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Differential response of TRHergic neurons of the hypothalamic paraventricular nucleus (PVN) in female animals submitted to food-restriction or dehydration-induced anorexia and cold exposure

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Abstract

TRH neurons of the hypothalamic paraventricular nucleus (PVN), regulate pituitary–thyroid axis (HPT). Fasting activates expression of orexigenic peptides from the arcuate nucleus, increases corticosterone while reduces leptin, and pro-TRH mRNA levels despite low serum thyroid hormone concentration (tertiary hypothyroidism). TRH synthesis is positively regulated by anorexigenic peptides whose expression is reduced in fasting. The model of dehydration-induced anorexia (DIA) leads to decreased voluntary food intake but peptide expression in the arcuate is similar to forced-food restriction (FFR), where animals remain hungered. We compared the response of HPT axis of female Wistar rats submitted to DIA (2.5% saline solution, food *ad libitum*, 7 days) with FFR (provided with the amount of food ingested by DIA) and naïve (N) group fed *ad libitum*, as well as their response to acute cold exposure. Pro-TRH and pro-CRH mRNA levels in the PVN were measured by RT-PCR, TRH content, serum concentration of TSH and thyroid hormones by radioimmunoassay. DIA rats reduced 80% their food consumption compared to N, decreased PVN pro-CRH expression, serum estradiol and leptin levels, increased corticosterone similar to FFR. HPT axis of DIA animals failed to adapt: FFR presented tertiary hypothyroidism and DIA, primary. Response to cold stimulation leading to increased pro-TRH mRNA levels and TRH release was preserved under reduced energy availability in FFR rats but not in DIA, although the dynamics of hormonal release differed: TSH release augmented only in naïve; thyroxine in all but highest in DIA, and triiodothyronine in FFR and DIA suggesting a differential regulation of deiodinases.

Keywords: TRH; Hypothalamic–pituitary–thyroid axis; Leptin; Thyroid hormones

Introduction

Neurons of the paraventricular nucleus (PVN) of the hypothalamus synthesize thyrotropin-releasing hormone (TRH) and corticotropin-releasing hormone (CRH), peptides that play crucial roles in energy homeostasis (Lechan and Fekete, 2006) or during chronic stress (Chrousos, 2000; Michel et al., 2005). Conditions of homeostatic challenge such as low nutrient availability and fasting depress the activity of the hypothalamic–pituitary–thyroid (HPT) axis in male and female rats: tertiary hypothyroidism develops, characterized by decreased pro-TRH mRNA expression in the PVN and low thyrotropin (TSH), thyroxine (T_4) and triiodotironine (T_3) levels (Blake et al., 1991; Shi et al., 1993; van Haasteren et al., 1995, 1996; de Gortari et al., 2000). The normal feedback regulation of thyroid hormones (TH) is impaired since, despite the low TH circulating levels, the biosynthesis of TRH in the hypophysiotropic neurons of the PVN remains inhibited (Blake et al., 1991). Negative energy balance conditions induce the expression of neuropeptides known to participate in the control of food intake: i.e. orexigenic peptide mRNA levels increase (in the arcuate nucleus [ARH]: neuropeptide Y [NPY], agouti related peptide [AgRP]; in lateral hypothalamus [LH]: orexins, melanin-concentrating hormone [MCH]) while anorexigenic's decrease (α-melanocyte-

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stimulating hormone [α-MSH], cocaine and amphetamine regulated-transcript [CART] in ARH) (Leibowitz and Wortley, 2004). TRHergic neurons have receptors for orexigenic and anorexigenic peptide signals arising from the LH, such as (CRH), and from the ARH (NPY, CART, AgRP and α-MSH (Mountjoy et al., 1994; Chalmers et al., 1995; Broberger, 1999; Fekete et al., 2002, 2004). Pro-TRH expression is restored by injecting either leptin, α-MSH or CART to fasted animals (Lechan and Fekete, 2006). Circulating levels of leptin, an anorexigenic protein released from adipose tissue, with re-activating functions on energy expenditure (Ahima and Osei, 2004), are also reduced during fasting; leptin induces pro-TRH expression directly (Nillni et al., 2000; Harris et al., 2001) or indirectly through its inhibitory effects on NPY and stimulatory on pro-opiomelanocortin [POMC] neurons of the ARH (Schwartz et al., 1996).

The synchrony between the fuel reservoir status and food intake that compensates the excess or deficit of energy in animals, can be disrupted by different psychological or physiological stimuli and may favor the appearance of an aberrant feeding behavior known as anorexia. Anorexia is defined as a loss of appetite despite low body weight and limited lipid deposits. It may result during illness, induced by different psychological factors, or as a response to homeostatic challenges like cellular dehydration; it is always accompanied by stress axis activation (Siegfried et al., 2003; Connan et al., 2003). In contrast to food restriction, anorexic patients present normal (or subnormal) serum levels of thyroxine (T_4) and thyrotropin (TSH), as well as thyroid atrophy (Stoving et al., 2001); TRH-induced TSH release is blunted or retarded (Tamai et al., 1986; Lesem et al., 1994; Muñoz and Argente, 2002; Troisi et al., 2005; Brambilla et al., 2006). The alterations of the HPT axis in anorexia nervosa are believed to be of a central origin due to reduced hypothalamic TRH secretion and are known as "low T_3 syndrome".

