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BASIC NEUROSCIENCES, GENETICS AND IMMUNOLOGY - ORIGINAL ARTICLE

Chronic variable stress induces oxidative stress and decreases butyrylcholinesterase activity in blood of rats

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Abstract Depressive disorders, including major depression, are serious and disabling, whose mechanisms are not clearly understood. Since life stressors contribute in some fashion to depression, chronic variable stress (CVS) has been used as an animal model of depression. In the present study we evaluated some parameters of oxidative stress [thiobarbituric acid reactive substances (TBARS), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx)], and inflammatory markers (interleukin 6, C reactive protein, tumor necrosis factor-alpha and nitrites), as well as the activity of butyrylcholinesterase in blood of rats subjected to chronic stress. Homocysteine and folate levels also were measured. Stressed animals were submitted to different mild stressors for 40 days. After CVS, a reduction in weight gain was observed in the stressed group, as well as an increase in immobility time in the forced swimming test as compared with controls. Stressed animals presented a significant increase on TBARS and SOD/CAT ratio, but stress did not alter GPx activity and any inflammatory parameters studied. CVS caused a significant inhibition on serum butyrylcholinesterase activity. Stressed rats had higher plasmatic levels of homocysteine without differences in folate levels. Although it is difficult to extrapolate our findings to the human condition, the alterations observed in this work may be useful to help to understand, at least in part, the pathophysiology of depressive disorders.

Keywords Depression · Oxidative stress · Inflammation · Butyrylcholinesterase · Homocysteine

Introduction

Major depression is a common, severe, chronic, and often life-threatening illness. There is a growing appreciation that, far from being a disease with purely psychological

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manifestations, major depression is a systemic disease with deleterious effects on multiple organ systems (Charney and Manji 2004). On the other hand, stressful life events have a substantial causal association with depression, and there is now compelling evidence that even early life stress constitutes a major risk factor for the subsequent development of depression (Charney and Manji 2004). The chronic variable stress (CVS) model of depression has high validity, since a large number of recent publications have confirmed that CVS causes behavioral changes in rodents that parallel symptoms of depression (Gamaro et al. 2008; Ni et al. 2008; Wilner 2005; Katz and Hersh 1981).

Despite extensive research, the current theories on serotonergic dysfunctions do not provide sufficient explanations for the nature of depression (Maes et al. 2009). In this context, both oxidative stress and inflammatory mediators have been suggested to contribute to the neuropathology of depression (Lucinio and Wong 1999; Cumurcu et al. 2009). Oxidative stress, characterized by the imbalance between production of free radicals and the antioxidant capacity of organism, has been implied in the pathogenesis of several psychiatric disorders such as schizophrenia, bipolar disorder, and depression (Ng et al. 2008). Free radicals are molecules that play physiological roles in cellular signaling, immunological responses, and mitosis. However, being highly unstable molecules with unpaired electrons, they have differential oxidative strengths and hence the potential to damage cellular proteins, lipids, carbohydrates and nucleic acids (Halliwell 2006). Chronic stress has been shown to cause oxidative damage in the central nervous system (CNS) (Lucca et al. 2009; Madrigal et al. 2001; Olivenza et al. 2000), but there is a lack of works investigating peripheral effects of CVS.

New developments in psychiatric research have led to the hypothesis that inflammatory processes and neural-immune interactions are involved in the pathogenesis of major depression (Maes et al. 2009). Studies have demonstrated that proinflammatory parameters such as interleukins (IL-1, IL-2, IL-6, IL-8 and IL-12), interferon-γ (IFNγ), and tumor necrosis factor-α (TNFα) are increased in patients with depression (Schiepers et al. 2005). However, other studies have failed to find an association between the immune system and depression (Haack et al. 1999; Steptoe et al. 2003), indicating the need for more investigations in order to confirm the activation of immune system as cause of depressive symptoms.

