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Changes in Antioxidant Defense Enzymes after D-amphetamine Exposure: Implications as an Animal Model of Mania

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Abstract Studies have demonstrated that oxidative stress is associated with amphetamine-induced neurotoxicity, but little is known about the adaptations of antioxidant enzymes in the brain after amphetamine exposure. We studied the effects of acute and chronic amphetamine administration on superoxide dismutase (SOD) and catalase (CAT) activity, in a rodent model of mania. Male Wistar rats received either a single IP injection of D-amphetamine (1 mg/kg, 2 mg/kg, or 4 mg/kg) or vehicle (acute treatment). In the chronic treatment rats received a daily IP injection of either D-amphetamine (1 mg/kg, 2 mg/kg, or 4 mg/kg) or vehicle for 7 days. Locomotor behavior was assessed using the open field test. SOD and CAT activities were measured in the prefrontal cortex, hippocampus, and striatum. Acute and to a greater extent chronic amphetamine treatment increased locomotor behavior and affected SOD and CAT activities in the prefrontal cortex, hippocampus and striatum. Our findings suggest that amphetamine exposure is associated with an imbalance between SOD and CAT activity in the prefrontal cortex, hippocampus and striatum.

Keywords Animal model · Amphetamine · Bipolar disorder · Catalase · Oxidative stress · Superoxide dismutase

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Introduction

There is an emerging body of data indicating that major neuropsychiatric disorders, such as bipolar disorder (BD) and schizophrenia, are associated with increased oxidative stress and changes in antioxidant enzymatic defense [1–3]. A recent genetic study found that BD was significantly associated with a single-nucleotide polymorphism in the TRPM2 gene, which is involved in intracellular calcium homeostasis in response to oxidative stress [4]. In addition, chronic administration of lithium and valproate (first-line mood stabilizers) demonstrated robust antioxidant properties against glutamate-induced oxidative stress in vitro [5].

It has long been recognized that the administration of dopaminergic drugs induces manic symptoms in individuals with BD [6, 7]. Further, the use of amphetamine in healthy volunteers induced manic symptoms, such as enhanced mood, racing thoughts, high energy and restlessness [8]. Considering the difficulty of modeling the highly complex mood swinging nature of BD, the psychostimulant-induced

hyperactivity is the best established animal model of mania [9–11]. We have recently found that longer administration of amphetamine was associated with increased protein and lipid oxidative damage in rat brain [12]. As an extension of this latter study, we assessed the effects of acute and chronic amphetamine administration on superoxide dismutase and catalase activity (two major antioxidant enzymes) in a rodent model of mania.

Experimental procedure

Adult male Wistar rats, obtained from our breeding colony, were maintained on a 12-h light/dark cycle, with free access to food and water. All experimental procedures involving animals were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behavior (SBNeC) recommendations for animal care. Rats received 1 mg/kg, 2 mg/kg, or 4 mg/kg IP injections of D-amphetamine (Sigma, St Louis, USA) either as an acute treatment (single injection) or chronic treatment (once daily injection for 7 days). Locomotor activity was measured 2 h after the last injection, and the rats were sacrificed by decapitation right after the behavioral experiment. The prefrontal cortex, hippocampus and striatum were dissected, rapidly frozen, and stored at −80°C until assayed.

Locomotor activity was assessed using the open-field task. This task was carried out in 40×60 cm open field surrounded by 50 cm high walls made of brown plywood with a frontal glass wall. The floor of the open field was divided into 12 equal rectangles by black lines. The animals were gently placed on the left rear rectangle, and allowed to explore the arena. Crossings of the black lines and rearings performed were counted for 5 min. To determine CAT activity, the brain tissue was sonicated in 50 mM phosphate buffer and the resulting suspension was centrifuged at 3,000g for 10 min. The supernatant was used for enzyme assay. CAT activity was measured by the rate of decrease in hydrogen peroxide absorbance at 240 nm [13]. SOD activity was assayed by measuring the inhibition of adrenaline auto-oxidation, as previously described [14]. Differences among groups were performed using one-way ANOVA and multiple comparisons were performed by a Newman-Keuls test. Behavioral data are presented as mean ± SEM. and biochemical data are presented as mean ± SD. In all comparisons, $P < 0.05$ was considered to indicate statistical significance.