Dehydration is a model of anorexia, induced in animals by the consumption of a hyperosmolar solution of NaCl (2.5%) (Watts et al., 1999). It provokes voluntarily reduced food intake with the associated changes of negative energy balance that are similar to those seen in pair-fed food-restricted animals: weight loss, increased corticosterone, lowered leptin and insulin serum levels, reduced expression of POMC and increased NPY expression in the arcuate nucleus (Brady et al., 1990; Watts et al., 1999). All these signals should stimulate eating, however feeding is increasingly suppressed suggesting the actions of inhibitory control networks (Watts, 2001, Watts et al., 2007). The anorexic behavior appears as an adaptation to the altered hydric balance, diminishing the need for water in digestion and contributing to a more adequate distribution in the organism; decreased feeding impedes the increase in the cellular osmolality of the organism (Watts et al., 2007). The diminished food intake in dehydration-induced anorexic (DIA) animals is accompanied by a similar pattern of neuropeptide expression in the arcuate nucleus as their pair-fed controls; however, activation of CRH neurons in the lateral hypothalamus (LH) that project to the PVN is observed only in DIA (Watts et al., 1999).

TRHergic cells contain CRH receptors (Hisano et al., 1993) and their activity can be modified by different kinds of stress (Cizza et al., 1995; Gutiérrez-Mariscal et al., submitted for publication), we therefore analyzed if pro-TRH expression of PVN neurons and parameters of the HPT axis function, could be differentially modified in dehydration-induced anorexia compared to paired-forced food restricted (hungered) animals, in particular, due to the anorexic effects of TRH. Central or peripheral TRH administration reduces food and water intake in rats and hamsters (Vijayan and McCann, 1977; Karydis and Tolis, 1998; Choi et al., 2002; Steward et al., 2003) and when injected directly into the medial hypothalamus the highest inhibitory effect is observed on feeding and drinking while into the lateral hypothalamus, only diminishes water intake (Suzuki et al., 1982).

Neuronal stimuli such as cold exposure increases in a fast and transient manner pro-TRH expression in the PVN (Uribe et al., 1993; Sánchez et al., 2001); therefore, we also studied whether forced-food restriction (FFR) or DIA affect this response. Given that the stress history of the animal can affect neuroendocrine responses, we evaluated the degree of anxiety-like behavior imposed by DIA or FFR.

Female Wistar rats were submitted up to 7 days of DIA and results compared to a pair-fed group (FFR) and naïve. Animals were exposed on day 7 for 1 h to 4 °C. Anxiety-like behavior was evaluated on day 3 in the defensive-burying test (DBT) (Treit et al., 1981). The activity of the HPT axis was evaluated measuring: the relative mRNA levels of TRH-R1 in the PVN and in adenohypophysis, the degrading enzyme of TRH: pyroglutamyl-aminopeptidase II (PPII) only in the later, TRH content in dorsal and mediobasal hypothalamus and, serum levels of TSH, T_3, and T_4 in serum. The stress response was analyzed measuring serum corticosterone concentration and the relative mRNA levels of pro-CRH in the PVN. Serum leptin, prolactin and estradiol levels were also quantified.

Materials and methods

Animals and housing

All diets and treatments were approved by the Ethics Committee of the Instituto Nacional de Psiquiatría (INPRFM) following the guidelines of the Neuroscience Society (USA). Wistar female rats [225–230 g body weight (b.w.)] from the Institute's (INPRFM) animal house were maintained with a 12 h light–dark cycle (7:00–19:00 h), 23 °C of temperature and water and food consumption *ad libitum.* Experiments of cold exposure were performed at the Instituto de Biotecnología, UNAM with Wistar female rats raised and kept in its animal house.

Experiments

Seven days before the experiment, animals were placed in individual cages and randomly selected to form 3 groups from the same lot. On the first day, the naïve group (N) received water and food *ad libitum*; the dehydrated group (DIA) received a 2.5% NaCl (SIGMA, St. Louis, MO, USA) solution as drinking liquid (Watts et al., 1999). Solid food (Lab rodent diet #5001, PMI feeds; Brentwood, MO, USA) and liquid ingestion were measured daily at 18:00 h in all groups. Food was administered at the end of the light cycle (18:30 PM); forced-food restricted animals (FFR) received the amount of solid food ingested by the DIA group on the previous day and tap water *ad libitum*. Six independent experiments were performed including 4–8 rats/group/experiment. By the 3rd trial, FFR received the calculated food consumed by DIA on the first day of the first 2 experiments. Total energy provided by the solid food ingested was calculated taking into account the amount of metabolizable energy in PMI food is 3.04 Kcal/g. Body weight was registered daily.