ISSN 1819-7124, Neurochemical Journal, 2014, Vol. 8, No. 2, pp. 125–128. © Pleiades Publishing, Ltd., 2014.

EXPERIMENTAL ARTICLES

Evaluation of Antioxidant Enzymes in Response to Predator Odor Stress in Prefrontal Cortex and Amygdala[1]

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Received December 30, 2013

Abstract—Conditions of stress can originate from diverse stimuli including physical, chemical, antigenic and psychological. The latter is processed in part via the hypothalamic-pituitary-adrenal (HPA) axis, with input from and communication between the amygdala (AM) and prefrontal cortex (PFC). The HPA axis generates an increase in circulating glucocorticoids, augmenting metabolism and, consequently, oxygen consumption, increasing the production of free radicals and reactive oxygen species (ROS). Exposure to predator odor as a model of non-invasive acute stress was used to evaluate the hypothesis that psychogenic stress can modify enzymatic antioxidant responses. The activities of various enzymes, catalase (CAT), cytosolic and mitochondrial superoxide dismutase (Cu, Zn-SOD and Mn-SOD, respectively) and glutathione S-transferase (GST), were determined in AM and PFC. Acute psychogenic stress inhibited CAT activity in the AM and PFC, and increased Mn-SOD activity in the PFC. These results demonstrate that different responses can be elicited by the same stressor in two separate brain regions involved in processing emotional stimuli, and that changes in specific antioxidant enzymatic responses can be seen with exposure to acute psychogenic stress.

Keywords: *catalase, superoxide dismutase, glutathione S-transferase, oxidative stress, psychogenic stress, amygdala, prefrontal cortex*

DOI: 10.1134/S181971241402007X

INTRODUCTION

The central nervous system has a high metabolic demand, consuming approximately 20% of total inspired oxygen, which makes it vulnerable to damage by free radicals and reactive oxygen species (ROS). Neurons are particularly sensitive to this damage, as they have high concentrations of polyunsaturated fatty acids, ascorbate, and iron, and intrinsically low quantities of the antioxidant enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione S-transferase (GST) [1–3]. Psychogenic stress is a significant contributor to altered metabolism, through changing vegetative physiological functions such as heart rate, muscle tone and sweating, and activation of the hypothalamic-pituitary-adrenal (HPA) axis and related structures such as the prefrontal cortex (PFC) and amygdala (AM) [4–6]. The PFC serves as a convergence zone for exogenous and endogenous stimuli, and can inhibit the activation of other brain regions when there is no reinforcement for a stimulus [7, 8]. The AM has reciprocal innervations with the hypothalamus and PFC, and functions in mediating HPA axis responses to external stimuli. Increased activation in the PFC and AM can ultimately regulate autonomic and HPA axis outputs, inducing catecholamine and glucocorticoid release, and subsequently increase oxygen metabolism and the production of ROS [5, 9]. Although ROS are normally eliminated from the cell by a number of molecular mechanisms, they are also important regulators of the endogenous cellular "REDOX" state, and thus have a role in controlling the expression of antioxidant enzymes [10, 11].

The antioxidant enzyme response has been explored previously using different physical stressors [12–14]. To the best of our knowledge, however, this response has not been explored following an acute episode of psychogenic stress, nor in the AM or PFC which are specific brain regions at least partly responsible for processing this type of stimulus. The aim of this study was to examine antioxidant enzyme responses, namely the antioxidant capacity of the AM

[1] The article is published in the original.

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and PFC as determined by the enzymes CAT, SOD and GST, to acute psychogenic stress. The purpose of this work was to assess the neurological consequences of the type of acute stress exposure that may reflect the daily emotional stress exposures often experienced by humans, with the goal of developing potential strategies for the prevention or treatment of stress-related illnesses. While not considered in this experiment, the effects of physical or social stressors, alone or in combination with psychological stressors, are also relevant and warrant further study.

MATERIALS AND METHODS

Adult male Sprague Dawley rats (230–280 g; Harlan, México) were housed individually in standard vivarium cages at 25 ± 1°C on a 12 hour light-dark cycle, food (rat chow) and tap water were given ad libitum. Rats were randomly assigned to control and acute stress groups (n = 6/group). Stress was carried out between 10 am and 12 pm (light cycle) in a Plexiglass cage [15]. Stressed rats were exposed for 1 h to a cloth that had been placed in the bed of a domestic cat for 24 h and then rubbed against the cat's fur before testing, while control rats were exposed to a similar cloth free of cat odor [15]. After the hour of exposure, all rats were immediately euthanized by rapid decapitation; brain tissues were harvested and frozen on dry ice. AM and PFC regions were dissected bilaterally according to bregma coordinates from the Rat Brain Atlas [16].

