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Sucrose Combined with L-carnitine or Desvenlafaxine does not Increase Hyperglycemia. Inhibition of Oxidative Stress may be Involved in this Effect

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Abstract: The effect of antidepressives drugs in brain as outcome bias oxidative stress combined with an antiobesity in hyperglycemic model is unknown and this is the core objective of the present study. L-carnitine (165 mg kg^{-1}) and desvenlafaxine (8.5 mg kg^{-1}) in the presence or absence of sucrose 20% were administered intraperitoneally to young rats for 5 consecutive days. The animals were sacrificed and fresh blood was collected from each to measure the glucose level. The measurement of dopamine, glutathione (GSH), ATPase and lipid peroxidation in brain regions were carried out using validated methods. The animals treated with sucrose combined with L-carnitine or desvenlafaxine did not show hyperglycemia. The levels of dopamine increased in cerebellum/medulla oblongata of the rats that received sucrose in combination with L-carnitine or desvenlafaxine. However, in cortex of groups treated with sucrose alone or combined, dopamine levels were decreased. GSH decreased in all groups treated with sucrose. However, in cerebellum/medulla oblongata GSH witnessed an increase in the absence of sucrose. Lipid peroxidation decreased in all regions of rats that received L-carnitine and desvenlafaxine combined with sucrose. Total ATPase decreased in cerebellum/medulla oblongata in all groups that received sucrose, but increased in cortex in groups that received sucrose in combination with L-carnitine or desvenlafaxine. The animals treated with sucrose combined with L-carnitine or desvenlafaxine did not show hyperglycemia, probably due to inhibition of fatty acid oxidation in brain regions. Reduction of oxidative stress may be involved in these effects.

Key words: Antidepressive agents, dopamine, hyperglycemia, oxidative stress

INTRODUCTION

A few years ago, obesity and depression were considered as health problems incident only in adults. Today however, they are recognized as common health problems among youths. Recent data of National Health and Nutrition Examination (USA) estimated that 17% of youths between 2 and 19 years old are overweight (Ogden *et al*., 2006), when compared with only 5% reported a few decades ago. Birmaher *et al*. (2007) in a study of depressive disorder reported a prevalence of 2% in children and 4.8% in adolescents. The vegetative symptoms of depression include changes in appetite. Depressive mood is also associated in a significant way with recurrent depression in adult age (Pettit *et al*., 2006). The above suggests that increase in appetite a feature of depression and could be treated with antidepressives. Desvenlafaxine is an antidepressive which acts by inhibiting serotonin recapture. The use in the treatment of major depressive disorder is common however, its application to pediatric patients and young adults is associated with some risks and must be evaluated depending on the clinical need (Septien-Velez *et al*., 2007). The connection between serotonin and food consumption is related to appetite, food preference and consumption. Winter is a season that is mostly accompanied by a decrease in serotonin in depressive people and this could explain their preference for carbohydrates in this time (Wurtman and Wurtman, 1995). Seasonal changes in gene expression of serotonin transporters have been demonstrated in adults with seasonal depression (Willeit *et al*., 2008). Besides, various studies indicate that dopaminergic and serotonergic systems strictly interact at neuropsychological level and that deterioration in serotonergic system could affect the regulation of

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dopaminergic system. It is known that dopamine is closely linked with the appearance of anxiety state and depression (Seo *et al*., 2008; Ryding *et al*., 2008). Additionally, it is well known that obesity as well as depression are accompanied by imbalance between pro and antioxidant systems and that this is associated with generation of free radicals which have been associated to a large extent with the appearance of neurodegenerative sicknesses. Until recent times, the only drug approved by FDA for long time use in adults has been sibutramine. Consequently, it is highly important to evaluate alternative treatments that could be effective with minimum adverse effects.

