Harmine and imipramine promote antioxidant activities in prefrontal cortex and hippocampus

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A growing body of evidence has suggested that reactive oxygen species (ROS) may play an important role in the pathogenesis of neurological and psychiatric diseases including bipolar disorder and major depression.¹⁻⁹

ROS are free radicals or reactive anions/molecules containing oxygen atoms, such as hydroxyl radical, superoxide, hydrogen peroxide and peroxynitrite. ROS can cause cell damage by enzyme inactivation, lipid peroxidation and DNA modification.¹⁰ Oxidative stress is well known to contribute to neuronal degeneration in the central nervous system (CNS) in the process of aging, as well, in neurodegenerative diseases. Studies have consistently reported increase ROS in plasma on patients with major depression, especially with melancholia associated.¹¹ Recent study showed evidences of oxidative stress in major depression as reflected in increased oxidative stress from frontal regions of patients compared to those of matched controls.¹² Moreover we showed that rats subjected to chronic mild stress (CMS) had increase in superoxide production in hippocampus, prefrontal cortex and cortex brain and thiobarbituric acid reactive in cortex.¹ In addition we demonstrated that stressed rats had increase protein (prefrontal, hippocampus, striatum and cortex) and lipid peroxidation (cerebellum and striatum), increase catalase (cerebellum, hippocampus, striatum and cortex) and a decrease in superoxide dismutase activity (prefrontal cortex, hippocampus, striatum and cortex).² Additionally, oxidative stress is able to affect a number of synaptic functions, resulting in impaired neurotransmission.³

The monoamine hypothesis posits that depression is caused by decreased monoamine function in brain.⁴ Actually, the clinically-used antidepressants increase the extracellular concentrations of monoamines serotonin or norepinephrine either by inhibiting their reuptake from the synapse or by blocking their degradation by inhibiting monoamine oxidase.⁵⁻⁷

Recently, studies have reported that β-carboline harmine possesses antidepressant properties.⁸⁻¹⁰ In fact, harmine interact with monoamine oxidase A (MAO-A) [21] and several cell-surface receptors, including serotonin receptor 2A (5-HT2A), which are involved in antidepressant pharmacotherapy.¹²⁻¹³

Because of these findings, we designed the present study to investigate the effects of acute and chronic administration of harmine, imipramine (standard antidepressant) and saline on lipid and protein oxidation levels (markers of oxidative stress) and on superoxide dismutase (SOD) and catalase (CAT) activities (the major antioxidant enzymes) in rat brain.

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imipramine at doses of 10 and 30 mg/kg and harmine in all doses reduced the lipid peroxidation in prefrontal cortex; and in all doses both harmine and imipramine decreased the lipid peroxidation in hippocampus ($F = 10.44; p < 0.05$; Fig. 1B).

**Protein carbonyls.** As depicted in Figure 2A, acute administration of imipramine in all doses decreased protein carbonilation in prefrontal cortex and in hippocampus; however, acute treatment with harmine reduced protein carbonilation at doses of 5 and 15 mg/kg in prefrontal cortex and at dose of 15 mg/kg in hippocampus ($F = 2.18; p < 0.05$). In the chronic treatment only the higher (15 mg/kg) of harmine decreased the protein carbonilation in prefrontal cortex and hippocampus and imipramine at dose of 20 mg/kg decreases protein carbonilation in hippocampus ($F = 28.8; p < 0.05$; Fig. 2B).

**Catalase activity.** The intraperitoneal acute treatment with imipramine in doses of 10 and 30 mg/kg and harmine in dose of 5 mg/kg increased catalase activity in prefrontal cortex; treatment with imipramine in all doses and harmine at doses of 5 and 10 mg/kg increased catalase activity in hippocampus ($F = 4.9; p < 0.05$; Fig. 3A). In the chronic treatment only imipramine at the dose of 20 mg/kg increased catalase activity in prefrontal cortex in comparison with control group ($p < 0.05$; Fig. 3B).