Results

As expected, both acute and repeated amphetamine administration significantly increased locomotor activity

(Figs. 1, 2). Figures 3 and 4 illustrate that a single amphetamine injection increased SOD activity in the prefrontal cortex, whereas repeated amphetamine exposure increased SOD activity in the hippocampus and decreased striatal SOD activity. No effects on CAT activity were observed after a single amphetamine injection in any brain region (Fig. 5). Repeated amphetamine administration increased CAT activity in the prefrontal cortex and decreased striatal CAT activity (Fig. 6). Higher amphetamine dosage (4 mg/kg) increased CAT activity, whereas a lower dosage (2 mg/kg) decreased CAT activity in the hippocampus after repeated amphetamine exposure.
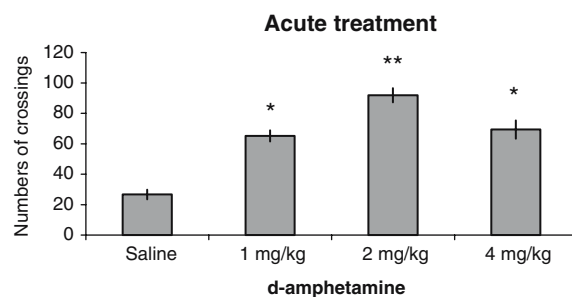

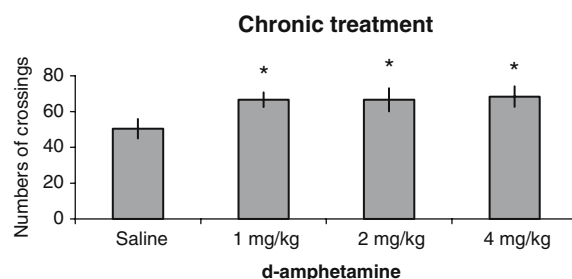Fig. 1 Numbers of crossings in the acute treatment

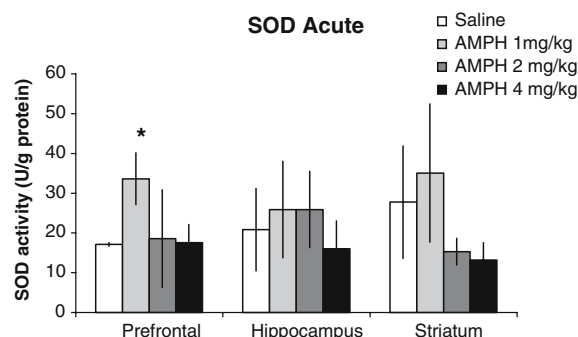Fig. 2 Numbers of crossings in the chronic treatment

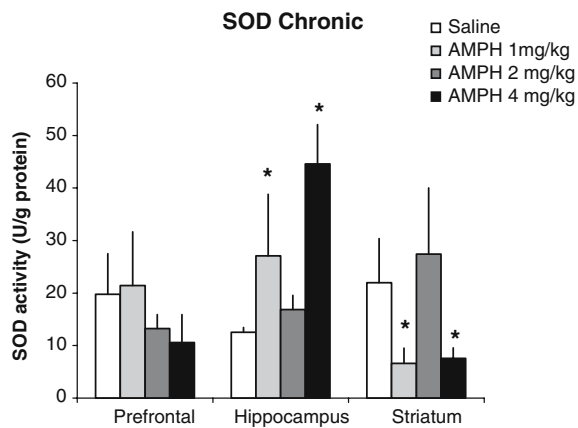Fig. 3 SOD activity in the acute treatment SOD: superoxide dismutase

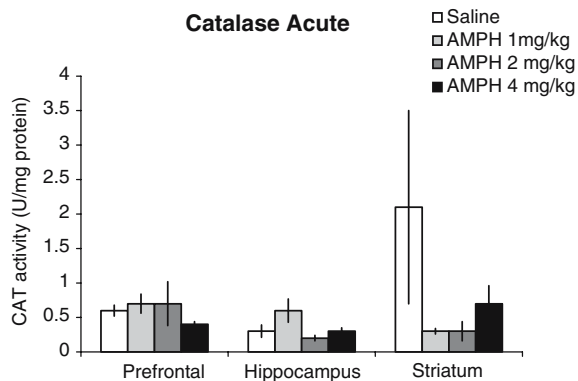Fig. 4 SOD activity in the chronic treatment SOD: superoxide dismutase

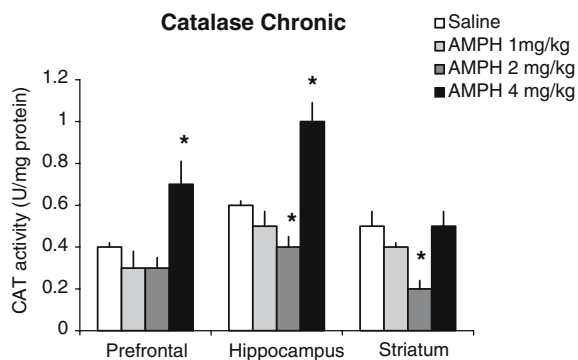Fig. 5 Catalase activity in the acute treatment

