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Full Length Research Paper

Effect of an anorexiant on levels of 5-HIAA and GSH in brain of well-nourished and malnourished rats

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In this study, the effect of sibutramine and carnitine on 5-HIAA and GSH levels in brain regions of malnourished rats was analyzed. Twenty-four rats were fed with a normal protein diet (23%) and another similar group with protein starvation diet (7%). The animals of both groups were re-assigned into four groups of 6 animals per group and treated for 10 days as follows: group 1, saline solution; group 2, sibutramine (10 mg/kg); group 3, carnitine (500 mg/kg); and group 4, sibutramine (10 mg/kg) + carnitine (500 mg/kg). The animals were sacrificed at the end of treatment and their cerebrum, cortex, cerebellum and medulla oblongata were extracted to determine the levels of glucose, glutathione (GSH), lipid peroxidation (TBARS), and 5-hydroxyindole acetic acid (5-HIAA). The corporal weights of animals fed with low protein diet were significantly lower than those on normal diet. The levels of 5-HIAA, TBARS, and GSH showed significant differences due to subitramine and carnitine effects, principally in cortex than in the rest of the brain areas of rats fed with low diet. Sibutramine and carnitine alter serotonergic metabolism in the brain regions of animals subjected to low protein diets. Reduction in oxidative stress may be involved in these effects.

Key words: Anorexiant, brain, glutathione, malnutrition, serotonin.

INTRODUCTION

Obesity and overweight have been implicated in increase in free radicals (FR) (Flora, 2007). It is related to central nervous system diseases and gives rise to complications that affect cognitive regions of the cerebrum which modulate the memory, or culminate in neurodegenerative diseases (ND) (Maiese et al., 2007) especially those induced by the presence of free radicals in central nervous system (CNS) (Tang et al., 1996) that make the cells of this system susceptible to oxidative damage caused by excessive production of oxygen reactive species implicated in pathologic process (Coyle and Puttfarcken 1993), as well as in inhibition of biomarkers that participate in mitochondrial metabolism (Virmani et al., 2005).

Free radicals are reactive species possessing unpaired electron that principally comes from nitrogen and oxygen metabolism. These substances have been implicated in oxidative stress mechanism and cerebral dysfunction (Driver et al., 2000). An alternative to combat endogenous free radicals induced by aging and

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neurodegenerative diseases requires the presence of free radical absorbers (Wu et al., 2004) which can be stimulated by using supplements with antioxidant activities (Arockia and Panneerselvam, 2001). Glutathione (GSH) is the principal regulator of redox equilibrium and collaborates in protection of tissues exposed to oxidizing agents (Beckman et al., 1990). The formation of excessive FR which could be interpreted like oxidizing damage have dual participation not only deleting, but also beneficial effects to maintain the physiological functions of every cell structure (Valko et al., 2007). Depletion of systemic glutathione levels has been reported in a number of stress conditions, including short-term food deprivation and chronic dietary protein deficiency (Grimble et al., 1992). Among the drugs presently used for weight reduction is the sibutramine and It could be an alternative for people suffering from obesity (James et al., 2000). Presently, carnitine is used as supplement for weight reduction in adults and adolescents (Onem et al., 2006; Rogovik and Goldman, 2009); however its effects on young subjects are still unknown.

The etiology of eating disorders as anorexia (AN) and bulimia (BN) which mainly occur in young women are consistently characterized by perfectionism, obsessive-compulsiveness and intake of drugs for weight loss. The etiology is unknown, but there are biological factors that play a relevant role in the pathogenesis (Ostuzzi et al., 2008). It was found that caloric restriction reduces inflammatory and oxidant effects in the brain (Ugochukwu et al., 2006) which is in conformity with the studies carried out in our laboratory where it was suggested that hypoproteic-normocaloric diet increases oxidative damage (Calderon et al., 2005). However, we cannot exclude the participation of leptine which is responsible for regulating satiety through the control of food ingestion and body weight directly in the hypothalamic nucleus and other regions of the brain (Kutlu et al., 2005). Hyperleptinemia is associated with oxidative stress and nitric oxide (NO) inactivation (Beltowski et al., 2004). This messenger has effect on serotonin (5-HT) which is influenced by hormonal status of the female rats (Diaz et al., 1997) and their metabolites (Benuck et al., 1995) are important neurotransmitters that actively participate in the control of appetite and body weight (Prunet et al., 2003). Based on this, the aim of this study is to evaluate the effect of sibutramine and carnitine on the levels of 5-hydroxyindole acetic acid, GSH and lipid peroxidation in an animal model subjected to hypoproteic as well as normocaloric diets.

