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Voluntary access to a warm plate reduces hyperactivity in activity-based anorexia

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Abstract

Activity-based anorexia (ABA) is considered an animal model of anorexia nervosa. In ABA, scheduled feeding in combination with voluntary wheel running leads to hyperactivity, reduced food intake, severe body weight loss and hypothermia. In this study it was investigated whether hyperactivity in ABA could be reduced by introducing a warm plate (which was voluntary accessible and did not influence ambient temperature) into a part of the cage.

In ad libitum fed rats, the presence of the warm plate did not influence body temperature, running wheel activity (RWA), body weight or food intake. During ABA, however, rats preferred the warm plate and hypothermia was prevented, while hyperactivity and body weight loss were significantly reduced when compared to ABA rats without a plate. Correlation analysis revealed a significant association between basal body temperature and RWA during the light phase in ABA rats. However, there was no evidence that initiation of light phase RWA was a result of hypothermia. These data suggest that ABA rats prefer to prevent hypothermia passively by choosing a warm plate rather than actively regulating body temperature by hyperactivity.

Keywords: Temperature; Anorexia; Hyperactivity; Animal model; Running wheel activity

1. Introduction

Anorexia nervosa is a psychiatric disorder often characterized by extreme hypophagia, body weight loss and hypothermia. Patients frequently show excessive exercising and can not avoid being active [1,2]. The activity-based anorexia (ABA) model is used to study anorectic behavior in rodents and serves as an animal model of anorexia nervosa. In ABA, voluntary access to a running wheel in combination with scheduled feeding leads to a paradoxical increase in running wheel activity (RWA) and a decrease in food intake, resulting in substantial body weight loss (>20%). Not only total RWA increases, but the distribution of activity throughout the day changes as well. ABA rats also show hypothermia, loss of estrous cycle, stomach ulceration and will eventually die of emaciation [3,4].

The biological trigger of hyperactivity in ABA rats remains unclear. Hyperactivity of ABA rats might be explained by anticipation to the feeding period [5]. Indeed, substantial activity takes place prior to food access (independent of light or dark phase), which is also known as food-anticipatory activity (FAA). Wheel running is also considered as foraging behavior and has rewarding properties [6,7] and recently it was hypothesized that reduced plasma leptin levels associated with body weight loss might trigger hyperactivity [8]. Furthermore, hyperactivity might be explained as a thermoregulatory behavior to prevent starvation-induced hypothermia [9], since activity levels of ABA rats are inversely related to core body temperature [10].

Thus far, only a few reports focused on whether body temperature affects ABA, by influencing ambient temper-

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ature. From these reports it appeared that ambient temperature has a strong influence on survival rate in ABA. Low ambient temperatures increase RWA and enhance the development of ABA, whereas high ambient temperatures, reduce (but do not prevent) RWA and thereby inhibit the development of ABA [9]. Although wheel running of ABA rats can be altered by manipulating ambient temperature, the effects obtained are probably not specific for ABA, since ad libitum fed running rats also decrease RWA when housed in a warmer environment and increase RWA in a colder environment [11–14].

The hypothermia observed in ABA rats might be perceived as unpleasant and this may trigger hyperactivity. Therefore, an experimental setting was designed in which rats could choose for a warm plate in order to passively raise body temperature, instead of active behavioral temperature regulation via RWA. In the present experiment rats had voluntary, instead of forced, access to a warm environment, which was provided by a warm plate in the cage that did not influence ambient temperature. It was hypothesized that ABA rats, but not ad libitum fed running rats, would prefer a warm environment and that the presence of a warm plate in the cage would subsequently reduce RWA in ABA rats.

2. Material and methods

2.1. Rats

Female outbred Wistar WU rats (Harlan, Horst, The Netherlands) weighing 180 g upon arrival were individually housed in a temperature and humidity controlled room (21±2 °C) under a 12:12 h light/dark cycle (ZT12=lights off). Rats were allowed to adapt to these housing conditions for 1 week under ad libitum food and water conditions. The ethical committee on use and care of animals of Utrecht University approved all described procedures.

2.2. Surgical procedures

One week after arrival all rats ($n=13$) received transmitters (TA10TA-F40 Data Sciences International, St. Paul, MN, USA) in the abdominal cavity under fentanyl/fluanisone (Hypnorm®, Janssen Pharmaceutica, Beerse, Belgium, 0.1 ml/100 g im) and midazolam (Dormicum®, Hoffman-LaRoche, Mijdrecht, The Netherlands, 0.05 ml/100 g, i.p.) anaesthesia. After surgery, rats were treated with buprenorphin (Temgesic®, Schering-Plough, Maarssen, The Netherlands, 0.05 ml/100 g, s.c.) and saline (1 ml, s.c.) and were allowed to recover for 2 weeks.

2.3. Experimental set-up

After 2 weeks of recovery from surgery, rats were housed in cages with running wheels for a free training period of 15 days (day-15–day 0) with ad libitum food and water access. RWA was continuously registered using a Cage Registration Program (Dep. Biomedical Engineering, UMC Utrecht, The Netherlands). On day-7 rats were divided into two groups (warm plate $n=7$/no plate $n=6$) matched for RWA (average 6077.7±977.0 revolutions at day-7) and aluminum plates (covering ±20% of cage area) were introduced into seven cages. Plates were separated from the sawdust by a Perspex layer and were heated to 37 °C by a continuous flow of hot water using a covered water bath. It was confirmed that ambient temperature in the cages was not affected using a (non-contact) thermometer. On day-2, transmitters were activated for baseline measurements of body temperature. Food was removed from all cages at the onset of the dark period of day 0 (ZT12). The next days (days 1–6) rats had 1 h access to food at ZT12, while water was continuously available. Body weight (just before ZT12) and food intake (ZT13) were measured daily. On day 6 (ZT11) rats were decapitated. Trunk blood was collected in lithium-heparin (Sarstedt, Nümbrecht, Germany) containing tubes after adding 83 μmol EDTA and 1 mg aprotonin. Plasma was frozen at −20 °C. Interscapular brown adipose tissue (BAT) and white adipose tissue (WAT) surrounding the ovaries and oviduct were weighed and stored at −80 °C.

2.4. Radioimmunoassay

Plasma levels of leptin were measured using a commercially available rat leptin RIA kit (sensitivity: 0.5 ng/ml), according to the manufacturer's protocol (Linco Research, St. Charles, Missouri, USA).

2.5. Quantitative PCR

Total RNA was prepared from BAT and WAT of ABA rats using Trizol Reagent (Invitrogen Gibco, Paisley UK). RNA was treated with DNAse I and was reverse transcribed into cDNA using oligodT_{12-18} primers and SuperScript II reverse transcriptase (Gibco). The Lightcycler real time PCR detection system (Roche Diagnostics, Mannheim, Germany) was used for amplification and quantification of uncoupling protein (UCP1) and leptin mRNA expression levels. Cyclophilin was used as a reference gene. An amount of cDNA corresponding to 40 ng of total RNA was amplified using the Lightcycler-Faststart DNA Master SYBR Green I kit (Roche Diagnostics) and the appropriate primers. Optimal $MgCl_2$ concentrations, annealing temperature and cDNA dilution (1:10) were determined (Table 1), resulting in PCR efficiencies >1.8. All cDNA samples were measured in duplicate. Expression levels of UCP1 and leptin were calculated as a normalized ratio relative to a calibrator sample (Rn). Thus, UCP1 and leptin expression were analyzed relative to cyclophilin and each sample was