Fig. 6 Catalase activity in the chronic treatment

Discussion

In the present study, we demonstrated that acute and, to a greater extent, repeated amphetamine exposure modified SOD and CAT activities in the prefrontal cortex, hippocampus and striatum. Using the same animal model, we have recently reported that repeated amphetamine admin-istration increased oxidative stress in a greater extent than single amphetamine use [12]. SOD acts by metabolizing the excess of superoxide anion (O_2^-) generation and by producing hydrogen peroxide (H_2O_2). CAT metabolizes the excess of H_2O_2 producing $O_2 + H_2O$, thereby decreasing the intracellular redox status. The brain is par-ticularly prone to oxidative damage due to its relative high content of peroxidizable fatty acids and limited antioxidant capacity [15]. Previous studies have demonstrated that alterations on the redox state can lead to an imbalance between SOD and CAT activities and to oxidative stress [16, 17]. In situations which SOD levels are increased without a concomitant CAT increase, the intermediate product H_2O_2 may accumulate and generate hydroxyl radicals, which may lead to lipid and protein oxidation (damage).

Even though there is compelling data indicating that oxidative stress plays a major role in amphetamine-induced neurotoxicity [18, 19], there is a paucity of data assessing the effects of amphetamine on antioxidant enzymes. In a model of neurotoxicity (4 × 10 mg/kg), Jayanthi et al. [20] found that methamphetamine exposure decreased SOD activity in the frontal cortex and decreased CAT activity in the striatum of CD-1 mice. Using a different model (20 mg/kg for 14 days), Carvalho et al. [21] showed that amphetamine decreased SOD activity in the striatum and increased CAT activity in the prefrontal cortex of Wistar rats. On the other hand, D'Almeida et al. [22] found no changes in SOD and CAT activity after chronic metham-phetamine treatment (2.5 mg/kg for 5 months) in Wistar rats. The profound methodological differences between studies make it difficult to draw conclusions, and therefore further studies are necessary to clarify the importance of these antioxidant changes. Interestingly, it has been reported that the dopamine D2 receptor agonist ropinirole protected mouse striatal neurons against 6-hydroxydop-amine (6-OHDA) toxicity, by increasing SOD, CAT, and glutathione activity [23], whereas the dopamine agonist cabergoline demonstrated robust antioxidant effects against 6-OHDA-neurotoxicity [24]. In addition, the stimulation of D2 presynaptic autoreceptors may exert neuroprotective effects by a negative feedback mechanism, reducing the release of dopamine for oxidation by monoamine oxidase [25]. Studies assessing the effect of D2 blockers on amphetamine-induced oxidative stress would help to increase our knowledge on the dopamine-mediated neuro-protection and neurotoxicity.

Acute and chronic amphetamine exposure modulated SOD and CAT activity with a distinct pattern for each brain region and dosage regimen. These findings may explain, in part, why different areas of the brain are differentially susceptible to the toxic effects of amphetamine [26, 27]. One reason for these discrepancies may be fact the basal

activities of various antioxidant enzymes, such as SOD, CAT, glutathione reductase, glutathione peroxidase, and glutathione-S-transferase are highly variable across brain regions [21]. It has been recently demonstrated that amphetamine regulates the expression of SOD mRNA via c-fos/c-jun activation in the hypothalamus [28]. Although speculative, it is possible that the regulation of antioxidant's gene expression by amphetamine in other brain regions may be relevant to the differences observed. Future studies addressing the genes regulated by amphetamine exposure may help us clarify this issue.

We also found that during acute treatment, 4 mg/kg of amphetamine increased the locomotor behavior to a lesser extent than 2 mg/kg. During chronic treatment all of the dosages induced the same level of locomotor activation. This is in line with previous reports showing that the locomotor activity after acute amphetamine challenge reduces with increasing dosage, due to the emergence of stereotypic behavior [29, 30]. In conclusion, our findings suggest that acute and, to a greater extent, chronic amphetamine administrations are associated with an imbalance between SOD and CAT activities. This is possibly due to changes in the intracellular redox state, in the prefrontal cortex, hippocampus, and striatum. Such an imbalance may increase the predisposition to the generation of free radicals and therefore increase the susceptibility to oxidative damage. Given the recent evidence that oxidative stress may play a role in the pathophysiology of BD [1–4], this animal model may be a useful tool to further test the molecular underpinnings underlying amphetamine-induced oxidative stress.

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