METHODOLOGY

Forty eight young Sprague Dawley rats were employed in the study. The animals were divided in two groups and fed either with normal diet (diet I) or hypoproteic diet (diet II). The animals fed with diet I and diet II were each divided into four sub-groups and in combination of the diet, a dose of the following substances was administered every 36 h in 10 occasions in the following form: group 1, NaCl 0.9% (control); group 2, sibutramine (10 mg/kg); group 3, carnitine (500 mg/kg); and group 4, sibutramine (10 mg/kg) + carnitine (500 mg/kg). All treatments were given intraperitoneally. The animals under diet I were fed with bioterium normal diet (23% protein) (Lab Rodent Diet 5001) and those on diet II were fed with hypoproteic diet (7% protein). Similar dosage and design have been used previously (Guzman et al., 2009). Body weight and food consumption registry were taken prior and after the treatment. All experimental procedures were done under rules of the Laboratory Animals Use and Care Committee of our Institution. 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 and 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 w/v so as to determine lipid peroxidation, GSH, and 5-HIAA. The samples were maintained at -20°C until analyzed.

Measurement of blood glucose concentration

The procedure to measure blood glucose was carried out in all groups of animals at the moment of sacrifice. 10 µl 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.

Measurement of lipid peroxidation (TBARS concentration)

The determination of TBARS was made using the modified technique of Gutteridge and Halliwell (1990). Each brain region (cortex, hemispheres, cerebellum and medulla oblongata) was homogenized in 2 ml of tris-HCl 0.05 M pH 7.4 buffer. From the homogenates, 250 µl was taken and was mixed with 1 ml of thiobarbituric acid (TBA) containing 1.25 g of TBA, 40 g of trichloroacetic acid (TCA) and 6.25 ml of concentrated chlorhydric acid (HCl) diluted in 250 ml of deionized H_2O were added to the homogenates. The mixtures were heated to boiling point for 30 min (Thermomix 1420). The samples were later placed in an ice bath for 5 min and were centrifuged at 3,000 g for 15 min (Sorvall RC-5B Dupont). The absorbances of the floating were read in duplicate at 532 nm in a spectrophotometer (Heλios-α of UNICAM). The concentration of substances reactive to thiobarbituric acid (TBARS) was expressed in µM of malondialdehyde/g of wet tissue for each region.

Measurement of 5-hydroxyindole acetic acid concentration

The levels of 5-hydroxyindole acetic acid (5-HIAA) were evaluated using the floating tissues of the brain regions previously mixed with $HClO_4$ (2:1 v/v) and centrifuged at 10,000 rpm for 10 min in a micro centrifuge (Hettich Zentrifugen, model Mikro 12-42, Germany). Aliquots of the brain regions were taken and processed in Perkin Elmer LS 55 fluorometer with wavelengths of 296 nm/333 nm of excitation and emission, using FL Win Lab version 4.00.02 software (Guzman et al., 2010). The values were inferred in a standard curve