Acetylcholine (ACh) is the principal vagus neurotransmitter and its action is finished by hydrolysis catalyzed by acetylcholinesterase (AChE) (E.C.3.1.1.7) and butyrylcholinesterase (BuChE) (E.C.3.1.1.8) (Darvesh et al. 2003). In humans, AChE is more abundant in the CNS, end plate of skeletal muscle, and erythrocytes membranes, while BuChE is more abundant in serum (Massoulié et al. 1993). Although the exact physiological function of BuChE is unclear, it has been shown that it can promptly hydrolyze acetylcholine and to substitute AChE in maintaining the structural and functional integrity of central cholinergic pathways (Mesulam et al. 2002). In addition, reports from the literature suggest a relationship between BuChE activity and risk factors for coronary artery disease (Alcantara et al. 2002) and that heart disease and depression are highly co-morbid (Johnson and Grippo 2006). In relation to stress, Rada et al. (2006) demonstrated that ACh levels are elevated in animals subjected to forced swimming and that this alteration is compensated by AChE activation. Otherwise, stress insults induce hyperexcitation of cholinergic circuits (Tracey 2002; Sapolsky 1996).

Another alteration that has been related to depressed patients concerns Hcy metabolism (Jendricko et al. 2009; Levine et al. 2008; Tolmunen et al. 2004). Homocysteine (Hcy) is a sulfurated amino acid derived from ingested methionine. It is directly toxic to neurons and blood vessels and can induce DNA strand breakage, oxidative stress and apoptosis (Mattson and Shea 2003; Lipton et al. 1997). On the other hand, the methionine–homocysteine metabolic pathway produces methyl groups required for the synthesis of catecholamines and DNA. This is accomplished by remethylating homocysteine—using B12 and folate as cofactors—back to methionine. A recent study demonstrated that serum homocysteine levels correlate positively with cortisol levels (Cascalheira et al. 2008). Furthermore, there are evidences supporting an association of depression with high blood homocysteine in humans (Bottiglieri et al. 2000; Tolmunen et al. 2004; Folstein et al. 2007) though the results are still controversial.

Thus, in line of the foregoing considerations, in the present study, we evaluated some parameters of oxidative stress [thiobarbituric acid reactive substances (TBARS), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx)], and inflammatory markers [interleukin 6 (IL-6), C reactive protein (CRP), tumor necrosis factor-alpha (TNF-α) and nitrite (NO)], as well as the activity of butyrylcholinesterase in blood of rats subjected to chronic stress. Homocysteine and folate levels were also measured.

Materials and methods

Animals and reagents

Fifty-two (20 for oxidative stress measurements; 20 for inflammatory markers and BuChE; 12 for Hcy and folate assay), male Wistar rats (60 days old; 200–270 g weight) were obtained from the Central Animal House of the Department of Biochemistry of the Federal University of

Rio Grande do Sul, Porto Alegre, Brazil. The experimentally naive animals were housed in groups of 4–5 in home cages made of Plexiglas material (65 × 25 × 15 cm) with the floor covered with sawdust. They were maintained under a standard dark–light cycle (lights on between 7:00 and 19:00 h) at a room temperature of 22 ± 2°C. The rats had free access to food (standard rat chow) and water, except for the stressed group during the period when the stressor applied required no water. After being randomized to assure all groups presented similar body weights, the animals were divided into two groups: control and stressed. Animal care followed the official governmental guidelines in compliance with the Federation of Brazilian Societies for Experimental Biology and was approved by the Ethical Committee of the Universidade Federal do Rio Grande do Sul, Brazil. All chemicals were purchased from Sigma Chemical Co., St Louis, MO, USA.