Presently, L-carnitine is used as supplement for weight reduction in adults and adolescents (Rogovik and Goldman, 2009), although, in this last group, its use is with precaution and limited for the mere fact that its effects on young subjects are still unknown. Also, carnitine could possibly be used in treatment of neuronal dysfunction symptoms due to waste accumulation (Fariello *et al*., 1988). Glutathione (GSH) is the principal regulator of redox equilibrium and collaborates in the protection of tissues exposed to oxidizing agents (Wu *et al*., 2004). Free Radical induces damage of the cell membrane lipids (Calderon-Guzman *et al*., 2005). Membrane lipids have a strong bond with lipid bilayer of structural protein (Swapna *et al*., 2006), such as Na+-K+ ATPase, which is responsible for ion interchange across the membrane ((Neault *et al*., 2001) and the inhibition of Na+-K+ ATPase promotes excitatory amino acids release in CNS (Hernandez, 1982). The present study is proposed to measure assays of glucose, dopamine, GSH, lipoperoxidation and ATPase activity, on rat brain regions treated with L-carnitine and desvenlafaxine, in the presence and absence of sucrose.

MATERIALS AND METHODS

Thirty young Wistar rats were deployed in the study (120 g). The animals were assigned in groups of 5 and received some of following treatment for 5 days either in presence or absence of sucrose 20% in water w/v at libitum. Group 1 (control), NaCl 0.9%; group 2, L-carnitine (165 mg kg^{-1}); group 3, desvenlafaxine (8.5 mg kg^{-1}); group 4, sucrose; group 5, L-carnitine + sucrose and group 6, desvenlafaxine + sucrose (Wikell *et al*., 1998). All treatments were given intraperitoneally. The animals were fed with bioterium normal diet (23% protein) (Lab Rodent Diet 5001). Body weight and food consumption registry were taken. At the end of the treatments, all the animals were sacrificed by decapitation and blood glucose concentration were immediately measured. The brains were dissected in cortex, hemispheres, cerebellum/medulla oblongata regions (Glowinski and Iversen, 1966) and each region was placed in a solution of NaCl 0.9% at 4°C and homogenized in 10 volumes of Tris-HCl 0.05 M pH 7.2 p/v so as to determine ATPase and lipid peroxidation. A sample of each of these tissues was centrifuged and mixed with $HClO_4$ 0.1M 50:50 to measure GSH and dopamine. The samples were maintained at -20°C until analyzed. This study was carried out within the procedures and roles approved by the international committees for animal protection.

Technique to measure blood glucose: The procedure to measure blood glucose was carried out in all groups of animals at the moment of sacrifice. Ten microlitter of non-anticoagulant fresh blood were obtained and smeared on a reactive filter paper in Accu-Chek active (Roche Mannheim Germany) equipment and the concentration was read in mg dL^{-1}.

Technique to measure dopamine (DA): The DA levels were measured in the supernatant tissue homogenized in $HClO_4$ after centrifugation at 9,000 rpm for 10 min in a microcentrifuge (Hettich Zentrifugen, model Mikro 12-42, Germany), with a version of the technique reported by Calderon-Guzman *et al*. (2008).

Technique to measure glutathione (GSH): The levels of GSH were measured from a sample of the floating tissue homogenized in $HClO_4$ which was gotten after being centrifuged at 9000 rpm for 5 min. (in a microcentrifuge Mikro 12-42, Germany), according to the technique reported by Hissin and Hilf (1976).

Technique to measure lipid peroxidation (TBARS): The measurement of lipid peroxidation by technique of thiobarbituric acid reactive substance (TBARS) was done using the technique reported by Gutteridge and Halliwell (1990).

Technique to measure ATPase: In the case of total ATPase activity, the measurement was carried out using the technique reported by Guzman *et al*. (2005). To measure inorganic phosphate (P_i) was based on method proposed by Fiske and Subbarow (1925).

Statistic analysis: Kruskal-Wallis statistical test and Analysis of Variance (ANOVA) with their respective contrasts with Dunnett test, after being subjected to variances homogeneity with χ^2 test were used. The values of $p<0.05$ were considered statistically significant (Castilla-Serna and Cravioto, 1999).

