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Paradoxical sleep deprivation activates hypothalamic nuclei that regulate food intake and stress response

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Summary A large body of evidence has shown that prolonged paradoxical sleep deprivation (PSD) results in hypothalamic—pituitary—adrenal (HPA) axis activation, and in loss of body weight despite an apparent increase of food intake, reflecting increased energy expenditure. The flowerpot technique for PSD is an efficient paradigm for investigating the relationships among metabolic regulation and stress response. The purpose of the present study was to examine the mechanisms involved in the effects of 96 h of PSD on metabolism regulation, feeding behaviour and stress response by studying corticotrophin-releasing hormone (CRH) and orexin (ORX) immunoreactivity in specific hypothalamic nuclei. Once-daily assessments of body weight, twice-daily measurements of (spillage-corrected) food intake, and once-daily determinations of plasma adrenocorticotropic hormone (ACTH) and corticosterone were made throughout PSD or at corresponding times in control rats (CTL). Immunoreactivity for CRH in the paraventricular nucleus of the hypothalamus and for ORX in the hypothalamic lateral area was evaluated at the end of the experimental period. PSD resulted in increased diurnal, but not nocturnal, food intake, producing no significant changes in global food intake. PSD augmented the immunoreactivity for CRH and plasma ACTH and corticosterone levels, characterizing activation of the HPA axis. PSD also markedly increased the ORX immunoreactivity. The average plasma level of corticosterone correlated negatively with body weight gain throughout PSD. These results indicate that augmented ORX and CRH immunoreactivity in specific hypothalamic nuclei may underlie some of the metabolic changes consistently described in PSD.

1. Introduction

Studies on the effects of sleep deprivation on food intake in rats have consistently shown a peculiar syndrome characterized by hyperphagia and loss of body weight—effects not dependent on the sleep deprivation method used (Bhanot et al., 1989; Elomaa, 1985; Kushida et al., 1989). Body weight loss takes place even in sleep-deprived rats fed high-calorie diets (Everson and Wehr, 1993; Koban et al., 2008; Suchecki et al., 2003). Numerous studies have addressed the mechanisms involved in such a major energy imbalance. On the one hand, total sleep or paradoxical sleep deprivation (PSD)

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reduces anabolic hormones such as leptin (Everson and Crowley, 2004; Koban and Swinson, 2005), testosterone (Andersen et al., 2005), and insulin (Hipolide et al., 2006). On the other hand, sleep deprivation increases sympathetic (Andersen et al., 2005; Meerlo et al., 2008) and hypothalamic—pituitary—adrenal (HPA) axis activity (Fadda and Fratta, 1997; Koban et al., 2006; Suchecki et al., 1998; Suchecki et al., 2002), increasing catabolic hormones such as corticosterone and adrenaline. Hyperphagia, in turn, can be explained by a progressive increase of neuropeptide Y (NPY) expression in the hypothalamic arcuate nucleus from 5 to 20 days of PS deprivation in addition to reduction of proopiomelanocortin, a well-known anorexic peptide (Koban et al., 2006). Recently, the same group used a high energy-containing liquid diet and reported a direct correlation between expression of NPY in the arcuate nucleus and food intake (Koban et al., 2008).

Orexin (ORX) is a recently identified orexigenic neuropeptide implicated in the regulation of food intake and energy metabolism (Bernardis and Bellinger, 1996; Date et al., 1999). This peptide is synthesized by neurons located in the lateral hypothalamic area (LHA), which project to waking-related areas, such as the locus coeruleus, dorsal raphe, and tuberomammilary nuclei indicating that ORX is involved in the regulation of waking (Hagan et al., 1999; Williams et al., 2004). Corticotropin releasing factor (CRH), in turn, is synthesized in neurons of the paraventricular nucleus of the hypothalamus (PVN) and orchestrates the neuroendocrine stress response, participates in metabolic pathways known to increase energy expenditure, decreases food intake (Woods et al., 1998) and, like ORX, also promotes waking (Chang and Opp, 2001; Sanford et al., 2008). CRH mRNA expression increases progressively from five to 20 days of PSD (Koban et al., 2006), whereas ACTH and corticosterone (CORT) levels are reported to be increased earlier—already by one day of sleep deprivation (Andersen et al., 2005; Suchecki et al., 1998).