Isolated AM and PFC tissues were thawed in 50 mM cold phosphate potassium buffer pH 7.4 with 10% PMSF (Sigma-Aldrich, China), and all procedures were performed on ice. Tissues were homogenized and sonicated 3 times at 30 w (6.9 kHz; Sonic Dismembrator, Model 100, Fischer Scientific) for 10 s at 1 min intervals. The homogenates were divided into two aliquots: the first was centrifuged at 52 g for 20 min at 0°C and the supernatant collected to evaluate CAT; the second was centrifuged at 20.800 g for 30 min at 0°C and the supernatant used to evaluate SOD and GST [17]. Supernatants were frozen and stored at –20°C until the time of assay, which was performed within 7 days.

To evaluate the specific enzyme activity of CAT, a kinetic protocol was followed [18]. This method evaluates the dismutation of hydrogen peroxide (H_2O_2) into water and molecular oxygen; the reaction was followed for 3 min at 240 nm. One unit of CAT was defined as the amount of enzyme necessary to dismutate one μmol of H_2O_2/min. To measure total activity of SOD (tSOD) a kinetic protocol was also used [19], which followed the auto-oxidation of pyrogallol (Sigma-Aldrich, Japan) for 3 min at 420 nm. One unit of SOD was defined as the amount of enzyme necessary to cause a 50% inhibition of the production of oxidized pyrogallol. To determine the specific activity of mitochondrial SOD that is Mn-dependent (Mn-SOD), 110 mM NaCN (Sigma-Aldrich, USA) was added to the reaction mixture and the enzyme assay was carried out as previously described. Differences between the values obtained for tSOD and Mn-SOD reflect the specific activity of Cu, Zn-SOD. GST activity was determined by using 1-chloro-2,4-dinitrobenzene (CDNB, Sigma-Aldrich, USA) [20], adjusted for use in a microassay [21]. This method evaluates the appearance of the compound GS-CDNB, catalyzed by GST at 340 nm. One unit of GST was defined as the amount of enzyme necessary to catalyze the formation of one μmol of GS-CDNB complex/min. All enzyme assays are reported as mg total protein as compared to a known protein standard (BSA; Sigma-Aldrich, USA) [22].

Comparisons were made between control and stressed groups for all variables; the data are expressed as the mean ± standard deviation for 6 rats per group. Significance was determined by Student's T-test with significance at the $p \leq 0.05$ level.

RESULTS

Acute exposure to predator odor led to changes in animal behavior at the time of euthanasia. Compared to the control group, animals exposed to predator odor displayed anxiety-like behaviors such as bristling back hair and increased release of fecal material. Specific enzyme activities for CAT, Cu, Zn-SOD, Mn-SOD and GST are shown in table. Overall, in the AM and PFC, a trend toward increasing enzyme activities was observed in response to acute predator odor exposure, as compared to the no odor control condition. CAT activity was decreased by 60% in the AM ($p \leq 0.05$), while Cu, Zn-SOD tended to show increases in activity of up to 40%, but this finding was non-significant ($p > 0.1$). Mn-SOD and GST activities were increased by 12 and 17%, respectively, in the AM, but again these results were not statistically significant ($p > 0.1$). A significant decrease in CAT activity was also observed in the PFC, although to a lesser degree (11%) than that seen in the AM. Cu, Zn-SOD showed a non-significant trend toward increased activity in the PFC, while Mn-SOD activity increased significantly by 57% and GST displayed a nearly-significant increase of 26% ($p = 0.06$).

