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Sleep deprivation alters energy homeostasis through non-compensatory alterations in hypothalamic insulin receptors in Wistar rats

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ABSTRACT

Studies have shown a gradual reduction of sleep time in the general population, accompanied by increased food intake, representing a risk for developing obesity, type II diabetes and cardiovascular disease. Rats subjected to paradoxical sleep deprivation (PSD) exhibit feeding and metabolic alterations, both of which are regulated by the communication between peripheral signals and the hypothalamus. This study aimed to investigate the daily change of 96 h of PSD-induced food intake, body weight, blood glucose, plasma insulin and leptin concentrations and the expression of their receptors in the hypothalamus of Wistar rats. Food intake was assessed during the light and dark phases and was progressively increased in sleep-deprived animals, during the light phase. PSD produced body weight loss, particularly on the first day, and decreased plasma insulin and leptin levels, without change in blood glucose levels. Reduced leptin levels were compensated by increased expression of leptin receptors in the hypothalamus, whereas no compensations occurred in insulin receptors. The present results on body weight loss and increased food intake replicate previous studies from our group. The fact that reduced insulin levels did not lead to compensatory changes in hypothalamic insulin receptors, suggests that this hormone may be, at least in part, responsible for PSD-induced dysregulation in energy metabolism.

Introduction

Obesity is a global concern; what once was seen only in rich countries, today is evidenced around the world, with the prevalence doubled between 1980 and 2008, regardless of gender, race and age (World Health Statistics 2012 — World Health Organization). Increased prevalence of obesity has high co-morbidity with cardiovascular disease and type II diabetes, and has been attributed to modern lifestyle, characterized by bad eating habits and less physical activity (Egger and Swinburn, 1997). This lifestyle also seems to have changed the sleep habits of the population, as more people undergo episodes of sleep deprivation, or even chronic voluntary sleep restriction, due to stress, work and even leisure (Broman et al., 1996), affecting not only the total sleep time, but also its architecture and quality (Shechter et al., 2012).

If on one hand, obesity may increase the likelihood to develop sleep apnea (Panossian and Veasey, 2012; Peppard et al., 2000), one of the most prevalent sleep disorders (Tufik et al., 2010; Young et al., 1993), on the other hand, reduction of sleep can increase the risk of obesity and type II diabetes (Patel and Redline, 2004; Van Cauter and Knutson, 2008; Vorona et al., 2005; Xu et al., 2010), establishing a vicious circle. Even individuals displaying naturally occurring short sleep (Taheri et al., 2004) and those submitted to short-term sleep restriction (Spiegel et al., 2004, 1999) exhibit hormonal and metabolic changes, including reduced leptin and increased ghrelin plasma concentrations, prolonged return to baseline cortisol levels at night and higher insulin resistance, that strengthens the impact of inadequate sleep on metabolic homeostasis. Spiegel et al. (2004) reported increased rates of hunger and appetite in subjects submitted to two nights of 4 h of sleep, compared to two subsequent nights of 10 h of sleep. In addition, volunteers sleep-restricted for 14 days (5.5 h in bed) increased their preference for high-carbohydrate foods during the night, compared to 14 days of 8.5 h of sleep (Nedeltcheva et al., 2009).

Similar to what is observed in humans, rats subjected to sleep deprivation also display increased food intake (Galvão et al., 2009; Koban et al., 2008; Martins et al., 2010; Rechtschaffen and Bergmann, 1995). Nevertheless, these animals show intense catabolism (Everson and Wehr, 1993; Hipolide et al., 2006; Suchecki et al., 2003) and energy expenditure, resulting in weight loss during the sleep deprivation period (Koban and Stewart, 2006; Martins et al., 2006; Suchecki et al., 2003). Furthermore, reduced plasma concentrations of anabolic hormones such as insulin (Hipolide et al., 2006), testosterone (Andersen et al., 2004), leptin and growth hormone (Everson and Crowley, 2004; Koban and Swinson, 2005) and increased plasma concentrations of catabolic hormones, such as ACTH, corticosterone, noradrenaline, and glucagon (Galvão et al., 2009; Martins et al., 2010; Suchecki et al., 1998) have been reported.

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Despite this evidence, the central mechanisms involved in the metabolic changes induced by sleep deprivation are not yet fully understood. The hypothalamus is the main regulation *locus* of feeding behavior and energy expenditure (Abizaid and Horvath, 2008) and the neuropeptides and neurotransmitters acting in some hypothalamic nuclei are responsible for inhibiting or stimulating feeding behavior (Beck, 2000; Klok et al., 2007; Lustig, 2001). Regulation of these neuropeptides is made in part by peripheral hormones, such as insulin and leptin, which modulate satiety signals that determine the start and end of food intake and energy balance (Klok et al., 2007; Lustig, 2001; Schwartz et al., 1996).

Thus, the aim of this study was to evaluate the time course of plasma concentrations of insulin and leptin and their hypothalamic receptors, correlating these values with food intake, body weight change and blood glucose, throughout four days of paradoxical sleep deprivation.

Methods

Animals

Male Wistar rats, 90 day-old (n = 112) were obtained from Centro de Desenvolvimento de Modelos Experimentais para Medicina e Biologia (CEDEME) of Universidade Federal de São Paulo (UNIFESP). They were housed in the animal facility of the Department of Psychobiology of UNIFESP in plastic cages (grouped in four/five animals) until the beginning of the experiments. The animals were kept under controlled temperature (21 ± 2 °C) and a 12 h/12 h light/dark cycle (lights on at 7:00 a.m.). All procedures were approved by the Ethics Committee in Research of UNIFESP (CEP: 1372/09).