Taken together, these data justify the investigation of ORX and CRH activities within the initial four days of PSD in rats. We sought to investigate ORX and CRH immunoreactivities (ORX-IR and CRH-IR) in the LHA and PVN, respectively, at the end of this period. In addition, we also evaluated food intake corrected for spillage, and daily ACTH and CORT plasma concentrations within the course of PSD.

2. Methods

2.1. Animals

Male Wistar rats (3—4 months of age) were bred and raised in the animal facility of the Department of Psychobiology of Universidade Federal de São Paulo (UNIFESP), housed in groups of four in plastic cages from weaning until the onset of the deprivation procedure. The animals were habituated to the sleep deprivation room for two weeks and handled every day for a week before the beginning of the experiment. Throughout the study, the animals were maintained under controlled temperature (21 ± 2 °C) and a 12 h/12 h light/dark cycle (lights on at 7:00 h). The Ethics Committee in Research of UNIFESP approved all procedures (CEP # 0638/05).

2.2. Groups

Rats were randomly distributed in two main groups: PSD and control (CTL). PSD was induced by the single platform technique. The animals were individually maintained for 96 h in water chambers (22.0 cm long, 22.0 cm wide, and 35.cm high), for proper assessment of food intake and spillage. Within the chambers, PSD rats were placed onto a 7.0 cm diameter platform immersed in water up to 1.0 cm below the platform upper surface. Whenever rats on the platforms lapse into paradoxical sleep, they lose muscle tone, make facial contact with or fall into the surrounding water, awakens, and the cycle begins again (Cohen and Dement, 1965). CTL rats were kept in a similar chamber filled with sawdust bedding instead of water. This was done because previous studies from our group have shown that rats placed onto large immersed platforms (14.0 cm) to purportedly control for PSD-related stress are actually also deprived of paradoxical sleep, albeit to a lesser extent (Machado et al., 2004; Suchecki et al., 2000).

All rats were habituated to their experimental environment for 1 h/day for the two consecutive days preceding the onset of the study. This routine is adopted so that each PSD animal is trained to balance on the platform to avoid excessive falling in the water, unless there is a decrease in muscle tone due to sleep onset. CTL and PSD chambers were cleaned twice a day, throughout the experimental period, at the same time of food intake assessment (see below).

2.3. Experimental procedure

Animals were weighed immediately before being placed in the chambers and at 8:00 h thereafter. At 8:00 h and at 17:00 h, pellet leftovers were removed and weighed, and the food container was refilled a predetermined amount of chow (150 g). At the same time-points, food crumbs spilled in the water were separated from faeces, dried overnight (at 50 °C) and weighed as reported in detail by Martins et al. (2006). Actual nocturnal and diurnal food intake was estimated at these respective time-points by subtracting the amounts of spilled food and pellet leftovers from 150 g.

2.4. Blood sampling

During the week preceding the onset of experiments, the animals were daily handled to get habituated to blood sampling. Blood samples of approximately 300 μl were collected from a small cut on the tail tip at 8:00 h before PSD (basal levels) and throughout the experiment. The tail skin was cleaned with an antiseptic solution before and after each sampling, and a neomycin-containing ointment (Nebacetin, Bayer ®) was applied topically to prevent water contact and local infection.

Blood samples were collected in pre-cooled Eppendorf tubes containing 0.05 ml of EDTA (60 mg/ml), centrifuged immediately at 2300 rpm at 4 °C for 20 min, and the resulting plasma samples were frozen in clean Eppendorf tubes at −20 °C for later determination of plasma ACTH and CORT. ACTH was assayed by a sequential chemiluminescence immunometric method (DPC Immulite, Los Angeles, CA, USA). The sensitivity of the method is 9 pg/ml, and intra- and interassay variations are 9.6% and 9.4%, respectively. CORT levels were