DISCUSSION

Psychogenic stressors raise blood pressure and elevate oxygen consumption and metabolism [23], increase dopamine (DA)-dependent signals [24], increase the release of adrenaline and noradrenaline, and increase catecholamine metabolism [25, 26]. Our findings show increased Mn-SOD activity in the PFC following psychogenic stress in the form of acute predator odor exposure. This increase could result from

Activity of antioxidant enzymes in amygdala and prefrontal cortex[a]

Enzyme	Group	Amygdala	Prefrontal Cortex
CAT	Control	178.44 ± 38.5	128.62 ± 4.4
	Stressed	68.94 ± 16.1**	114.03 ± 4.8**
Cu, ZnSOD	Control	4.59 ± 0.77	4.27 ± 0.53
	Stressed	6.41 ± 0.76	5.06 ± 0.41
Mn-SOD	Control	3.93 ± 0.56	3.98 ± 0.38
	Stressed	4.39 ± 0.51	6.24 ± 0.30***
GST	Control	5.89 ±0.84	4.51 ± 0.36
	Stressed	6.89 ± 0.97	5.65 ± 0.42*

[a] Control (rats not exposed to cat odor); stressed (rats exposed to cat odor). Catalase (CAT) expressed as mU per mg of protein; cytosolic superoxide dismutase (Cu, Zn-SOD) and mitochondrial superoxide dismutase (Mn-SOD) are expressed as U per mg of protein; glutathione S-transferase (GST) expressed as mU per mg of protein. Values are mean ± standard deviation of 6 animals. Significantly different from control at T-Test * $P \leq 0.1$; ** $P \leq 0.05$; *** $P \leq 0.001$.

enhanced mitochondrial respiratory chain activity, causing an overproduction of superoxide anion ($O_2^{-\bullet}$) and giving rise to elevated Mn-SOD activity as a compensatory mechanism [27, 28]. Another explanation could be that post-translational modifications occurred in response to elevated ROS production [29, 30], resulting in an elevation of Mn-SOD activity.

Dismutation of $O_2^{-\bullet}$ produces H_2O_2, as does the oxidation of biogenic amines by monoamine oxidase [31]. Together, Mn-SOD and monoamine oxidase activities could potentially lead to an elevation of H_2O_2 in the cytosol, which should lead to an elevation in CAT activity. However, as our group and a few others have shown in various brain structures [14, 26, 32], CAT activity decreased in response to acute stress exposure, therefore allowing H_2O_2 to accumulate in the cytosol where it may readily convert to hydroxyl radicals (OH^-) through the Fenton reaction [33]. The stress-associated inhibition of CAT activity in the AM and PFC seen in our study is supported by previous experiments demonstrating that high concentrations of hydroxyl radicals, superoxide anions and even hydrogen peroxide can inhibit CAT [34]. The only significant change seen in the AM in our study was a decrease in CAT activity; this inhibition was likely due to increased catecholamine metabolism, and increased H_2O_2 production and conversion to OH^-.

To the best of our knowledge, enzymatic antioxidant activities in response to an acute episode of psychogenic stress have not yet been explored in the AM and PFC. Several models of stress have been used to examine the antioxidant response to stressful events in diverse brain structures [14, 32, 35–37]. Few of these, however, have evaluated antioxidant enzyme activities in the principal neurological regions responsible for emotion processing [38, 39]. Specifically, no study to date has assessed enzymatic antioxidant responses in the AM and PFC following exposure to predator odor. Similar to our findings of CAT inhibition in both of these structures, decreased CAT activity has also been seen in the rat hippocampus after acute immobilization or cold stress [14, 26], and in whole brain under sub-acute cyanide exposure [32]. Our increases in Mn-SOD activity in the PFC may indicate that this enzymatic response differs in conditions of psychogenic stress, compared to physical stress, as 2 h of immobilization or cold has been shown to decrease Mn-SOD activity [14] or cause no change at all [26].

CONCLUSIONS

The AM and PFC, two primary central nervous system regions responsible for emotional processing, respond to acute psychogenic stress by inducing antioxidant (increased Mn-SOD in the PFC) and prooxidative (decreased CAT in the AM) states. We have shown that this type of negative emotional state can trigger oxidative damage, and alter the REDOX state of neurons, which are novel findings of importance to the scientific community and have broad implications for health and disease.

ACKNOWLEDGMENTS

CONACyT (Fondo 10013) grants 91191 and 89791; Universidad Autónoma de Ciudad Juárez and Escuela Nacional de Medicina y Homeopatia-Instituto Politécnico Nacional. Mejia-Carmona GE acknowledges a fellowship by CONACyT and support by UACJ.

This work was supported by Grant 5G12MD007592 from the National Institutes on Minority Health and Health Disparities (NIMHD), and Grant 1R24DA029989 from the National Institute on Drug Abuse (NIDA), components of the National Institutes of Health (NIH).

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