Stress model

The CVS protocol was applied as described by Gamaro et al. (2003) with some modifications in the stressors applied, such as inclination of the home cages instead of food deprivation and damp bedding instead of forced swimming. Control animals were handled daily. A variable-stressor paradigm was used for the animals in the stressed group. This protocol differs from other chronic stress protocols that use only one stressor in that the different stressors used diminish adaptation to stress (Marin et al. 2007). Animals were subjected to one stressor per day, at different times each day, in order to minimize predictability. The following stressors were used: (a) 24 h of water deprivation, (b) 1–3 h of restraint, as described below, (c) 1.5–2 h of restraint at 4°C, (d) flashing light during 120–210 min, (e) isolation (2–3 days), (f) inclination of the home cages at a 45° angle for 4–6 h, (g) damp bedding (300 mL water spilled onto bedding during 1.5–2 h). Restraint was carried out by placing the animal in a 25 × 7 cm plastic tube and adjusting it with plaster tape on the outside, so that the animal was unable to move. There was a 1-cm hole at the far end for breathing. Exposure to flashing light was made by placing the animal in a 50-cm high, 40 × 60 cm open field made of brown polywood with a frontal glass wall. A 40-W lamp, flashing at a frequency of 60 flashes per minute, was used. Rats were submitted to chronic variate stress for 40 days as described in Table 1.

Table 1 Schedule of stressor agents

Day of treatment	Stressor applied
1	Cold restraint (1.5 h)
2	Inclination of home cages (4 h)
3	Flashing light (2 h)
4	Restraint (2 h)
5	Isolation
6	Isolation
7	Isolation
8	Damp bedding (2 h)
9	Inclination of home cages (6 h)
10	No stressor applied
11	Flashing light (2 h)
12	Water deprivation (24 h)
13	Restraint (3 h)
14	Damp bedding (3 h)
15	Inclination of home cages (4 h)
16	Cold restraint (2 h)
17	Flashing light (3 h)
18	Restraint (2.5 h)
19	Damp bedding (3 h)
20	Isolation
21	Isolation
22	Isolation
23	Cold restraint (1.5 h)
24	Water deprivation (24 h)
25	Inclination of home cages (4 h)
26	Restraint (3 h)
27	Flashing light (3 h)
28	Restraint (1 h)
29	Damp bedding (2 h)
30	No stressor applied
31	Water deprivation (24 h)
32	Inclination of home cages (6 h)
33	Flashing light (2 h)
34	Cold restraint (2 h)
35	Isolation
36	Isolation
37	Isolation
38	Flashing light (3 h)
39	Damp bedding (2 h)
40	Restraint (3 h)

After 40 days of stress, forced swimming test was performed according to Porsolt et al. (1977), in order to confirm the ability of this stress to increase the immobility time, an indicative of depressive behavior. The test involves two individual exposures to a cylindrical tank with water in which rats cannot touch the bottom of the tank or escape. The tank is made of clear Plexiglas, 50 cm tall, 30 cm in diameter, and filled with water (22–23°C) to a depth of 30 cm. Water in the tank was changed after each rat swimming test section. For the first exposure, rats were placed in the water for 15 min (pre-test session). Twenty-four hours later, rats were placed in the water again for a 5-min session (test session), and the immobility time of rats was recorded in seconds.

Body weight was measured at different times during treatment, since several works reported that chronic stress-induced significant reduction in body weight gain (Konarska et al. 1990; Harro et al. 2001).

Erythrocyte and plasma preparation

Erythrocytes and plasma were prepared from whole blood samples obtained from rats (controls and stressed rats) after decapitation.

Whole blood was collected and transferred to heparinized tubes for erythrocyte separation. Blood samples were centrifuged at 1,000×*g*, plasma was removed by aspiration and frozen at −80°C until determination of TBARS, HCy, and folate levels. Erythrocytes were washed three times with cold saline solution (0.153 mol/L sodium chloride). Lysates were prepared by the addition of 1 mL of distilled water to 100 µL of washed erythrocytes and frozen at −80°C until determination of the antioxidant enzyme activities.

For antioxidant enzyme activity determination, erythrocytes were frozen and thawed three times, and centrifuged at 13,500×*g* for 10 min. The supernatant was diluted in order to contain approximately 0.5 mg/mL of protein.

