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# High ambient temperature reverses hypothalamic MC4 receptor overexpression in an animal model of anorexia nervosa

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**Summary** The potential involvement of the melanocortin system in the beneficial effects of heat application in rats submitted to activity-based anorexia (ABA), an analogous model of anorexia nervosa (AN), was studied. Once ABA rats had lost 20% of body weight, half of the animals were exposed to a high ambient temperature (HAT) of 32 °C, whereas the rest were maintained at 21 °C. Control sedentary rats yoked to ABA animals received the same treatment. ABA rats (21 °C) showed increased Melanocortin 4 (MC4) receptor and Agouti gene Related Peptide (AgRP) expression, and decreased pro-opiomelanocortin (POMC) mRNA levels (Real Time PCR), with respect to controls. Heat application increased weight gain and food intake, and reduced running rate in ABA rats, when compared with ABA rats at 21 °C. However, no changes in body weight and food intake were observed in sedentary rats exposed to heat. Moreover, heat application reduced MC4 receptor, AgRP and POMC expression in ABA rats, but no changes were observed in control rats. These results indicate that hypothalamic MC4 receptor overexpression could occur on the basis of the characteristic hyperactivity, weight loss, and self-starvation of ABA rats, and suggest the involvement of hypothalamic melanocortin neural circuits in behavioural changes shown by AN patients. Changes in AgRP and POMC expression could represent an adaptative response to equilibrate energy balance. Moreover, the fact that HAT reversed hypothalamic MC4 receptor overexpression in ABA rats indicates the involvement of brain melanocortin system in the reported beneficial effects of heat application in AN. A combination of MC4 receptor antagonists and heat application could improve the clinical management of AN.


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## 1. Introduction

AN is an eating disorder characterized by hypophagia, body weight loss, hypothermia and hyperactivity (Birmingham and Beumont, 2004). Thus, AN patients usually display elevated physical activity levels despite severe weight loss and emaciation, in great contrast to the loss of energy and fatigue characteristic of other starvation states associated with weight loss (Hebebrand et al., 2003). AN, which has proved difficult to treat, is associated with frequent relapses and is thought to have the highest mortality rate of any psychiatric disorder (Birmingham et al., 2005).

ABA is an animal model of anorexia nervosa (Epling and Pierce, 1991, 1996; Epling et al., 1983) and is a result of previous evidence from the second half of twentieth century which showed the progressive escalation of running activity and subsequent self-starvation displayed by food-restricted rats given the opportunity to run in a wheel (Hall and Hanford, 1954; Routtenberg and Kuznesof, 1967). ABA, also known as activity—stress or activity—anorexia, is based upon the combination of scheduled feeding and free access to a running wheel. In this model, rats given restricted access to food but unrestricted access to activity wheels, run excessively while reducing food intake, lose a sizeable percentage of body weight, become hypothermic, and can fail to recover unless removed from these conditions (Routtenberg and Kuznesof, 1967). In fact, it has been proposed that excessive physical activity could be central to the pathogenesis of anorexia nervosa by contributing to extreme weight loss (Epling and Pierce, 1985; Davis et al., 1997).

At variance with other hypotheses that interpret the hyperactivity in ABA rats as an expression of foraging behavior (Spatz and Jones, 1971), or as rewarding in itself (Sherwin, 1998), increased running produced by food restriction could be related to thermoregulation (Campbell and Lynch, 1967, 1968; Paré, 1977; Gutiérrez et al., 2002). Preliminary evidence for this last hypothesis came from the strong modulating effect of ambient temperature (AT) observed in the context of research on ulcer formation (Lambert, 1993; Morrow et al., 1997). However, until recently (reviewed in Gutiérrez et al., 2002), ABA research remained unaware of this modulating effect of AT. It has been shown that voluntary access to a warm plate in rats reduces hyperactivity in activity-based anorexia (Hillebrand et al., 2005d), and that conducting the experiment at thermo neutral temperature (29 °C) prevents animals from excessive running, allowing them to survive the experimental procedure in contrast with animals exposed to the ABA procedure at standard 21 °C AT (Gutiérrez et al., 2006, exp 1). Furthermore, increasing AT when the animals have lost 20% of body weight and running has become excessive reverses excessive running and weight loss allowing the recovery of one hundred per cent of the rats, a rate never before reported in the literature (Gutiérrez et al., in press). In summary, heat supply both inhibits the development of ABA outcome, but more importantly heat supply reverses the consequences of previous exposure to ABA procedure. Moreover, a relationship between AN and AT has been described (Vazquez et al., 2006), and heat is becoming a component in the treatment of anorexia nervosa as preliminary evidence is accumulated by case studies (Gutiérrez and Vazquez, 2001) and by randomized clinical trials (Bergh et al., 2002; Birmingham et al., 2004). Given both that ABA is at present the animal model which best mirrors the human disorder and the striking coincidence in the beneficial role of heat in both the animal and human disorder, it is necessary to examine further the effect of HAT on this animal model as it could allow a better understanding of the mechanisms underlying this phenomenon and of the possible clinical implications of its use in the treatment of human AN.

Molecular changes in the melanocortin system (MS) are interesting candidates in the search for those mechanisms. MS plays a pivotal role in the central nervous system control of food intake and body weight (Schwartz et al., 2000; Morton et al., 2006) and is one of the main neuropeptidergic systems involved in the physiological regulation of energy balance during the anorexia model (Hillebrand et al., 2005b; Adan et al., 2006). Thus, neurons that express AgRP or POMC in the hypothalamic arcuate nucleus (ARC) project to the paraventricular nucleus (PVN) and the lateral hypothalamic area (LHA), as well as to a variety of other nuclei (Cone, 1999; Baile et al., 2000; Williams et al., 2001; Cone, 2005). MC4 receptors are the most abundant and most widely distributed melanocortin receptor subtypes in the brain. A high density of these receptors is found in several hypothalamic regions involved in the regulation of energy balance (Mountjoy et al., 1994; Vergoni and Bertolini, 2000). α-melanocyte stimulating hormone (α-MSH), a POMC-derived peptide, decreases food intake and body weight through its action on MC4 receptors (Adan et al., 1994; Marsh et al., 1999), whereas AgRP is a high-affinity antagonist of these receptors (Williams et al., 2001; Neary et al., 2004; Butler, 2006).

It has been shown that during the development of ABA, the expression of hypothalamic appetite-regulating neuropeptides changes in a predictable way in response to negative energy balance. Thus, expression of AgRP is upregulated, whereas that of POMC is downregulated, in these food-restricted running rats (Kas et al., 2003; De Rijke et al., 2005).

This being the case, the aim of the present work was to study the potential involvement of hypothalamic melanocortin system in the beneficial effects of exposure to HAT in ABA rats, a model of AN.

## 2. Methods

### 2.1. Experimental animals

A total of 24 male Sprague—Dawley rats (40-day old, weight range 164—186 g) acquired from the University Animal Resources Centre of the University of Santiago de Compostela were kept for 3 days in the colony room on a 12 h light—dark cycle, with lights on from 0800 h to 2000 h and AT was set at 21 °C. The ethics committee on the use and care of animals of Santiago de Compostela University approved all the procedures described. All experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 and D.L. of 27 January 1992 no. 116 (86/609/EEC). All efforts were made to minimize animal suffering and to reduce the number of animals used.

### 2.2. Running wheels

The laboratory contained 8 Wahman-type activity wheels (1.12 m circumference, 10 cm-wide running surface of 10 mm wire mesh bounded by clear Plexiglas walls). These