Groups

Initially the animals were distributed into two groups: control (CTL — n = 56) and paradoxical sleep deprivation (PSD — n = 56). Each of these groups was then subdivided equally into four new groups, according to the day of euthanasia: CTLD1, CTLD2, CTLD3, CTLD4, PSDD1, PSDD2, PSDD3 and PSDD4. Each group was composed of 14 animals.

Paradoxical sleep deprivation

Animals were submitted to PSD by the single platform technique, by housing them in water containers (22 cm long × 22 cm wide × 35 cm high) onto a platform 6.5 to 7.0 cm in diameter. The platform remained immersed in water up to 1.0 cm from its upper surface. Control animals were housed in similar containers lined with corn cob. For each container, water and food were provided ad libitum in removable compartments. The animals were habituated to the sleep deprivation facility for two weeks and to the experimental environment for 60 min/day during the three days before the beginning of the experiments. The protocol of PSD lasted 96 h.

Assessment of body weight and food consumption

All animals underwent this evaluation. Body weights were recorded daily in the morning (7:30 a.m.) after we made sure that the animals were not wet, from the adaptation period until the last day of PSD. Since we sought to determine the daily changes induced by PSD, the percent weight change was calculated by the following equation: [(current weight − previous weight) / previous weight] × 100. For determination of food intake, chow pellets were weighed daily at two different times: at 7:00 a.m., to assess intake during the dark phase, and at 7:00 p.m., to assess food intake during the light phase.

Measurements of glucose, insulin and leptin

Half of the animals in each group (n = 7/group/day) was used for biochemical receptor expression assessment. The animals were euthanized by decapitation (9:00 a.m.), 2 h after removal of the food containers for the purpose of reducing the variability of results. One drop of blood was used to determine glucose levels by OneTouch™ device Johnson & Johnson (system measuring range: 20 to 600 mg/dL). Trunk blood was also collected in dry tubes containing 0.1 mL of a 6% EDTA solution and centrifuged at 2400 rpm for 20 min at 4 °C. Plasma was stored in two separate aliquots and frozen (−20 °C) for later determination of hormone concentrations. Insulin and leptin levels were determined by radioimmunoassay (Millipore EMD Chemicals, Germany). The sensitivity of the insulin assay is 0.081 ng/mL; intra- and inter-assay variabilities of 1.4 to 4.6% and 8.5 to 9.4%, respectively. The sensitivity of the leptin assay is 0.639 ng/mL, intra-assay variability of 2.8 to 3.6% and inter-assay variability of 6.5 to 8.7%.

Expression of insulin and leptin receptors in the hypothalamus

After decapitation, the brains were removed from the skull and the hypothalamus was dissected, frozen and stored at −80 °C for posterior Western blotting assays. The total cell lysates of the hypothalamus were homogenized in lysis buffer (T-PER Tissue Protein Extraction Reagent, Thermo Scientific, USA and protease and phosphatase inhibitors cocktails, Pierce, USA) and total protein concentration was determined by Lowry's method (Lowry et al., 1951). The samples were subjected to electrophoresis on SDS-polyacrylamide gel. The separating gel of 10% polyacrylamide was prepared in 0.4 M Tris–HCl buffer (pH 8.8), containing 0.1% SDS, 0.01% N, N, N′, N′-tetra metiletilenodiamina (TEMED) and 0.05% ammonium persulfate. After polymerization of the gel, the stacking gel was prepared containing 3% polyacrylamide in 0.1 M Tris–HCl buffer (pH 6.8), 0.1% SDS, 0.01% TEMED and 0.05% ammonium persulfate. An aliquot of 50 μg of total protein hypothalamic extract was combined with denaturant and reductant buffer containing 0.125 M Tris–HCl (pH 6.8), 4% SDS, 20% glycerol, 0.002% bromophenol blue and 4% mercaptoethanol. Samples were heated at 95 °C for 5 min and applied on the gel. 0.025 M Tris–HCl, containing 0.18 M glycine, pH 8.3, and 1% SDS, was used as a running buffer. The runs were performed at 100 V for 2.5 h at room temperature. Then the samples were transferred by electroblotting to nitrocellulose membrane (Amersham GE, Little Chalfont, UK). The membranes were blocked with 2% non-fat milk for 2 h and then incubated with primary antibodies overnight at the indicated dilutions: rabbit anti-insulin receptor Santa Cruz Biotechnology (Santa Cruz, CA, USA) 1:1000 and rabbit anti-LepRb (Abcam, Cambridge, UK) 1:2000, respectively. After three washes of 5 min, the membranes were incubated for 45 min with Alexa-680-conjugated anti-rabbit IgG (1:10,000, Invitrogen, Carlsbad, CA, USA). After five washes of 5 min, digital images of the membranes were acquired and quantified, by pixel density, using the Odyssey Infrared Image System (LI-COR, Baltimore, MD, USA).

Statistical analysis

Statistical analysis of daily food intake was performed by the General Linear Model (GLM) for repeated measures, with Group (CTL, PSD), Day (D1, D2, D3, D4 — repeated measures) and Phase (light, dark — repeated measures) as main factors. Analysis of body weight was carried out by GLM for repeated measures with Group (CTL, PSD) and Day (D1, D2, D3, D4 — repeated measures) as main factors. GLM univariate test was applied to blood glucose, plasma insulin and leptin levels, and expression of insulin and leptin receptors, with main factors: Group (CTL, PSD) and Day (D1, D2, D3, D4). When required, Bonferroni post hoc test was used, and the significance level was established at $p < 0.05$. Finally, Pearson's correlation test was applied to evaluate the