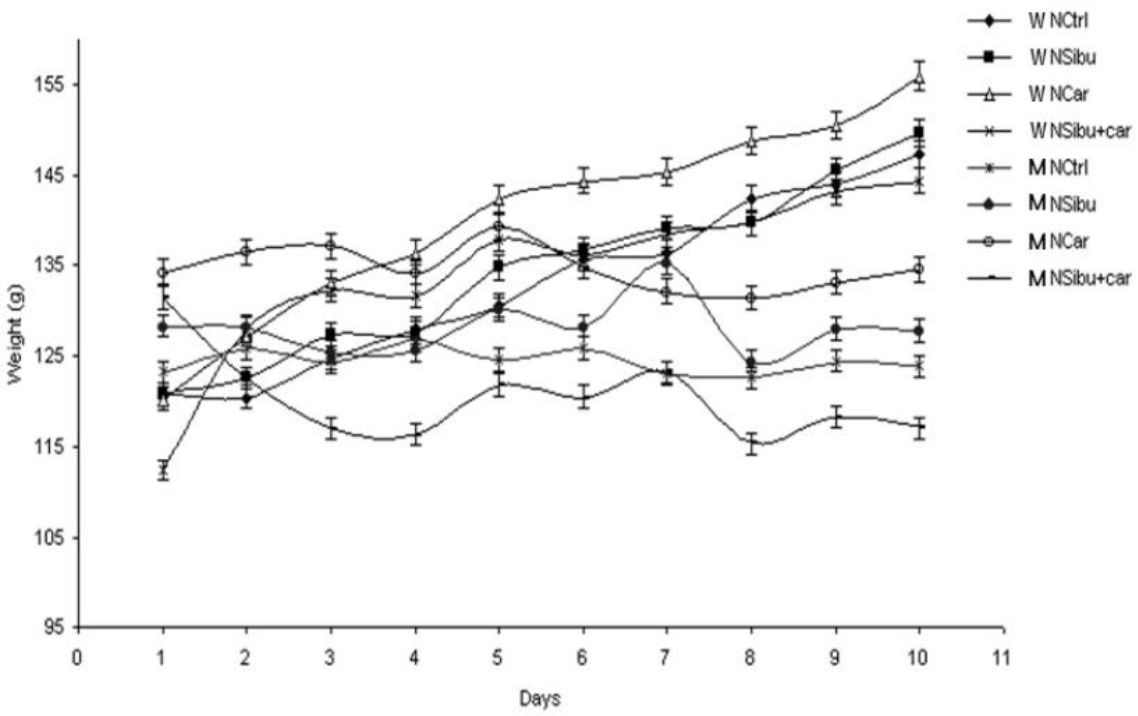

Figure 1. Register of body weight of rats under normal and low protein diets treated with sibutramine and carnitine. Mean value ± S.D. WNCtrl = Well-nourished control, WNSibu = Well-nourished sibutramine, WNCarni = Well-nourished carnitine, WNSibu + carni = Well-nourished sibutramine + carnitine. MNCtrl = Mal-nourished control, MNSibu = Mal-nourished sibutramine, MNCarni = Mal-nourished carnitine, MNSibu + carni = Mal-nourished sibutramine + carnitine. ANOVA two ways, Well-nourished vs Mal-nourished *p < 0.05.

previously standardized and reported in nM/g of wet tissue.

Measurement of glutathione levels

The floating tissue homogenized in $HClO_4$ and obtained after being centrifuged at 10000 rpm for 10 min (in Mikro 12-42, Germany, microcentrifuge) was used to measure the levels of GSH employing a modified technique reported by Hissin and Hilf (1976). This entails putting 1.8 ml of phosphate buffer pH 8.0 with EDTA 0.2%; an aliquot of $HClO_4$ floating; and 100 µl of ortho-phtaldialdehyde (OPT) in concentration of 1 mg/ml in methanol in an assay tube and incubating the mixture without shaking for 15 min at atmospheric temperature in total darkness. At the end of the incubation, the samples were read in a Perkin Elmer LS 55 spectrofluorometer, with a longitude of 350 nm of excitation and 420 of emission. An FL Win Lab version 4.00.02 Software was used. The values were inferred in a standard curve previously standardized and were reported as nM/g of wet tissue.

Analysis of results

Results were analyzed by analysis of variance (ANOVA) two-way test, with Dunnett contrasts, which was employed prior to homogeneity variance test determined by Bartlett X^2 test. The values of $p < 0.05$ were considered statistically significant (Castilla and Cravioto, 1999). Version 6.0.0 JMP Software for academic purposes was used in the analysis.

RESULTS

The body weights of animals treated with normal diet (23% protein), sibutramine and carnitine significantly increased ($p = 0.001$) (Figure 1) in comparison with those treated with low protein diet (7% protein). Food consumption in the animals treated with different diets (Table 1) and carnitine increased for both diets and decreased in the group of animals under low protein diet that received a combination of sibutramine and carnitine, however, without significant differences. The concentration of glucose in the rats under different nutritional condition that were treated with sibutramine and carnitine (Table 2) partially reduced in the group of animals fed with low protein diet that received only sibutramine and sibutramine + carnitine with respect to those under normal diet. The animals under both kinds of diet treated with only carnitine did not witness any changes in their glucose concentration.