RESULTS

Table 1 shows blood glucose levels of rats treated with L-carnitine 136.0±6.96 mg dL^{-1} (Mean±Standard Deviation) and desvenlafaxine 142.4±8.53 in the absence (134.0±13.2) and presence of sucrose (138.0±5.47) which were found without statistically significant changes when compare with the control group. The same table shows food consumption per group of rats treated with L-carnitine and desvenlafaxine in absence and presence of sucrose during study. There was a slight decrease in food intake during treatment in all groups treated with sucrose or desvenlafaxine.

Figure 1 depicts dopamine levels in brain regions of rats treated with L-carnitine and desvenlafaxine in the absence or presence of sucrose. Dopamine concentration in cerebellum and medulla oblongata was found to increase significantly ($p<0.001$ Kruskal-Wallis test) in rat groups that received sucrose plus L-carnitine 1.8±0.41 (Mean±Standard Deviation) nM g^{-1} wet tissue or sucrose plus desvenlafaxine 2.2±0.44 with respect to the control group 1.45±0.32. Dopamine level in cortex region significantly decreased ($p<0.05$) in groups that received only sucrose or sucrose in combination with desvenlafaxine or L-carnitine.

With respect to hemisphere regions, there was a significant decrease in this biomarker ($p<0.001$) in groups that received only sucrose or only desvenlafaxine in comparison with the control group.

The levels of glutathione (GSH) in brain regions of rats treated with L-carnitine and desvenlafaxine in the absence and presence of sucrose (Fig. 2) decreased significantly ($p<0.001$ Kruskal-Wallis test) in all groups combined with sucrose, however, the rats, in the same group but not treated with sucrose 191±13 (Mean±Standard Deviation) nM g^{-1} wet tissue, witnessed an increase of this biomarker in cerebellum/medulla oblongata 351±35 and a decrease in cortex and hemispheres regions 139±11 nM g^{-1} wet tissue.

In the case of lipid peroxidation concentration in brain regions of rats treated with L-carnitine plus desvenlafaxine in the presence of sucrose (Fig. 3), there was significant decrease ($p<0.001$) in cortex

Table 1: Glucose levels of rats treated with L-carnitine and desvenlafaxine in absence and presence of sucrose and amount of food consumed in each group (g) during treatment days with bioterium normal diet (23% protein)

		Days of treatment				
Groups *	Glucose (mg dL^{-1})±SD**	1	2	3	4	5
Control (Vehicle)	134.0±13.2	35	60	45	70	70
L-carnitine	136.0±6.96	50	70	50	75	80
Desvenlafaxine	142.4±8.53	45	50	45	45	50
Control + Sucrose	138.0±5.47	35	38	28	20	40
L-carnitine + Sucrose	132.2±8.79	45	40	70	70	65
Desvenlafaxine + Sucrose	132±14.40	38	38	48	55	80