**Superoxide dismutase activity.** The superoxide dismutase activity increased in prefrontal cortex after acute treatment with imipramine at dose of 20 mg/kg and harmine at doses of 5 and 15 mg/kg. In hippocampus the superoxide dismutase activity increased only harmine at dose of 5 mg/kg ($F = 2.45; p < 0.05$; Fig. 4A). Figure 4B showed the superoxide dismutase activity after chronic treatment with imipramine and harmine. Only the higher dose (30 mg/kg) of imipramine increased superoxide dismutase activity in prefrontal cortex; and only harmine at dose of 5 mg/kg increased superoxide dismutase activity in hippocampus, compared to the control group ($p < 0.05$).

**Discussion**

In the present study we showed that both acute and chronic treatments with imipramine and harmine reduce lipid and protein peroxidation and increased superoxide and catalase activities, respectively. These findings suggest a potential role of these compounds in the modulation of oxidative stress and may have implications for the development of novel therapeutic strategies for the treatment of oxidative stress-related disorders.
compared to saline group in hippocampus and prefrontal cortex.

The hippocampus is one of several limbic structures that have been implicated in mood disorders. In addition, the hippocampus has connections with the prefrontal cortex, region that is more directly involved in emotion and cognition and thereby contributes to other major symptoms of mood disorders.24,25

The brain is particularly vulnerable to reactive oxygen species (ROS) production because it metabolizes 20% of total body oxygen and has a limited amount of antioxidant capacity.10

The oxidative stress in rat brain structures may play a role in the pathogenesis of anxiety26,27 and depression.28 In fact, our group very recently showed that rats subjected to chronic mild stress (CMS) had an increase in superoxide production and thiobarbituric acid reactive protein and lipid peroxidation and catalase activity, and a decrease in superoxide dismutase activity in rat brain.5,6 Moreover, a previous study using an animal model of repeated restraint stress showed that this model induced an increase in TBARS levels in hippocampus.29 In another study, it was demonstrated that animal model of immobilization stress causes significant increases in lipid peroxidation, which was found in cerebral cortex, cerebellum and hippocampus compared to the unstressed controls; significant increases in levels of protein oxidation were also found in cortex, hypothalamus and striatum; oxidative nuclear DNA damage increased after stress in all brain regions, although only the cerebral cortex showed a significant increase.30 In humans elevated ROS in plasma of patients with major depression, especially in those with melancholic type and increased oxidative stress in depressive woman, was demonstrated.31 Additionally, Galecki et al.32 demonstrated that combined fluoxetine antidepressant and acetylsalicylic acid therapy improvement of oxidative stress parameters in patients with depression. Moreover, exogenous administration of 5-hydroxytryptophan prevented depletion of serotonin concentration and antioxidant status induced by p-chlorophenylalanine in rat brain.32

In this study we showed that acute and chronic treatment with imipramine antidepressant improvement of oxidative stress parameters in rat brain. Several studies have reported the role of imipramine in oxidative stress parameters. In fact, imipramine treatment reversed the lipid peroxidation in brain of Sprague Dawley rats induced by chronic ozone.33 In addition, acute treatment with imipramine (10 and 20 mg/kg) and venlafaxine (5 and 10 mg/kg) reversed immobilized stress-induced behavioral and biochemical (such as malondialdehyde level, nitrite, glutathione and catalase enzyme) alterations in mice; in some study was showed that L-NAME and/or methylene blue potentiated the effect of both imipramine and venlafaxine, suggesting the involvement of nitric oxide mechanism in the protective effect of imipramine and venlafaxine.34 In other hand, acute
Nevertheless, a significant recovery in the activities of superoxide dismutase, catalase, glutathione S-transferase, glutathione reductase and glutathione levels by fluoxetine, imipramine and venlafaxine treatments following a restraint stress-induced decline of these parameters, and accumulated lipid peroxidation product malondialdehyde and protein carbonyl contents in stressed animal were normalized by some antidepressants.37

Moreover, we also demonstrated that β-carboline harmine improvement of oxidative stress parameters. Kim et al.38 reported that harmine has a role in oxidative stress. In fact, they showed that β-carbolines (harmaline, harmalol and harmine) attenuated the dopamine or 6-hydroxydopamine-induced alteration of brain mitochondrial and synaptosomal functions, and viability loss in PC12 cells, by a scavenging action on reactive oxygen species and inhibition of thiol oxidation.38