Thiobarbituric acid reactive substances

Usually, lipid peroxidation is quantified by measuring malondialdehyde (MDA), which is formed by the degradation products of polyunsaturated fatty acid hydroperoxides (Halliwell and Gutteridge 2006). The main source of MDA in biological samples is the peroxidation of polyunsaturated fatty acids. TBARS is a widely adopted method for measuring lipid oxidation (Ferreira et al. 2010; Kunz et al. 2008; Del Rio et al. 2005; Draper and Hadley 1990); however, the TBARS assay is not specific for MDA. This way, we expressed the results in terms of the amount of thiobarbituric acid reactive substances formed per unit of time instead of the amount of malondialdehyde produced. TBARS was determined according to the method described by Ohkawa et al. (1979) for in vivo studies. TBARS measures malondialdehyde (MDA), a product of lipoperoxidation caused mainly by hydroxyls free radicals. Plasma diluted in 1.15% KCl was mixed with 20% trichloroacetic acid and 0.8% thiobarbituric acid and heated in a boiling water bath for 60 min. TBARS were determined by the absorbance at 535 nm. Calibration curve was performed using 1,1,3,3-tetramethoxypropane and each curve point was subjected to the same treatment as that of the plasmas. TBARS was calculated as nanomoles of malondialdehyde formed per milligram of protein.

Catalase assay

CAT activity was assayed by the method of Aebi (1984). H_2O_2 disappearance was continuously monitored with a spectrophotometer at 240 nm for 90 s. One unit of the enzyme is defined as 1 mmol of hydrogen peroxide consumed per minute and the specific activity is reported as units per mg protein.

Superoxide dismutase assay

This method for the assay of SOD activity is based on the capacity of pyrogallol to autoxidize, a process highly dependent on $O_2^{-\cdot}$, which is a substrate for SOD (Marklund 1985). The inhibition of autoxidation of this compound occurs in the presence of SOD, whose activity can be then indirectly assayed spectrophotometrically at 420 nm. A calibration curve was performed with purified SOD as standard, in order to calculate the activity of SOD present in the samples. The results were reported as units/mg protein.

Glutathione peroxidase

GSH-Px activity was measured by the method of Wendel (1981), except for the concentration of NADPH, which was adjusted to 0.1 mM after previous tests performed in our laboratory. Tert-butylhydroperoxide was used as substrate. NADPH disappearance was continuously monitored with a spectrophotometer at 340 nm for 4 min. One GSH-Px unit is defined as 1 mmol of NADPH consumed per minute and specific activity is reported as units per mg protein.

Serum preparation

After decapitation, the blood was collected and centrifuged for 10 min at 1,000×*g*. The serum was used for the inflammatory marker assays and enzymatic (BuChE) analyses.

Cytokines (TNF-α and IL-6) assay

TNF-α and IL-6 levels in serum were quantified by rat high-sensitivity enzyme-linked immunoabsorbent assays (ELISA) with commercially available kits (Biosource®, Camarillo, CA).

Nitrite assay (NO)

Nitrite levels were measured using the Griess reaction; 100 µL of supernatant of hippocampus and cerebral cortex was mixed with 100 µL Griess reagent (1:1 mixture of 1% sulfanilamide in 5% phosphoric acid and 0.1%

naphthylethylenediamine dihydrochloride in water) and incubated in 96-well plates for 10 min at room temperature. The absorbance was measured on a microplate reader at a wavelength of 543 nm. Nitrite concentration was calculated using sodium nitrite standards (Green et al. 1982).

Acute-phase protein assay (CRP)

CRP levels in serum were determined by a colorimetric assay with commercially available kits (BioSystems® and Bioclin®, Brazil).

