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Research report

# Increased oxidative stress in prefrontal cortex and hippocampus is related to depressive-like behavior in streptozotocin-diabetic rats

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## HIGHLIGHTS

- Diabetic rats exhibited a more pronounced depressive-like behavior.
- Prefrontal cortex and hippocampus from diabetic rats presented an increase of oxidative stress.
- Treatment of diabetic rats with insulin and vitamin E induced an antidepressant-like behavior.
- Imipramine induced an antidepressant-like behavior in both normoglycemic and diabetic rats.
- Prevention of hyperglycemia by Insulin induced the most effective control of oxidative stress.

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## ABSTRACT

Depression is a common comorbid in diabetic patients. The pathophysiologic mechanisms that relate this comorbidity is not completely elucidated yet, although several lines of evidence point out that increased oxidative stress resulting from hyperglycemia may have a crucial role. Thus, the effect of prolonged treatment with insulin (INS), the antioxidant vitamin E (VIT E) or the antidepressant imipramine (IMI) was evaluated in animals submitted to forced swimming test. Oxidative stress parameters (lipid peroxidation product levels, reduced gluthatione levels and catalase and superoxide dismutase activities) were also evaluated in brain areas related to depression, prefrontal cortex (PFC) and hippocampus (HIP). Our data show that treatment of streptozotocin-induced diabetic (DBT) rats with INS (6 UI/day, s.c.) prevented the blood glucose increase, reduced the immobility time, an antidepressant-like behavior, and normalized the reduced weight gain. Although the VIT E treatment (300 mg/kg, p.o.) had not altered the blood glucose levels, this treatment was able to reduce the immobility time and to reestablish the reduced weight gain in DBT rats. Differently, treatment with IMI (15 mg/kg, i.p.) induced antidepressant-like behavior in normoglycemic besides DBT animals. While VIT E and IMI treatments restored only specific oxidative stress parameters, INS was able to prevent all changed parameters evaluated in both PFC and HIP from DBT animals. Therefore, our data provide further evidence of the importance of oxidative stress in PFC and HIP in the pathophysiology of depression related to diabetes.



## 1. Introduction

Depression is the most common psychiatric disorder identified in patients with diabetes. Besides, the prevalence of major depressive disorder has been recognized to be much higher among people with diabetes compared to non-diabetic populations [1–3]. Furthermore, depression, in turn, may increase the risk of hyperglycemia, leading to a poorer management of plasma glucose and an increase in diabetes-related complications, in health care expenses and in medical morbidity and mortality [4,5]. Also noteworthy is that depression induces activation of various integrated biological systems that can increase insulin resistance as well as facilitating the development of diabetes [6].

It has recently become clear that the central nervous system (CNS) is not spared from the deleterious effects of diabetes, since diabetic encephalopathy was recognized as a complication of this heterogeneous metabolic disorder [7,for a review, see 8]. In both human and animal models, diabetes is associated with pathological changes in the CNS that lead to cognitive and affective deficits, and to an increased risk of brain vascular complications [9].

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Given the high costs of treatment for depression and diabetes, studies have been extensively growing in an attempt to meet an effective treatment to avoid the symptoms and damage caused by both diabetes and depression. In this regard, it is known that maintenance of glucose homeostasis prevents the harmful effects of the disease on the CNS, heart, eyes, kidneys, nerves and peripheral vasculature [10,11]. As rigid glycemic control is not always clinically possible, the antidepressants have been recommended as the first-choice drugs for treating depression associated with diabetes [12]. However, evidence shows the treatment with antidepressant drugs is effective in only a subset of patients with depression (only 30% respond to treatment effectively), and it requires a continuous therapy (weeks to months) to achieve a therapeutic response [13,14]. Moreover, antidepressant drugs can also directly affect plasma glucose and insulin levels besides interact with hypoglycemic drugs [15]. Thus, the understanding of the pathophysiological mechanisms responsible for the development of diabetes and depression is urgent and it may help to propose alternative treatments which aim an increase of effectiveness and reduction of latency to the therapeutic effect.

The persistent hyperglycemia appears to have a major role in the onset of cognitive and affective disorders associated with diabetes [16]. It has been suggested that hyperglycemia causes tissue damage through several mechanisms, such as an increase in glucose flux and other sugars through the polyol pathway, increase of advanced glycation end-products (AGEs) synthesis, increase of AGEs receptor expression, activation of protein kinase C isoforms and overactivity of the hexosamine pathway (reviewed by [17]). Furthermore, evidence indicates that these mechanisms are activated by increased oxidative stress [18,19], which results from increasing of reactive species of oxygen or nitrogen (ROS/RNS) production and/or impairment of antioxidant defenses [20]. In fact, the oxidative stress can lead to damage of the main components of the cellular structure, including nucleic acids, proteins, amino acids and lipids [21], affecting several cell functions, such as metabolism and gene expression, which in turn can precipitate or impair other pathological conditions [22]. The persistence of oxidative stress also leads to a cascade of events resulting in neurodegenerative apoptotic injury [23].