With regard to lipid peroxidation levels in brain regions of rats subjected to different nutritional condition that were treated with sibutramine and carnitine (Figure 2), there were changes in this indicator in cortex, cerebellum and medulla oblongata of the animals fed with both diets

Table 1. Food consumption of rats under normal and low protein diets treated with sibutramine and carnitine. Mean value per group (g).

Parameter	Days of treatment								
Groups	2	3	4	5	6	7	8	9	10
WNCtrl (6)	45	48	52	54	54	42.5	55	52.5	62.5
WNSibu (6)	42	52	38	61	47	42.5	48	54	56
WNCarni (6)	38	68	49	58	53.5	42.5	58	55.5	64.5
WNSibu+Carni (6)	40	51	40	56.5	40.5	37.5	72	51	46
MNCtrl (6)	23.5	39.7	62	67	59	50	42.5	57.5	60
MNSibu (6)	31.5	28.5	51.5	27.2	49.3	36.5	37	60.5	56
MNCarni (6)	30	43.5	52.5	30	45	45.5	42	70	67
MNSibu+Carni (6)	15.5	25.5	41	39.3	52.7	48	33	68.5	57.5

( ) Number of animals, WNCtrl = Well-nourished control, WNSibu = Well-nourished sibutramine, WNCarni = Well-nourished carnitine, WNSibu + carni = Well-nourished sibutramine + carnitine. MNCtrl = Mal-nourished control, MNSibu = Mal-nourished sibutramine, MNCarni = Mal-nourished carnitine, MNSibu + carni = Mal-nourished sibutramine + carnitine.

Table 2. Glucose concentration of rats under normal and low protein diets treated with sibutramine and carnitine. Mean Values ± S.D.

Groups	Mean	SD
WNCtrl	131.5	19.91
WNSibu	136.75	18.08
WNCarni	138.75	12.37
WNSibu + carnitine	131.5	12.77
MNCtrol	120.75	10.63
MNSibu	135.0	25.76
MNCarni	138.75	11.87
MNSibu + carnitine	122.5	11.73

S.D.=Standard Deviation. WNCtrl = Well-nourished control, WNSibu = Well-nourished sibutramine, WNCarni = Well-nourished carnitine, WNSibu + carni = Well-nourished sibutramine + carnitine. MNCtrl = Mal-nourished control, MNSibu = Mal-nourished sibutramine, MNCarni = Mal-nourished carnitine, MNSibu + carni = Mal-nourished sibutramine + carnitine.

and treated with sibutramine and carnitine. In the region of medulla oblongata, the administration of sibutramime and carnitine combined or alone in the group of animals with protein deficiency diet showed a dual effect with significant differences ($p < 0.05$) when compared with the groups under normal diet.

For the levels of 5-HIAA in cerebral regions of rats under different nutritional conditions which were treated with sibutramine and carnitine (Figure 3), the animals with normal diet and carnitine treatment presented significant differences ($p = 0.0005$) in brain cortex with respect to the group of animals in the same diet and those with low protein diet that were treated with carnitine. In the region of cortex, the group of animals with malnutrition and treated with sibutramine showed differences in comparison with the rest of the groups in the same diet. In brain cortex of the animals with normal diet subjected to sibutramine + carnitine treatment, an increase of 5-HIAA with significant differences ($p = 0.0005$) was observed when compared with the rest of the groups in the same diet. In the region of cerebellum, the animals that received a combination of sibutramine and carnitine witnessed an increase in the levels of 5-HIAA with respect to the animals under low protein diet that received the same treatment. In the region of medulla oblongata, the animals with normal diet that were treated with a combination of sibutramine and carnitine showed a significant difference ($p = 0.0011$) in the levels of 5-HIAA with respect to the rest of the groups on the same diet.

The levels of GSH in the cerebral regions of rats with different nutritional conditions which were treated with sibutramine and carnitine (Figure 4) significantly increased ($p = 0.05$) in the cortex of animals with protein deficiency diet that were treated with carnitine when compared with animals on normal diet. In the medulla oblongata, there was a decrease in the levels of GSH with significant differences ($p < 0.05$) in the group of animals under normal diet that received sibutramine plus carnitine with respect to the groups that received only sibutramine or carnitine that were subjected to the same diet.