For each group n = 5 rats*. There was not significant difference ANOVA p>0.05**

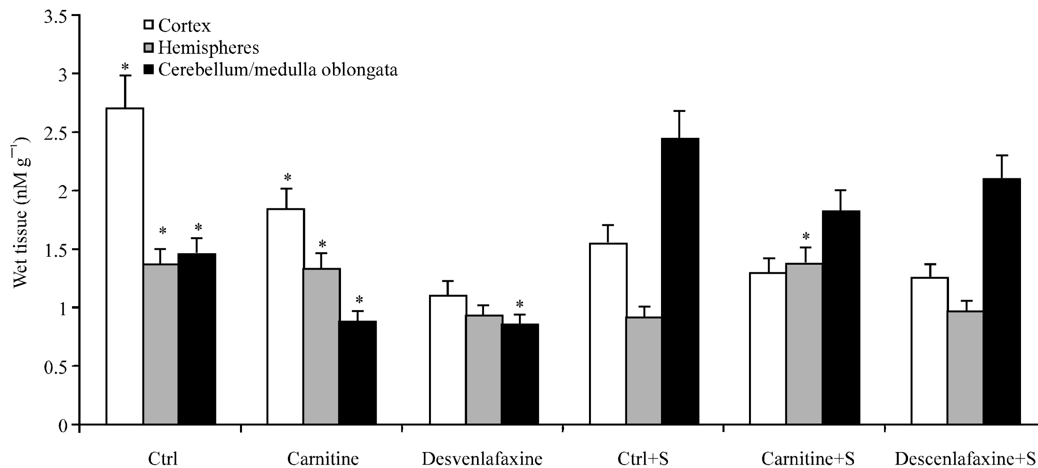

Fig. 1: Dopamine levels in brain regions of rats treated with L-carnitine and desvenlafaxine in the absence and presence of sucrose (s). Ctrl = control. Kruskal-Wallis test. *$p<0.05$; Cortex: Control vs. Desvenlafaxine, Desvenlafaxine + S, L-carnitine + S, Sucrose, L-carnitine. $p<0.0001$, L-carnitine vs. Desvenlafaxine, Desvenlafaxine + S, L-carnitine + S. $p<0.05$; Hemispheres: Control, L-carnitine, L-carnitine + S vs. Desvenlafaxine, Sucrose, Desvenlafaxine + S. $p<0.05$; Cerebellum/Medulla Oblongata: Control, L-carnitine, Desvenlafaxine vs. Sucrose, L-carnitine + S, Desvenlafaxine + S. $p<0.0001$. Bars represent each tissue

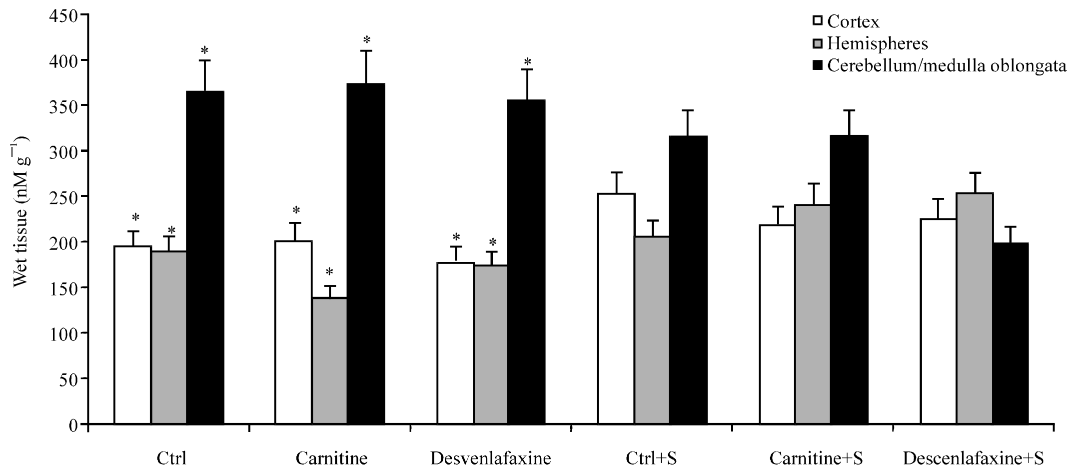

Fig. 2: GSH in brain regions of rats treated with L-carnitine and desvenlafaxine in the absence and presence of sucrose (s). Ctrl = control. Kruskal-Wallis test. * $p<0.05$, Cortex: Control, L-carnitine, Desvenlafaxine vs. Sucrose, L-carnitine + S, Desvenlafaxine + S. p=0.0001; Hemispheres: Control vs. Desvenlafaxine + S. $p<0.05$, L-carnitine vs. Desvenlafaxine + S, L-carnitine + S. $p<0.0001$, Desvenlafaxine vs. Desvenlafaxine + S, L-carnitine + S. p = 0.02; Cerebellum/Medulla oblongata: Control, L-carnitine, Desvenlafaxine vs. Control + S, Desvenlafaxine + S, L-carnitine + S. p=0.0003. Bars represent each tissue