Butyrylcholinesterase activity assay

BuChE activity was determined by the method of Ellman et al. (1961) with some modifications. Hydrolysis rate was measured at acetylthiocholine concentration of 0.8 mM in 1 mL assay solutions with 100 mM potassium phosphate buffer pH 7.5 and 1.0 mM 5,5-dithiobis (2-nitrobenzoic acid) (DTNB). Fifty microliters of rat diluted serum was added to the reaction mixture and preincubated for 3 min. The hydrolysis was monitored by formation of the thiolate dianion of DTNB at 412 nm for 2 min (intervals of 30 s) at 25°C. All samples were run in duplicate. Specific enzyme activity was expressed as mmol acetylthiocholine hydrolyzed per hour per milligram of protein.

Homocysteine level determination

Hcy levels in plasma were determined as described by Magera et al. (1999), using liquid chromatography electrospray tandem mass spectrometry (LC–MS/MS). After reduction and deproteinization of samples, Hcy concentration was detected through the transition from the precursor to the product ion (*m*/*z* 136 to *m*/*z* 90). Homocysteine-d was added as internal standard.

Folate levels determination

For folate determination, heparinized blood was collected and plasma was separated. Plasma folate concentration was measured by an automated chemiluminescence system (ACS: 180, Siemens). The method is based on a competitive immunoassay with acridinium ester-labeled folate in solid phase.

Protein determination

Protein was measured according to Bradford (1976) for butyrylcholinesterase assay and according to Lowry et al. (1951) for all others techniques. Serum bovine albumin was used as standard.

Statistical analysis

Data were analyzed by unpaired Student's *t* test. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 15.0 in a PC-compatible computer. The results were expressed as mean ± SEM and differences were considered statistically significant if $P < 0.05$.

Results

The body weight of animals was evaluated before and after CVS. We verified that the control group gained more weight after 40 days than the stressed group [$t(18) = 2.763$; $P < 0.01$]. Chronic stress also increased immobility time in the forced swimming test when compared with controls [$t(18) = 3.066$; $P < 0.01$].

The effect of chronic stress upon the levels of TBARS in plasma was measured. As can be observed in Fig. 1, chronic stress increased TBARS levels [$t(14) = 5.100$; $P < 0.001$]. Figure 2a shows a significant increase in SOD/CAT ratio in the stressed group [$t(18) = 3.363$; $P < 0.05$]. To verify whether other antioxidant enzyme was compensating the imbalance verified between SOD and CAT, we determined glutathione peroxidase activity. As shown in Fig. 2b, stress did not alter GPx activity [$t(18) = 0.644$; $P > 0.05$].

We also investigated some inflammatory parameters in serum of rats subjected to CVS. Table 2 shows that these inflammatory parameters studied were not affected in stressed group when compared with controls [IL-6: $t(17) = 1.433$, $P > 0.05$; TNF: $t(18) = 1.644$, $P > 0.05$; NO: $t(18) = 3.363$; $P > 0.05$; PCR: $t(16) = 1.490$; $P > 0.05$].

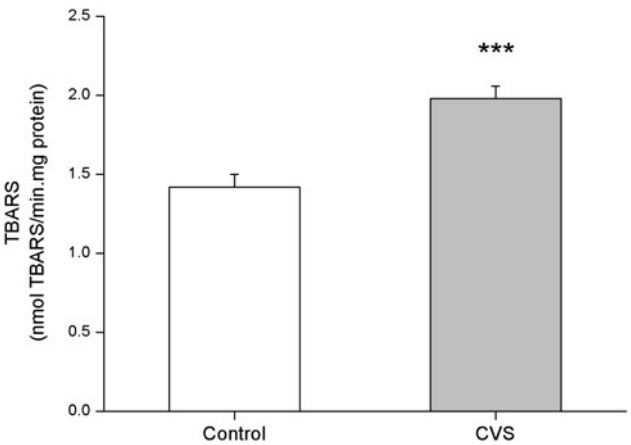

Fig. 1 Effect of chronic variable stress (CVS) on thiobarbituric acid reactive substances (TBARS) in plasma of rats. TBARS is expressed as nmol of thiobarbituric acid reactive substances per mg protein. Results are expressed as mean ± SEM for eight independent experiments performed in duplicate. ***$P < 0.001$ compared with control group (Student's *t* test)