DISCUSSION

In the face of the present problems of obesity, studies evaluating different protein diets in combination with weight reducing drugs are indispensable. This is because

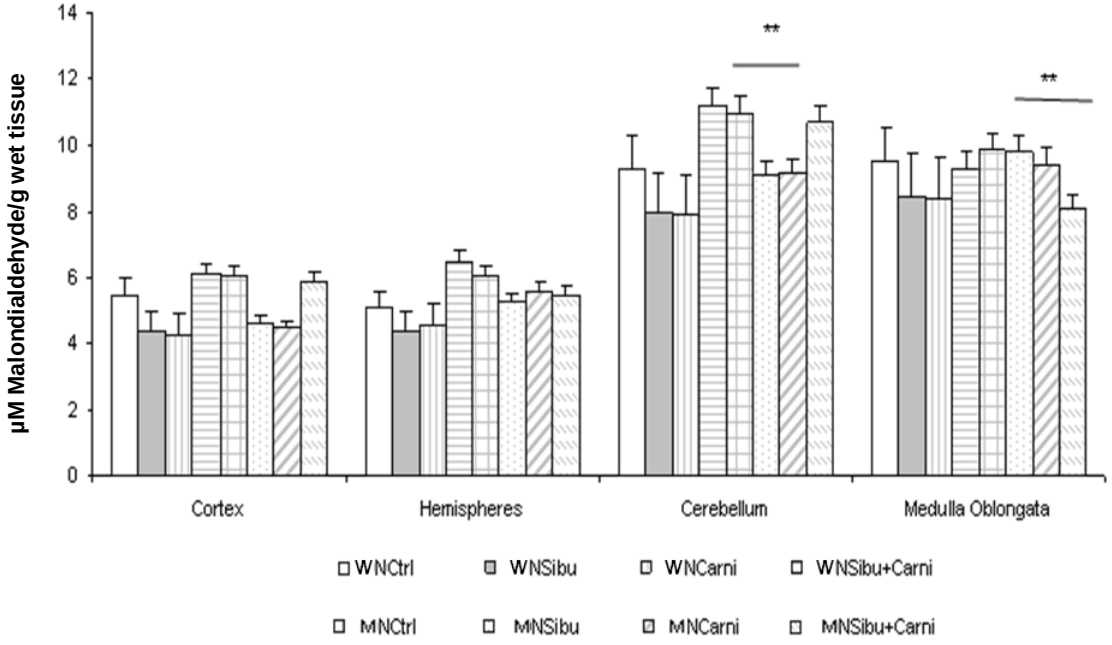

Figure 2. Lipid peroxidation levels in cerebral regions of rats under normal and low protein diets treated with sibutramine and carnitine. WNCtrl = Well-nourished control, WNSibu = Well-nourished sibutramine, WNCarni = Well-nourished carnitine, WNSibu + carni = Well-nourished sibutramine + carnitine. MNCtrl = Mal-nourished control, MNSibu = Mal-nourished sibutramine, MNCarni = Mal-nourished carnitine, MNSibu + carni = Mal-nourished sibutramine + carnitine. Mean value ± S.D. **p<0.05.