Our group has shown antidepressant properties of harmine.19,20 In fact, treatment with harmine at doses of 10 and 15 mg/kg and imipramine at doses of 20 and 30 mg/kg decreased immobility time of rats, and increased both climbing and swimming time of rats compared to saline group, and were also showed that imipramine and harmine did not affect spontaneous locomotor activity in the open-field test.19 In this study were demonstrated that harmine (15 mg/kg) increased brain-derived neurotrophic factor (BDNF) protein levels in rat hippocampus. In other study from our group showed that harmine reverted stress parameters induced by chronic mild stress model.20 In addition, Farzin et al.18 demonstrated that treatment with harmane, norharmane and harmine dose-dependently reduced the immobility time in the mouse forced swimming test. These studies suggest antidepressant-like effects of harmine could be due to interactions of harmine with several receptor systems involved in the modulation of behavioral and molecular actions of antidepressants.

In conclusion, considering that oxidative stress is probably involved in the pathophysiology of depression, the modulation by antidepressants could be an important mechanism of action of these drugs and harmine could be a positive effect in oxidative stress parameters, which may play a role in the pathogenesis of depression.

Figure 3. Effects of acute (A) and chronic (B) administration of imipramine (10, 20 and 30 mg/kg, i.p.) and harmine (5, 10 and 15 mg/kg, i.p.) on catalase activity in rat brain. The catalase activity increased in prefrontal cortex after acute (A) and chronic (B) treatments with imipramine and in prefrontal cortex and hippocampus after chronic treatment (B) with harmine, compared to control group. Bars represent means ± S.E.M. of 5 rats. * p <0.05 vs. saline according to ANOVA followed by Tukey post-hoc test.
Materials and Methods

Animals. Male Adult Wistar rats (60 days old) were obtained from UNESC (Universidade do Extremo Sul Catarinense, Criciúma, Brazil) breeding colony. They were housed five per cage with food and water available ad libitum and were maintained on a 12 h light/dark cycle (lights on at 7:00 AM). 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 and with approval by local Ethics Committee under protocol number 325/2008.

Figure 4. Effects of acute (A) and chronic (B) administration of imipramine (10, 20 and 30 mg/kg, i.p.) and harmine (5, 10 and 15 mg/kg, i.p.) on superoxide dismutase activity in rat brain. The superoxide dismutase activity increased after acute treatment (A) in prefrontal cortex with imipramine and harmine and in hippocampus with harmine and after chronic treatment with imipramine in prefrontal cortex and harmine in hippocampus, compared to control group. Bars represent means ± S.E.M. of 5 rats. * p <0.05 vs. saline according to ANOVA followed by Tukey post-hoc test.
Drugs and treatments. Harmane was obtained from THC-Pharm/STI-Pharm (Frankfurt, Germany) and imipramine, the standard antidepressant, from Novartis Pharmaceutical Industry (Criciúma, Brazil). Different groups of rats (n = 5 each) were administered intraperitoneally (i.p.) with saline or different doses of harmane (5, 10 and 15 mg/kg) or imipramine (10, 20 and 30 mg/kg) once only (acute treatment) or once a day for 14 days (chronic treatment).48-51 Imipramine and harmane were dissolved in saline immediately before the injections. All treatments were administered in a volume of 1 mL/kg. After 60 min acute and chronic treatment, the animals were killed by decapitation. The hippocampus and prefrontal cortex were quickly isolated by hand dissection using a magnifying glass and a thin brush, dissection was based on histological distinctions described by Paxinos and Watson.40 Samples were stored at 70°C for subsequent analysis of oxidative stress.

Oxidative stress parameters. In order to assess oxidative damage, we measured the formation of thiobarbituric acid reactive species (TBARS) during an acid-heating reaction, as previously described by Esterbauer and Cheeseman.41 The samples were extracted by hand dissection using a magnifying glass and a thin brush, dissection was based on histological distinctions described by Paxinos and Watson.40 Samples were stored at 70°C for subsequent analysis of oxidative stress.

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