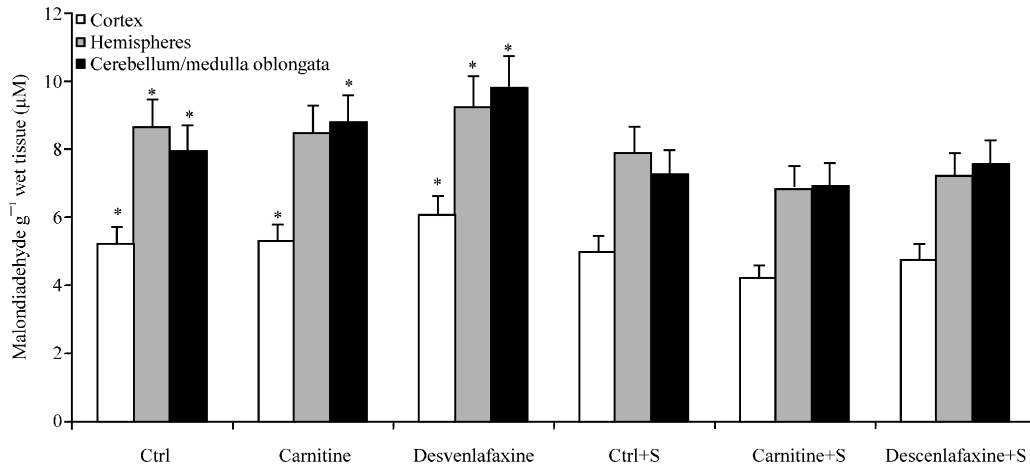

Fig. 3: Lipid peroxidation in brain regions of rats treated with L-carnitine and desvenlafaxine in the absence and presence of sucrose (s). Ctrl = control. Tukey test. * $p<0.05$; Cortex: Control vs. L-carnitine + S. p = 0.02, Desvenlafaxine vs. L-carnitine + S, Desvenlafaxine + S, Control + S. $p<0.05$, L-carnitine vs. L-carnitine + S. p = 0.01, Hemispheres: Desvenlafaxine vs. L-carnitine + S, Desvenlafaxine + S. $p<0.05$, Cerebellum/Medulla oblongata: Desvenlafaxine vs. Control, Control + S, L-carnitine + S, Desvenlafaxine + S. $p<0.003$, L-carnitine vs. Control + S, L-carnitine + S. $p<0.04$. Bars represent each tissue

8.3±0.9 µM g^{-1} wet tissue, hemispheres 7.1±0.8 and cerebellum/medulla oblongata 7.2±0.9 on application of Tukey test.

Total ATPase activity under the same treatments and condition and in the same regions is shown in Fig. 4. It could be seen that there was a decrease 104±9 µM g^{-1} wet tissue/min (Mean±SD) in the concentration of total ATPase activity in cerebellum/medulla oblongata in all groups that received sucrose 67±8 and 68±7 for L-carnitine or desvenlafaxine, respectively. However, in cortex this activity was found to increase in groups that received sucrose combined with