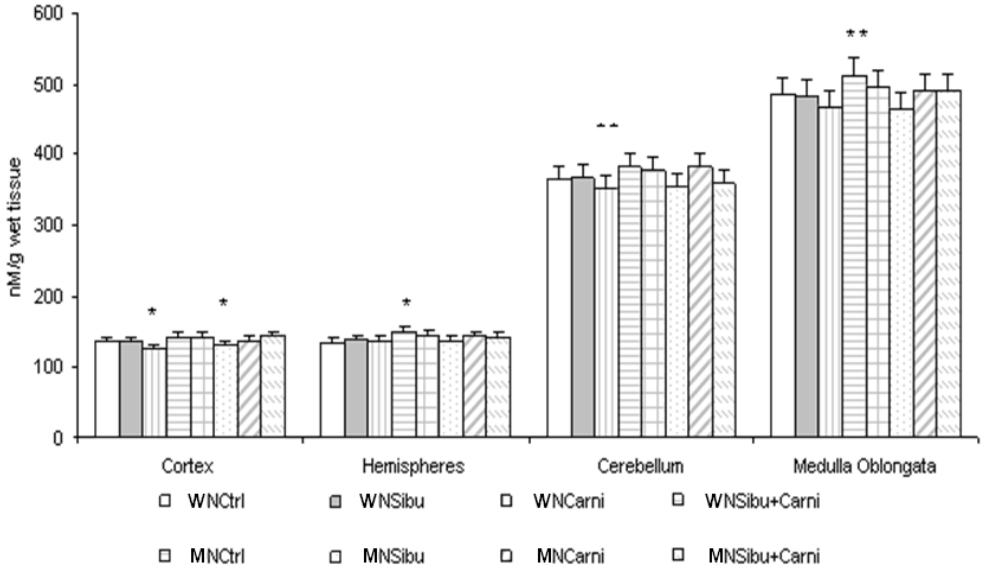

Figure 3. Levels of 5-Hidroxyindole acetic acid in cerebral regions of rats under normal and low protein diets treated with sibutramine and carnitine. WNCtrl = Well-nourished control, WNSibu = Well-nourished sibutramine, WNCarni = Well-nourished carnitine, WNSibu + carni = Well-nourished sibutramine + carnitine. MNCtrl = Mal-nourished control, MNSibu = Mal-nourished sibutramine, MNCarni = Mal-nourished carnitine, MNSibu + carni = Mal-nourished sibutramine + carnitine. Mean value ± SD. **p < 0.05.

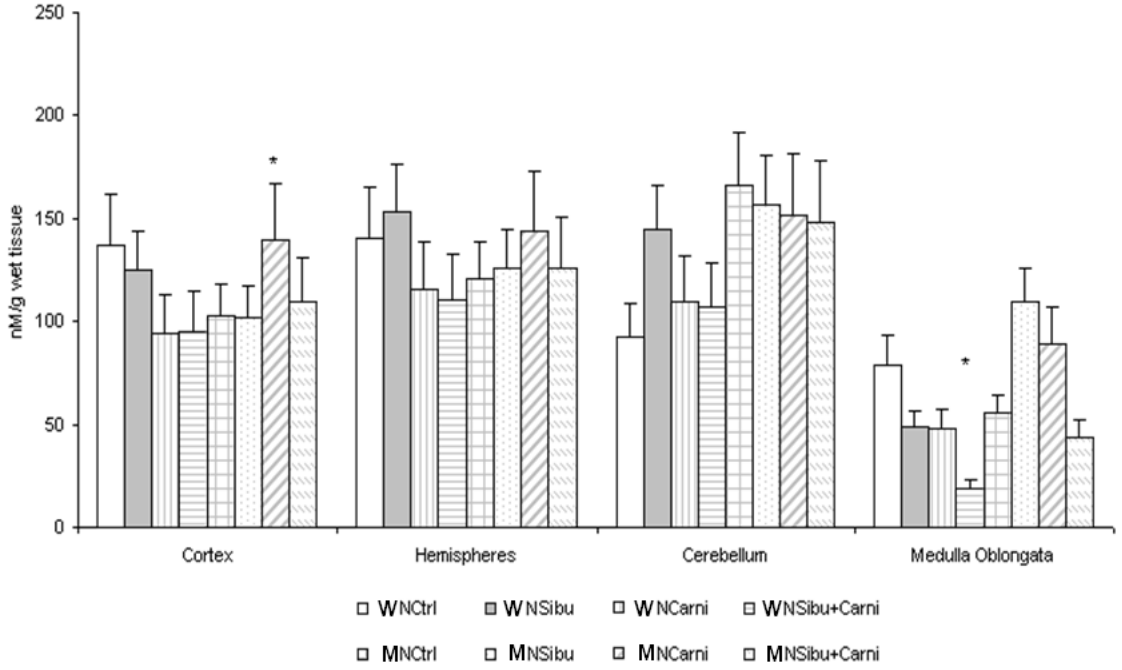

Figure 4. Levels of GSH in cerebral regions of rats under normal and low protein diets treated with sibutramine and carnitine. WNCtrl = Well-nourished control, WNSibu = Well-nourished sibutramine, WNCarni = Well-nourished carnitine, WNSibu + carni = Well-nourished sibutramine + carnitine. MNCtrl = Mal-nourished control, MNSibu = Mal-nourished sibutramine, MNCarni = Mal-nourished carnitine, MNSibu + carni = Mal-nourished sibutramine + carnitine. Mean value ± SD. **p<0.05.

