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Activity-based anorexia in C57/BL6 mice: Effects of the phytocannabinoid, $\triangle^9$-tetrahydrocannabinol (THC) and the anandamide analogue, OMDM-2

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Abstract

The activity-based anorexia (ABA) paradigm is one of the few animal models of human anorexia nervosa. We present here the translation of this approach to C57/BL6 mice, a common background for genetically modified mice, and investigate the effects of the cannabinoid agonist, Δ^9-tetrahydrocannabinol (THC) and the endocannabinoid uptake inhibitor, OMDM-2 in this model. The ABA paradigm was optimised so that food-restricted wheel-running mice displayed anorexia, reduced body weight and disrupted activity and circadian cycles. These conditions produced a murine ABA model with a defined stage and stability to allow for pharmacological intervention. Daily Δ^9-THC (0.5 mg/kg) decreased survival in the ABA animals but increased feeding in the survivors, OMDM-2 (3 mg/kg) increased food intake, but not sufficiently to reverse weight loss. The effects of this model on endocannabinoid tone in the brain remain to be determined. Since the endocannabinoid system may be implicated in anorexia nervosa and in view of the positive modulation by cannabinoids of some aspects of ABA in this study, further investigation of the effects of cannabinoids in ABA is warranted.

1. Introduction

Anorexia nervosa is a disorder of complex aetiology, in which genetic, biological, psychological and socio-cultural factors appear to contribute to susceptibility. No single risk factor appears necessary or sufficient to express the disorder. This has led to difficulties in developing an appropriate model for anorexia nervosa and consequently effective pharmacotherapies have remained elusive. Anorexia is currently managed by hospitalisation with cognitive behavioural therapy; however prognosis for patients remains poor (Steinhausen, 2002). Paradigms have focussed on mimicking aspects of the human condition. The main characteristics of anorexia nervosa are: decreased food intake, decreased body weight, increased activity, abnormal endocrine function, adolescent onset and predominance in females. Of the models, activity-based anorexia (ABA) seems to mirror these

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facets most closely. The conditions required to produce ABA are the simultaneous restriction of feeding time and availability of a running wheel. In this setting, rodents will paradoxically self-starve at a time when they exercise excessively ultimately leading to starvation and death. Meanwhile non-exercising controls can maintain their body weight on the restricted feeding schedule. This model has been reproduced many times in the literature, the majority of authors using rats in their studies (Atchley and Eckel, 2005; Dixon et al., 2003; Epling et al., 1983; Gelegen et al., 2007; Hillebrand et al., 2006; Hillebrand et al., 2005; Routtenberg and Kuznesof, 1967). The use of genetically modified animals as a tool to investigate underlying disease mechanisms has created a demand for clinically relevant mouse models. However, to date, there have been only a few attempts to translate this paradigm from the rat to the mouse species (Gelegen et al., 2007; Gelegen et al., 2008).

Compounds that have proven efficacy in ABA include, α_2-agonists (Wilckens et al., 1992b), 5-HT_{1C} agonists (Wilckens et al., 1992a), the opioid antagonist, naloxone (Boer et al., 1990), the dopamine antagonist, *cis*-flupenthixol (Verhagen et al., 2009) and benzodiazepines such as chlordiazepoxide (Lett et al., 1998). However, while these drugs mediate a modest improvement in one or more facets of ABA, none of them successfully reverse the condition. More importantly, none of these drugs have successfully crossed into the clinic as potential supportive drug treatment in anorexia nervosa.

