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Difference in susceptibility to activity-based anorexia in two inbred strains of mice

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Abstract Food restricted rodents develop activity-based anorexia in the presence of a running wheel, characterised by increased physical activity, weight loss and decreased leptin levels. Here, we determined trait differences in the development of activity-based anorexia between C57BL/6J and DBA/2J inbred mouse lines previously reported as having low and high anxiety, respectively. C57BL/6J mice housed with running wheels and exposed to scheduled feeding reduced their wheel activity, in contrast to DBA/2J mice which exhibited increased behavioural activity under these conditions. Food restriction induced hypoleptinemia in both strains, but the decline in plasma leptin was stronger in DBA/2J mice and correlated with increased activity only in that strain. These data suggest that plasma leptin level dynamics rather than hypoleptinemia alone influences the development of activity-based anorexia and that recombinant inbred panels based on these progenitor lines offer opportunities for the identification of molecular determinants for anorexia nervosa related behavioural traits.

1. Introduction

Anorexia nervosa (AN) is characterised by a severe restriction of food intake resulting in an extreme loss in body weight. The disorder occurs most commonly in young women, often beginning at around the age of puberty. As in the case of other psychiatric disorders, the aetiology of AN is likely to involve a complex interaction between psychosocial and genetic risk factors. Although the disease has an approximately 60% estimated heritability (Bulik et al., 2000), little is known about the contribution of specific genetic factors to the development and maintenance of the disease, although associations with a number of genes have been reported (Hu et al., 2003; Ribases et al., 2004; Vink et al., 2001).

Behavioural hyperactivity is common in AN, with an estimated frequency varying from 31% to 80% (Hebebrand et al., 2004). It is therefore a trait with a significant role in the pathogenesis and progression of the disease, despite not being included in the formal diagnostic criteria. Although different psychobiological mechanisms may underlie the

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occurrence of this behavioural phenotype in AN (e.g. a conscious attempt to accelerate body weight loss, or restlessness associated with distress or anxiety), some studies have shown that biological factors related to the regulation of energy balance may underlie the excessive physical activity. For example, Holtkamp and colleagues have reported that physical activity levels in AN are directly related to serum concentrations of leptin (Holtkamp et al., 2003) an important satiety-signalling hormone in the brain that is released from peripheral adipocytes (Zhang et al., 1994; Halaas et al., 1995). Since this seemingly paradoxical behavioural hyperactivity and hence energy loss during food restriction is characteristic of and detrimental to a proportion of the AN patient population, understanding its aetiology, for example by finding predisposing genetic factors, is important.

Activity-based anorexia (ABA) or semi-starvation induced hyperactivity (SIH) is an experimental model in which rodents are placed on a restricted-feeding schedule and given unlimited access to running wheels. Rodents on a restricted-feeding schedule, without access to a running wheel are able to maintain their energy balance and survive over an extended period. However, when they have voluntary access to the running wheel they show rapid loss of body weight and would eventually die without intervention because of the presence of behavioural hyperactivity in combination with food restriction. This model (Hall et al., 1953; Routtenberg and Kuznesof, 1967) mimics several symptoms observed in AN patients such as increased behavioural activity, extreme body weight loss and low plasma leptin levels (Kas et al., 2003), providing a possibility to study mechanisms important to pathophysiological aspects of the disease.

Most studies of ABA have been performed in rats in combination with pharmacological interventions. The use of different inbred strains of mice offers the opportunity to examine strain differences, and to elucidate the genetic basis for the trait under study. These strains can then be used for the creation of chromosome substitution strains (CSS) and recombinant inbred strains of mice (RIS), which provide a permanent resource for studying the genetic control of phenotypic variation (Singer et al., 2004; Williams et al., 2001b). C57BL/6J and DBA/2J inbred strains which are used for the generation of one of the currently available RI panel differ in their behaviour related to anxiety (Crawley et al., 1997; Ohl et al., 2003), which is a common feature in AN (Kaye et al., 2004) and this may be related to the expression of hyperactivity during food restriction (Binder et al., 2004; Holtkamp et al., 2004). The aim of the study is to identify behavioural and physiological differences between two genetically different inbred strains of mouse (C57BL/6J and DBA/2J) to confirm the effectiveness of using RI panels based on these strains, for the identification of molecular determinants, which contribute to the development of pathophysiological processes observed in AN.

2. Materials and methods

2.1. Animals

Female C57BL/6J ($n=14$) and DBA/2J ($n=15$) mice were used in this study. Initial breeding pairs for these strains were obtained from The Jackson Laboratory (Bar Harbor, ME, USA). All mice were bred in the Rudolf Magnus Institute of Neuroscience animal facility and were 4–6 months old at the start of the experiment. Following weaning at 3–4 weeks, female and male mice were separately housed in groups in cages (2–4 animals per cage; Macrolon Type II; # 1284 L) in a room maintained on a 12 h dark/12 h light cycle (lights on at 2 a.m.), with an ambient temperature of 22.0±2 °C. The mice were given unrestricted access to food and water.

2.2. Experimental procedure

To adapt the animals to running wheel cages, all mice were individually housed in cages (26 cm×12 cm×16 cm (length×width×height)) with running wheels (diameter: 14 cm; width: 9 cm) for 3 days before the start of the experiment. After the adaptation period all mice were maintained in the same running wheel cages for 5 additional days. This period is termed "Baseline Conditions". Under baseline conditions, mice had unrestricted access to food, water and the running wheel. Body weight and food intake were measured daily just before the beginning of the dark phase. Individual wheel running revolutions were continuously registered using Cage Registration Software (Department of Biomedical Engineering, UMC Utrecht, The Netherlands). At the end of the Baseline Period mice from each strain were randomly divided into two groups and the first group of mice, which was defined as "Baseline Animals" ($n=7$ from each strain) were decapitated and the blood was taken to measure plasma leptin levels. The second group of mice were placed on a restricted feeding schedule for five consecutive days (2 h of daily access to food) and this group of mice was defined as "Restriction Animals" ($n=7$ from the strain C57BL/6J; $n=8$ from the strain DBA/2J). During the restriction period, the food was given during the first two hours of the dark phase (the habitual activity phase of this nocturnal species). Body weight and food intake were measured before and after food access and running wheel revolutions were registered continuously. At the end of the restriction period mice were decapitated and the blood was taken for the measurement of plasma leptin level.

2.3. Statistical analysis

Body weight, food intake and running wheel activity data are expressed as mean±SEM. Differences in body weight, food intake and physical activity were assessed by a general linear model (GLM) repeated measures procedure, using a between subject factor (STRAIN) and within subject factor (DAYS). In case of a significant difference between the two strains, independent t-test was performed on each day. Differences were considered significant at $p<0.05$. The plasma leptin level was analyzed by two-way ANOVA with STRAIN and EXPERIMENTAL CONDITION (Baseline Conditions and Restricted Food Access) factors. Pearson's correlation analysis was performed to investigate a correlation between baseline/restriction running wheel activity and plasma leptin level. Data were analyzed using SPSS 11.5 for Windows.

3. Results

3.1. Body weight loss across strains

Body weight in DBA/2J and C57BL6/6J mice was similar under baseline conditions (23.3±0.3 and 22.45±0.66, respectively). However, as can be seen in Fig. 1, due to substantial weight loss, the body weight in DBA/2J mice during restricted food access was significantly lower compared to C57BL/6J ($F(1,13)=27.579$; $P=0.0001$).