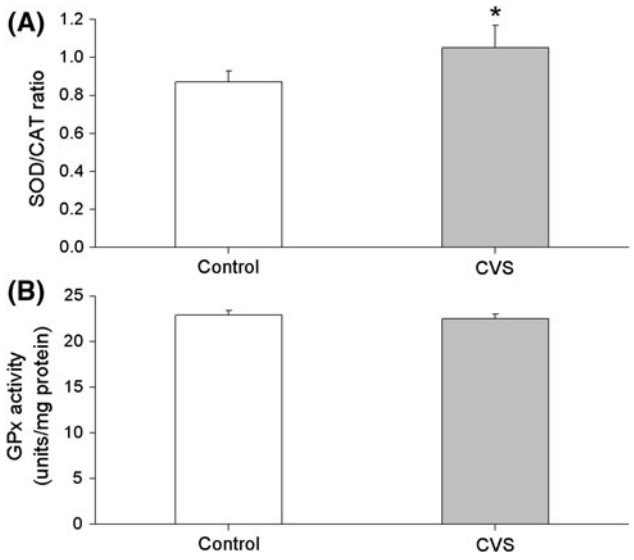

Fig. 2 Effect of chronic variable stress (CVS) on superoxide dismutase/catalase ratio (**a**) and on glutathione peroxidase activity (**b**). Results are expressed as mean ± SEM for ten independent experiments performed in duplicate. $^{*}P < 0.05$ compared with control group (Student's *t* test). *SOD* superoxide dismutase, *CAT* catalase, *GPx* glutathione peroxidase

Table 2 Effect of chronic variable stress (CVS) on proinflammatory cytokines levels in serum of rats

Parameters	Control	CVS
IL-6 (pg/mL)	6.4 ± 0.95	6.1 ± 1.10
TNF (pg/mL)	1.5 ± 0.09	1.7 ± 0.11
NO (μM)	0.50 ± 0.08	0.59 ± 0.06
CRP (mg/L)	0.30 ± 0.06	0.17 ± 0.06

Results are mean ± SEM for 8–10 independent experiments (animals), $P > 0.05$

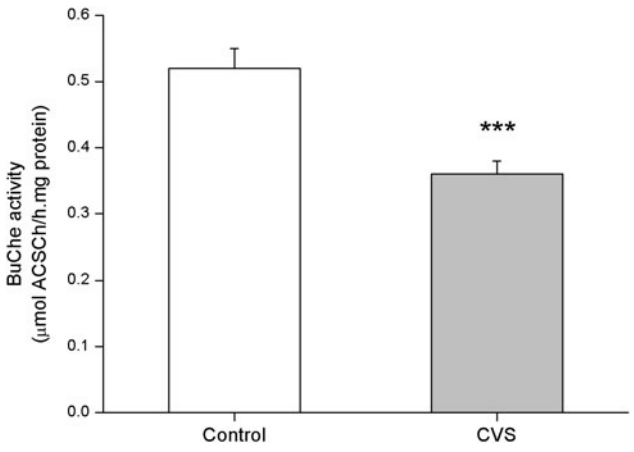

Fig. 3 Effect of chronic variable stress (CVS) on butyrylcholinesterase activity in serum of rats. Results are mean ± SEM for eight independent experiments performed in duplicate. $^{***}P < 0.001$ compared with control (Student's *t* test)

The effect of CVS on the activity of BuChE in serum of rats was also studied. Figure 3 shows that this enzyme was significantly inhibited in the stressed group [$t(14) = 4.793$; $P < 0.001$] as compared with the control group.

Plasma levels of Hcy and folate in animals submitted to CVS are demonstrated in Table 3. Results showed that the stressed group had higher plasma Hcy than the control group [$t(10) = 5.433$; $P < 0.001$], while no significant difference in folate concentration was detected between groups [$t(9) = 1.078$; $P > 0.05$].