The mechanisms underlying ABA are not fully understood. Leptin administration suppresses the development of rodent ABA and low plasma leptin levels have been implicated in the hyperactivity observed in rats with ABA and in patients with anorexia nervosa (Exner et al., 2000; Hebebrand et al., 1995; Hillebrand et al., 2005). However, leptin also causes a reduction in food intake and increases in thermogenesis, resulting in body weight loss in leptin-treated ABA rats (Hillebrand et al., 2005). In addition to alterations in leptin there is evidence for endocannabinoid dysregulation in anorexia. Indeed, plasma levels of the endocannabinoid anandamide are increased in anorexia nervosa and are inversely correlated with leptin (Monteleone et al., 2005). Likewise elevated hypothalamic endocannabinoids are associated with defective leptin signalling in rodents (Di Marzo et al., 2001). In mice, hypothalamic levels of 2-AG are decreased by prolonged diet restriction and are increased by short-term fasting (Hanus et al., 2003). The endocannabinoid system is also activated by moderate intensity exercise (Sparling et al., 2003). Moreover in a recent study, circulating cannabinoid receptor 1 (CB_1) mRNA was found to be elevated in anorexic patients compared to controls, the mRNA levels correlating with eating disorder symptomatology (Frieling et al., 2009). Evidence from genetic linkage studies have established that the restricting and bingeing/purging subtypes of anorexia are associated with different alleles of the CB_1 receptor (Siegfried et al., 2004).

These data taken together with the known orexigenic effects of cannabinoid agonists in rodent and human models (Haney et al., 1999; Mattes et al., 1994; Wiley et al., 2005; Williams et al., 1998), imply involvement of the endocannabinoids in the pathophysiology of activity anorexia and suggest that treatment may be possible by manipulation of this system. Here we describe development of a variant on the rat ABA model often described in the literature demonstrating its expression in C57/BL6 mice and investigate the effects of the exogenous cannabinoids, Δ^9-tetrahydrocannabinol (THC; a CB_1 and CB_2 partial agonist) and OMDM-2 (an anandamide reuptake inhibitor) in this paradigm.

2. Experimental procedures

2.1. Animals and housing

All animals used in these studies were C57/BL6J strain male mice (Harlan UK Ltd). Mice were acclimatised to the new environment before allocation into two groups. One group was singly housed in activity cages (Lafayette Instrument Co., IN, USA) with a running wheel, linked via a counter and interface to a computer-based monitoring system. Data was collected using the Lafayette Activity Wheel Monitor software (ver 5.5). The second ('control') group was singly housed in home cages (M3; 48×15×13 cm; North Kent Plastics, UK) provided with environmental enrichment in the form of plastic huts (North Kent Plastics, UK) and nesting material (Sizzlenest; Datesend Ltd., UK). Experiments were carried out in a temperature (21 °C±2 °C) and humidity regulated environment, maintained on a 12-hour light/dark cycle (lights on at 2200 h).

2.2. Activity-based anorexia protocol

Body weights were measured daily at the start of the dark phase before the feeding period (±0.1 g). Any animals that reached 70% baseline body weight were killed by cervical dislocation due to ethical considerations. Pre-weighed rodent pellets (CRM(P), Special Diets Services, UK) were provided in food hoppers at the start of the dark phase and removed after a variable feeding interval. To prevent competition between food and wheel running, wheels were blocked while food was available. Water was available *ad libitum* throughout the course of the studies. Remaining food pellets plus spillage were weighed at the end of the feeding period and food intake was calculated (±0.1 g). All procedures were in compliance with the requirements of UK Animals (Scientific Procedures) Act 1986.

2.3. Expt 1: initial optimisation of the anorexia-based nervosa paradigm

2.3.1. Expt 1A: acute induction

The aim of this study was to translate the ABA model demonstrated by Epling et al. (1983) from rats to mice. 16 mice (23 to 29 g; 9 to 11 weeks) were randomly allocated in activity cages (n=8) or home cages (n=8), for a habituation period of 9 days with food *ad libitum*. Subsequently both groups were placed on a restricted feeding schedule, with access to food for 3 h/day.

2.3.2. Expt 1B: progressive induction

Based on the initial results, the remaining mice from Expt 1A (activity cage n=6; home cage n=8) were fed freely over a period of 17 days until food intake, body weight (22 to 29 g; age range 12 to 14 weeks) and wheel-running activity stabilised. The protocol was refined to incorporate a progressively restrictive feeding schedule beginning with a low severity restriction (6 h/day) decreasing by increments over a protracted period (21 days) to 2 h/day. Advancing severity of food restriction proceeded cautiously depending on attaining stability at the preceding interval with the aim of insidiously developing ABA. Mice were kept in the same housing. The Expts (1A and 1B) were ended after 3 months from the initial food restriction.