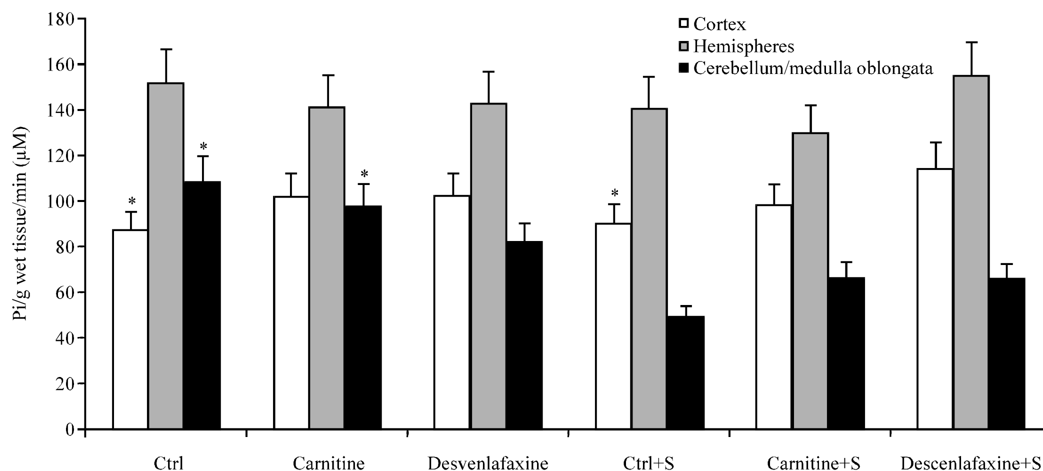

Fig. 4: Total ATPase in brain regions of rats treated with L-carnitine and desvenlafaxine in the absence and presence of sucrose (s). Ctrl = control. Kruskal-Wallis test. *$p<0.05$, Cortex: Control, Control + S vs. L-carnitine, Desvenlafaxine, L-carnitine + S, Desvenlafaxine + S. $p<0.05$; Cerebellum/Medulla oblongata: Control vs. Control + S, L-carnitine + S, Desvenlafaxine + S. $p<0.05$, L-carnitine vs. Control + S. $p<0.0$

L-carnitine or desvenlafaxine, even when Tukey or Kruskal-Wallis test showed significant difference ($p<0.05$).

DISCUSSION

The metabolism of brain glucose is crucial for CNS function mainly because glucose is critical for the brain (Shi and Liu, 2006). In the present study, glucose levels in blood increased slightly by the consumption of sucrose in water, probably as a consequence of less food intake or due to satiety. These results are in accordance with Koren *et al.* (2007) who suggest that an increase in either carbohydrate or protein intake increases satiety and leads to significant weight loss.

The changes in homeostasis of glucose are associated with the action of ATPase enzymes (Torlinska *et al.*, 2006). These enzymes decreased due to the effect of sucrose in combination with L-carnitine and desvenlafaxine in cerebellum/medulla oblongata regions, probably as a consequence of changes in the structural composition of the enzyme in the brain (Neault *et al.*, 2001). Studies carried out by Isaev *et al.* (2007), indicate that the effect of hypoglycemia is to increase calcium charge in the nerve tissue and this in turn alters the effect of ATPase enzymes. Other studies suggest that ATPase decreased in crude synaptosomal membranes of diabetic mice. These animals exhibit a decreased capacity for glucose oxidation and increased capacity for fatty acid oxidation (Makar *et al.*, 1995), because glucose determines fatty acid oxidation by controlling the rate of long-chain fatty acid entrance into the mitochondria (Sidossis *et al.*, 1996). These results are not in accordance with the present study, due to the fact that lipid peroxidation in brain regions (cortex, hemispheres, cerebellum/medulla oblongata), decreased by consumption of sucrose in combination with L-carnitine and desvenlafaxine.

In the present study, the concentration of dopamine increased in cerebellum/medulla oblongata of rats that received sucrose alone or sucrose plus L-carnitine or desvenlafaxine. Opposite effect were found on cortex and hemispheres regions, where sucrose intake alone or combined decreased dopamine. These results agreed with the results of Johnson *et al.* (2011), who suggest that the chronic effects of excessive sugar intake may lead to alterations in mesolimbic dopamine signaling, even though the mechanism of action is not clearly known.

The concentration of GSH increased in cortex and hemisphere regions principally in the administration of sucrose combined with L-carnitine or desvenlafaxine and decreased in cerebellum/medulla oblongata. These effects could be due to changes in NO levels, because, it is an extremely important system in cellular free radical detoxification (Zhu *et al.*, 2006).

CONCLUSION

The results of the present study suggest that the administration of sucrose in combination with L-carnitine or desvenlafaxine in subjects significantly reduces hyperglycemia, due to the inhibition of fatty acid oxidation in brain regions. Reduction of oxidative stress

may be involved in these effects. Further studies are necessary to investigate the possible relationship between chronic sugar intake and L-carnitine or desvenlafaxine in different animal models.

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