Interestingly clinical studies indicate that increased oxidative stress may also contribute to depressive states [24–26]. Therefore, antioxidants such as vitamin E and C were observed to be reduced in serum of the depressed patients [27,28]. Corroborating these data, studies show that antioxidant treatment can induce antidepressant effects [29–31].

Considering that the association between diabetes and depression may be a direct consequence of hyperglycemia and biochemical changes such as oxidative stress, in an attempt to understand the mechanisms related to the favoring of comorbidity diabetes/depression, the aim of our study was to investigate the effect of prolonged treatment with insulin, vitamin E (a potent antioxidant) or antidepressant drug imipramine on behavioral responses related to depression and on oxidative stress parameters evaluated in brain areas associated to depression, the prefrontal cortex and the hippocampus.

## 2. Materials and methods

### 2.1. Animals

Male *Wistar* rats (200–250 g), provided by the Federal University of Paraná colony, were used. The animals were housed in plastic cages (41 × 32 × 16.5 cm) with four rats per cage and food and water available *ad libitum*. They were maintained in a temperature-controlled room (22 ± 2 °C) under 12-h light:12-h dark cycle (lights on at 7 a.m.). The experiments were carried out according to Brazilian Society of Neuroscience and Behavior guidelines for care and use of Laboratory animals and all efforts were made to minimize animal suffering. The experimental protocol was approved by the local Ethical Committee (CEUA/BIO-UFPR; #576).

### 2.2. Drugs

The following drugs were used: human NPH Insulin (INS; Humulin®, Lilly, USA), Streptozotocin (STZ; Santa Cruz Biotechnology Inc., USA), sodium citrate (Merck S.A., Brazil), tricyclic antidepressant drug Imipramine (IMI; Novartis Pharmaceutical Industry, Brazil) and antioxidant drug Vitamin E (VIT E; Pharma Nostra, Brazil). STZ was dissolved in citrate buffer (10 mM, pH 4.5), IMI and INS were dissolved in saline and VIT E was dissolved in corn oil. The doses and treatment schedules were based on previous studies [32,33] and pilot experiments in our laboratory. Although all experiments were carried out by an observer blind to drug treatments, the experimenter could not be blind to normoglycemic and diabetic groups.

### 2.3. Diabetes induction

Experimental diabetes was induced following an overnight fast by a single intraperitoneal (i.p.) injection of STZ at a dose of 50 mg/kg freshly dissolved in citrate buffer (10 mM, pH 4.5). Hyperglycemia was confirmed 72 h after STZ administration by a strip operated reflectance meter in a blood sample obtained by tail prick and confirmed again at ending of the behavioral tests. Animals with fasting blood glucose levels ≥250 mg/dL were maintained in the study [34]. All animals were observed daily and weighed regularly during the experiment.

### 2.4. Open-field test

The open-field test was conducted according to Santiago et al. [35]. Briefly, animals were placed in the center of a rectangular open field (40 cm × 50 cm × 63 cm) with a floor divided into 6 rectangular units. The exploratory activity was recorded during 5 min and the number of squares crossed with all four paws was quantified.

### 2.5. Forced swimming test (FST)

Independent groups of rats were submitted to FST as described by Porsolt et al. [36] with minor modifications. The test was conducted in two sessions. First, in the pre-test session rats were placed individually to swim in a tank (30 cm × 40 cm height containing 25 cm of water at 24 ± 1 °C) for 15 min. Twenty four hours later, animals were submitted to a 5 min session of forced swim (test). During this session, total time of immobility (except the small movements necessary to float) and the latency to the first immobility episode were evaluated [37]. After each session (pre-test and test session), the animals were removed and allowed to dry in a separate cage before being returned to their home cages.

### 2.6. Preparation of subcellular fractions of brain

Prefrontal cortex (PFC) and hippocampus (HIP) from NGL and DBT animals were dissected and homogenized in 200 mM of potassium phosphate buffer (pH 6.5). The homogenate was used to determine the reduced glutathione (GSH) and lipid hydroperoxides (LOOH) levels and then centrifuged at 9000 × *g* for 20 min. The supernatant was used for the determination of superoxide dismutase (SOD) and catalase (CAT) activities.