their results can be extrapolated to older subjects, on the bases that their protein requirements are similar to that of young subjects (Kurpad and Vaz, 2000). Moreover, in infectious process; there is a synergic interaction between nutrition and infection for the mere fact that adverse nutritional state carries with it a higher susceptibility to infection (Kurpad, 2006). Eating disorders (ED) as anorexia (AN) and bulimia (BN) both by stress, are important psychiatric and somatic conditions that mainly occur in young women. Individuals with AN and BN are consistently characterized by perfectionism, obsessive-compulsiveness and intake of drugs for weight loss.

In the present study, the administration of sibutramine did not affect the body weight of animals under normal diet (23% protein) and under low protein diet (7% protein). These results coincided with that of Esposito et al. (2008) who hypothesized that sibutramine, through its effect on neurotransmitters, may induce antidiuretic hormone secretion and may lead to a syndrome of inappropriate antidiuretic hormone secretion, as a consequence of liquid retention in the body. These authors advised careful monitoring of water-electrolyte balance during sibutramine therapy. In contrast to the findings of Stroubini et al. (2008) who proposed that sibutramine, apart from having preferential effect on body fat attributed to its sympatomimetic properties on adiponectine increases the loss of body fat in subjects with high carbohydrate diet (Stroubini et al., 2009).

In the present study, the animals that received low protein diet which were treated with sibutramine in combination with carnitine witnessed a higher weight loss. This is probably because the rest of the experimental groups with low protein diet consumed a higher quantity of food. These findings did not coincide with the studies of Clifton (2008), who suggested that the combination of sibutramine with hypocaloric diet increases weight loss. Control animals under the same diet showed lower levels of glucose than the animals under normal protein diet, however, the blood glucose level of the former normalized with the administration of sibutramine and carnitine even when they had a higher consumption of food. These results partially coincide with the findings of Irat et al. (2003), who suggested that carnitine normalizes glucose levels and substances reactive to thiobarbituric acid in the blood of diabetic rats. This last effect was contrary to the present study because the levels of lipoperoxidation highly reduced in the

animals fed with low protein diet due to sibutramine and carnitine administration, particularly in cerebellum and medulla oblongata regions. This suggests that the nutritional condition was the cause in medulla oblongata. This effect coincides with previous studies carried out in our laboratory where the products of lipoperoxidation were found to reduce in the cerebrum after the sibutramine treatment, especially in female rats. This suggests that the effect could be attributable to the chemical structure of sibutramine which possesses certain functional groups like dimethylamine and chlorine heteroatom all found in the aromatic ring which confers to them electrophilic character that facilitates their interaction with lipidic structure of the cerebrum (Calderon et al., 2009).

The levels of 5-HIAA in the cortex and cerebellum of animals on normal diet reduced due to treatment with carnitine, but increased in cortex and medulla oblongata regions when the drug was combined with sibutramine. This is probably because the cortex is a region rich in cholinergic neurons and in the presence of carnitine, the concentration of serotonin is increased as suggested by some authors (Juliet et al., 2003).

The concentration of GSH in animals with low protein diet that were treated with carnitine increased in the cortex. This is in accordance with previous studies (Sun, 1990) and suggests a decrease in the vulnerability of CNS in the presence of hydroxyl and hydrogen peroxide radicals coming from normal metabolism of CNS. On the other hand, GSH levels in medulla oblongata decreased with the combination of sibutramine and carnitine in animals fed with normal diet, suggesting a lesser adverse effect. The fall in GSH levels under these conditions implies that a persistent oxidative load leads to the net consumption of reduced GSH in excess of the ability of the body to re-synthesize the molecule (Jahoor et al., 1995).

Conclusion

Obesity and overweight have been implicated in the increase of free radicals. Depletion of systemic glutathione levels has been reported in a number of stress conditions, including short-term food deprivation and chronic dietary protein deficiency. The results of the present study suggest that the administration of sibutramine and L-carnitine to animals under normal and low protein diets induces dual antioxidant effect and alter serotonergic metabolism. Reduction in oxidative stress may be involved in these effects

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