluconeogenesis, and lipolysis, resulting in increased metabolic substrate for production of ATP from cellular respiration via oxidative phosphorylation (Teague et al., 2007).

A metabolic by-product from the stress-induced increased in energy production is the formation of reactive oxygen species (ROS) such as superoxide radical from the mitochondrial electron transport chain (Du et al., 2009). One other potential source of superoxide production is through the tightly regulated multi-protein enzyme, nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase) (Brennan et al., 2009). Active oxidase consists of two membrane proteins, gp91phox and p22phox, and four cytosolic proteins, p40phox, p47phox, p67phox, and Rac. Upon stimulation, the cytosolic components translocate to the membrane to complex with the membrane proteins, resulting in production of superoxide via reduction of molecular oxygen. Moreover, in non-phagocytic cells, NADPH oxidase has been shown to generate ROS for the regulation of many cellular activities including transcription and intracellular signalling (Jiang et al., 2011).

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The endogenous antioxidant system systematically attempts to reduce superoxide to water through a series of intermediate ROS, each of which are capable of causing oxidative damage to proteins, lipids via peroxidation, and nucleic acids (Liu et al., 1996; Spiers et al., 2014). Traditionally, this is achieved by enzymatic and non-enzymatic redox cycling of the glutathione redox couple, in addition to other members of the antioxidant response pathway governed by the transcription factor, nuclear factor (erythroid-derived 2)-like 2 (Nrf2). Non-traditional antioxidants such as metallothionein also possess potent reducing and recycling capability (Oteiza, 2012). Although the antioxidant defence system is extremely effective, in rodents there is still an estimated 100,000 oxidative 'hits' to DNA per cell per day, the majority of which occur in the mitochondria (Ames et al., 1993). Liu and Zhou (2012) exemplified this using both chronic corticosterone administration and chronic unpredictable stress models to show increases in neural mitochondrial ROS production, in addition to decreased antioxidant activities of superoxide dismutase and the glutathione redox couple. The reduction/oxidation (redox) state of glutathione is crucial for maintaining oxidant reducing capability, as this has been demonstrated to govern progression of the cell cycle and the fate of differentiating stem cells (Wang et al., 2011). In regions of high neurogenesis and synaptic plasticity such as the hippocampus, this is particularly relevant as small perturbations in redox state may have long term implications.

We have recently demonstrated that peripheral measures of oxidative status and glutathione are significantly affected by an acute psychological stress (Spiers et al., 2013; Chen et al., 2014). These peripheral markers give an indication of the general redox state of the individual and have been suggested as useful biomarkers of chronic pathological redox-related conditions including the central neurodegenerative Alzheimer's, Parkinson's, and Huntington's diseases (Klepac et al., 2007; Zhou et al., 2008; Skoumalova and Hort, 2012). However, the acute physiology of the central redox system has only sparsely been studied and generally focuses on a single treatment or time-point. In the present study, we have examined the redox profile in the hippocampus and striatum, regions of the brain that display differential redox responses following acute stress exposure (Liu et al., 1996). This was additionally characterized to demonstrate the importance of glucocorticoid receptor signalling in these responses. Furthermore, we have assessed the mRNA levels of the canonical antioxidants glutathione peroxidase 1 (Gpx1) and 4 (Gpx4), Nrf2, and the non-canonical heavy-metal binding antioxidant, metallothionein 1a (Mt1a), all of which may play important physiological roles in neuroprotection following oxidative challenges induced by acute stress.

2. Materials and methods

2.1. Experimental animals

Outbred male Wistar rats (*Rattus norvegicus*) aged 6–8 weeks postnatal were housed individually in a colony room and given *ad libitum* access to standard rat chow and water. This room was on a 12:12 h light-dark cycle (lights off at 12.30 h) and maintained under standard laboratory conditions ($22 \pm 2\,^{\circ}C$; $55 \pm 5\%$ humidity). Experimental procedures were performed in strict adherence to the conditions approved by The University of Queensland Animal Ethics Committee with approval numbers 018/11 and 456/14.

2.2. Acute restraint stress

Restraint stress was applied using custom made adjustable wire mesh rat restrainers equipped with a rear tail aperture to facilitate tail bleeds described previously (Spiers et al., 2013). In order to isolate the effects of restraint, control animals were housed under similar conditions with no access to food and water during the treatment period.

2.3. Experimental protocol

2.3.1. Experiment part A

One week prior to experimentation, all animals were habituated to human handling for 10 min each day. On the experimental day, animals were transported to an experimental room under low light, noise, and other experimental disturbances one hour prior to the beginning of the experiment. Food and water were removed during this period and animal home cages were housed within larger external isolation boxes to further reduce disturbances. Animals were randomly allocated to control or restraint stress for 60, 120, or 240 min ($n = 6$–7 per group) during which blood was collected via tail-tipping immediately prior to treatment and following restraint stress. All blood samples were collected in sodium heparin (20 IU/mL blood) as an anticoagulant and a small aliquot was removed for blood glucose determination using a standard glucometer (Accu-Chek® Performa®, Roche Diagnostics Aust. Pty. Ltd.). Following each stress treatment, animals were overdosed with 100 mg/kg of sodium pentobarbital (IP injection; Lethabarb, Virbac, Peakhurst, Australia) and the brain removed and frozen on powdered dry ice for storage at $-80\,^{\circ}C$. Frozen brains were sectioned on a cryostat and the hippocampus and striatum were cryodissected from sections according to a rat brain atlas (Paxinos and Watson, 2007) on a dry ice-embedded metal platform. Regionalized neural tissues were stored at $-80\,^{\circ}C$ for later determination of neural oxidative status, glutathione/glutathione disulphide (GSH/GSSG), lipid peroxidation, and relative gene expression. All blood samples were centrifuged at $2000 \times g$ for 5 min immediately following collection and the resulting plasma supernatant was aliquoted and stored at $-80\,^{\circ}C$ for corticosterone, general oxidative status, and lipid peroxidation analysis.

2.3.2. Experiment part B

A separate cohort of male Wistar rats was habituated to human handling for 10 min per day one week prior to experimentation. On each experimental day, rats were transported in individual home cages to an experimental room and randomly allocated to 4 treatment groups with control or restraint stress for 60 min receiving either vehicle or RU-486 (mifepristone) injections ($n = 8$/group). The glucocorticoid receptor antagonist, RU-486 (10 mg/kg), was dissolved in 5% DMSO in normal saline and injected intraperitoneally 60 min before control or stress treatment at a final injection volume of 2 mL/kg. At the end of each treatment, rats were overdosed with 100 mg/kg of sodium pentobarbital (IP injection; Lethabarb, Virbac, Peakhurst, Australia) and the brain rapidly removed and frozen on powdered dry ice for storage at $-80\,^{\circ}C$. Frozen brains were cryodissected from sections according to a rat brain atlas (Paxinos and Watson, 2007). Regionalized neural tissues were stored at $-80\,^{\circ}C$ for later determination of neural oxidative status, glutathione/glutathione disulphide (GSH/GSSG), and lipid peroxidation.

2.4. Plasma corticosterone assay

Corticosterone was determined in samples using a previously described radioimmunoassay utilising a highly specific ovine anti-rat corticosterone polyclonal antibody (Sapphire Bioscience Pty. Ltd.) and tritiated [1,2,6,7-3H]-corticosterone tracer (Spiers et al., 2013). The intra-assay and inter-assay coefficients of variation were 4.34% and 4.59% respectively.