Discussion

Major depressive disorder has traditionally been considered to have a neurochemical basis, but despite the devastating impact of the illness, little is known about its etiology or pathophysiology. However, several studies have demonstrated that although the monoaminergic neurotransmitter systems may be involved in this disease, they are limited in elucidating the pathogenesis of depression. It has been demonstrated that depression arises from the complex interaction of multiple susceptible (and likely protective) genes and environmental factors, and disease phenotypes include not only episodic and often profound mood disturbances, but also a range of cognitive, motoric, autonomic, endocrine, and sleep/wake abnormalities (Manji et al. 2001). These observations have led to the appreciation that although dysfunction within the monoaminergic neurotransmitter systems is likely to play important roles in mediating some facets of the pathophysiology of depression, there are other possible mechanisms involved (Maletic et al. 2007; Belmaker and Agam 2008).

The HPA axis and its final effector system, glucocorticoids, are essential components of an individual's capacity to cope with stress, and in fact, a hyperactivity of the HPA axis is observed in the majority of patients with depression (Gillespie and Nemeroff 2005; Bao et al. 2008; Swaab et al. 2005). Considering that life stressors contribute in some fashion to depression and are an extension of what occurs normally, chronic stress has been used as an animal model of depression (Gamaro et al. 2008; Ni et al. 2008) since animals displayed typical changes in hedonic status.

Table 3 Homocysteine (Hcy) and folate levels in plasma of rats submitted to chronic variable stress (CVS)

Parameters	Control	CVS
Homocysteine (μmol/L)	5.93 ± 0.5	9.93 ± 0.5***
Folate (μg/mL)	47.33 ± 4.0	52.5 ± 2.2

Results are mean ± SEM for 5–6 independent experiments (animals)
*** $P < 0.001$ compared with control (Student's *t* test)

Using this model, in the present study we evaluated some parameters of oxidative stress in plasma and erythrocytes of rats. We observed a significant increase in TBARS, a method that evaluates the oxidative stress assayed for malondialdehyde, the last product of lipid breakdown caused by oxidative stress (Halliwell and Gutteridge 2006). Beyond generating pathways, in this study, we pay attention to consuming pathways of free radicals, namely SOD, CAT, and GPx, the major enzymatic system responsible for protecting cells against free radical attacks (Halliwell and Gutteridge 2006). Animals exposed to stress presented an imbalance between SOD and CAT, expressed by increased SOD/CAT ratio. When a cell has decreased activity of CAT, a large amount of H_2O_2 (the product of SOD action) becomes available to react with transition metals and generates the radical hydroxyl, which is the most harmful radical (Kelner et al. 1995; Matés et al. 1999). On the other hand, stress did not alter the activity of GPx; this result reinforces the view that oxidative stress responses do not always involve a coordinated regulation of all antioxidant enzymes and that their activities are regulated by different mechanisms (Röhrdanz et al. 2000; Wilson and Johnson 2000).

These data are consistent with evidences that indicate that oxidative stress is a major pathological mechanism in the maladaptation to chronic stress in rats (Lucca et al. 2009; Madrigal et al. 2001; Olivenza et al. 2000). In this line, clinical studies also demonstrate an induction of oxidative stress in serum of depressed patients (Cumurcu et al. 2009; Khanzode et al. 2003; Bilici et al. 2001). Oxidative stress induction caused by chronic stress could be explained by several pathways, for example, through over-stimulation of glucocorticoids receptors (You et al. 2009; Zafir and Banu 2009), inhibition of mitochondrial electron transport chain complexes (Tagliari et al. 2010), and alterations on homocysteine metabolism (de Souza et al. 2006).

Recent studies have demonstrated that inflammatory and neurodegenerative processes play an important role in depression and that enhanced neurodegeneration in depression may—at least partly—be caused by inflammatory processes (Maes et al. 2009; Miller et al. 2009; Dantzer 2006; Schiepers et al. 2005). Based on these studies, in the present study, we evaluated the effects of stress on some inflammatory markers such as IL-6, TNF-α, NO, and PCR. Results showed that chronic stress did not alter any of the inflammatory parameters studied. Although clinical studies showed an increase of cytokines levels in blood of depressed patients, animal models using stress as model of depression have inconsistent results. In this context, Kubera et al. (1996) demonstrated increased blood levels of IL-1 and IL-2 after 8 weeks of mild stress. On the other hand, mice exposed to a 3-week chronic mild stress had decreased expression of peripheral IL-1beta and IL-6 and an increased expression of brain IL-6 (Mormède et al. 2003). In addition, another study has also reported elevated cytokine levels in brain of mice subjected to chronic mild stress for 5 weeks (Goshen et al. 2008). These inconsistent results concerning blood interleukins may be due to different stress protocols or different periods of stress exposure.

We also measured the activity BuChE in serum of animals submitted to chronic stress. Results showed that this enzyme was inhibited in stressed animals as compared with the control group. Moreover, since the results of the present study show an imbalance between CAT and SOD activities, what could result in increased levels of H_2O_2, our results are in agreement with other studies demonstrating that hydrogen peroxide can inhibit serum cholinesterase (Schallreuter and Elwary 2007).

Since there are data from literature showing that Hcy metabolism can be altered in depression and/or stressed patients (Jendricko et al. 2009; Levine et al. 2008; Tolmunen et al. 2004) and that this amino acid induces oxidative stress (Matté et al. 2009; Faraci and Lentz 2004; Wyse et al. 2002), we investigate the plasma levels of homocysteine and folate in control and stressed rats. Our results showed an increase in homocysteine levels in the stressed group; however, there were no differences in folate levels between control and stressed groups. Previous studies regarding Hcy metabolism in depression have provided contradictory results. Several works suggest that Hcy levels are increased in depressed patients (Wilhelm et al. 2010; Alexopoulos et al. 2010; Resler et al. 2008; Tolmunen et al. 2004; Reif et al. 2003; Bottiglieri et al. 2000); however, the lack of correlation between Hcy and depression has been demonstrated by Kelly et al. (2004). In most cases, however, increased levels of Hcy were observed in depressed patients with vitamin B12 or folate deficiency (Kim et al. 2008; Bottiglieri et al. 2000; Refsum et al. 2006). Our results are in agreement with studies that demonstrated increased levels of Hcy in the absence of folate deficiency after restraint stress in rats (de Souza et al. 2006). In addition, Triantafyllou et al. (2008) showed that multiple sclerosis patients that present elevated Hcy levels are particularly prone to develop depressive symptomatology. Previous studies from our laboratory have shown that BuChE can be inhibited by Hcy (Scherer et al. 2007; Matté et al. 2006), and this inhibition is mediated by the generation of free radicals (Stefanello et al. 2005). Therefore, the reduced BuChE activity could be related to the altered Hcy observed in the present study. Besides, elevated levels of Hcy increase oxidative stress (Matté et al. 2009; Wyse et al. 2002).

Besides augmenting the probability of oxidative damage, increased Hcy levels in stressed animal may ultimately

be related to an imbalance at the monoamine or neurotransmitter level, since the rise in Hcy levels could be ascribed to failure of methylation of Hcy to methionine. Methionine, in turn, is the precursor of *S*-adenosylmethionine, the methyl donor in a host of methylation reactions in the CNS involving monoamines and various neurotransmitters, amongst other cellular constituents.

In conclusion, we found in the present study that the CVS model of depression provokes an increase in oxidative stress, an inhibition of BuChE, and an increase in Hcy levels in blood of rats. Since the pathophysiology of depression still is poorly understood, if confirmed in humans, our results could be useful to explain some symptoms observed in patients.

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