Brain, Behavior, and Immunity 60 (2017) 151–160

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Full-length Article

Leptin resistance elicits depressive-like behaviors in rats

V.A. Macht [a,b,1], M. Vazquez [a,1], C.E. Petyak [a], C.A. Grillo [a], K. Kaigler [a], R.T. Enos [c], J.L. McClellan [c], T.L. Cranford [c], E.A. Murphy [c], J.F. Nyland [c], G. Solomon [d], A. Gertler [d], M.A. Wilson [a,e], L.P. Reagan [a,e,*]

[a] Department of Pharmacology, Physiology and Neuroscience, University of South Carolina School of Medicine, Columbia, SC 29208, USA
[b] Department of Psychology, University of South Carolina, Columbia, SC 29208, USA
[c] Department of Pathology, Microbiology and Immunology, University of South Carolina School of Medicine, Columbia, SC 29208, USA
[d] Institute of Biochemistry, Food Science and Nutrition, The Hebrew University of Jerusalem, Rehovot 76100, Israel
[e] W.J.B. Dorn VA Medical Center, Columbia, SC 29208, USA

ARTICLE INFO

Article history:
Received 7 July 2016
Received in revised form 22 September 2016
Accepted 10 October 2016
Available online 12 October 2016

Keywords:
Obesity
Adiposity
Pegylated leptin receptor antagonist
C-reactive protein
Triglycerides
Forced swim test
Inflammation
Activated T cells
Spleen

ABSTRACT

There is a growing appreciation that the complications of obesity extend to the central nervous system (CNS) and include increased risk for development of neuropsychiatric co-morbidities such as depressive illness. The neurological consequences of obesity may develop as a continuum and involve a progression of pathological features which is initiated by leptin resistance. Leptin resistance is a hallmark feature of obesity, but it is unknown whether leptin resistance or blockage of leptin action is casually linked to the neurological changes which underlie depressive-like phenotypes. Accordingly, the aim of the current study was to examine whether chronic administration of a pegylated leptin receptor antagonist (Peg-LRA) elicits depressive-like behaviors in adult male rats. Peg-LRA administration resulted in endocrine and metabolic features that are characteristic of an obesity phenotype. Peg-LRA rats also exhibited increased immobility in the forced swim test, depressive-like behaviors that were accompanied by indices of peripheral inflammation. These results demonstrate that leptin resistance elicits an obesity phenotype that is characterized by peripheral immune changes and depressive-like behaviors in rats, supporting the concept that co-morbid obesity and depressive illness develop as a continuum resulting from changes in the peripheral endocrine and metabolic milieu.

Published by Elsevier Inc.

1. Introduction

Obesity is defined as a body mass index (BMI) of greater than 30 and is associated with a host of co-morbidities, including cardiovascular disease, type 2 diabetes mellitus (T2DM) and the metabolic syndrome (MetS). In addition to peripheral complications, clinical and epidemiological studies indicate that there is an association between obesity and neuropsychiatric disorders such as depressive illness, illustrating that the complications of obesity extend to the CNS (Simon et al., 2006; Luppino et al., 2010; Fabricatore and Wadden, 2006; McElroy et al., 2004; Andersen et al., 2010; Stunkard et al., 2003). Moreover, there is growing appreciation that while a complete obesity phenotype may provide the greatest risk for the development of neuropsychiatric disorders (Onyike et al., 2003), the pathological features associated with obesity develop as a continuum. While a number of factors have been suggested as mechanistic links in this continuum between obesity and mood disorders (Raison et al., 2006; Reagan, 2012), leptin resistance may be an initiating factor in this co-morbidity.

Leptin is synthesized and secreted by adipocytes and is transported across the blood-brain barrier (BBB) via a saturable transport system (Banks, 2004). In the hypothalamus, the actions of leptin are well characterized and include the regulation of food intake, metabolism, body weight, and body composition (For review, see (Schwartz et al., 2000)). Beyond the hypothalamus, leptin is also known to regulate hippocampal synaptic plasticity (For reviews, see (Harvey, 2007; Fadel et al., 2013)). However, in obesity phenotypes leptin transport across the BBB is impaired (Banks, 2004; Burguera et al., 2000; Banks et al., 1999), leading to a leptin-deficient state in the CNS. These findings have led to the suggestion that reduced CNS leptin activity may be a mechanistic link between obesity and major depressive illness (Lu, 2007). In support of this hypothesis, *ob/ob* mice, which lack the gene coding for leptin, exhibit increased immobility time in the forced swim test (FST) compared to wild-type controls, a behavioral change that

* Corresponding author at: Department of Pharmacology, Physiology and Neuroscience, University of South Carolina School of Medicine, 6439 Garners Ferry Road, D40 Columbia, SC 29208, USA.
E-mail addresses: lawrence.reagan@uscmed.sc.edu, Lawrence.reagan@va.gov (L.P. Reagan).
[1] Both authors contributed equally to this study.

http://dx.doi.org/10.1016/j.bbi.2016.10.008
0889-1591/Published by Elsevier Inc.

is indicative of behavioral despair in rodents (Collin et al., 2000; Yamada et al., 2011). Additionally, *db/db* mice, which lack functional leptin receptors, exhibit increased immobility time in the FST and altered anxiety-like behaviors (Sharma et al., 2010). Our recent studies have demonstrated that an obesity/MetS phenotype also induces depressive-like behaviors (Grillo et al., 2011b) and that these behavioral deficits are reversed by food restriction paradigms that restore leptin sensitivity (Grillo et al., 2014). Collectively, these studies suggest that leptin resistance may be a key initiating factor in the development of neuropsychiatric disorders in obesity. However, a causal relationship between leptin resistance and the increased risk of co-morbid depressive illness in obesity remains to be established.

In addition to leptin resistance, our previous studies illustrated that increases in peripheral inflammation are associated with depressive-like behaviors (Grillo et al., 2014), supporting the concept that a pro-inflammatory state in obesity may elicit depressive symptoms (For reviews, see (Shelton and Miller, 2010; Soczynska et al., 2011)). As leptin may also play an important role in signaling cytokine production (Lord et al., 1998), leptin resistance could be an important initiating factor in peripheral inflammation which potentially leads to the development of depressive-like behaviors. To test this hypothesis, the goal of the current studies was to determine whether leptin resistance or central and peripheral blockage of leptin action induced by chronic administration of a pegylated leptin receptor antagonist (Peg-LRA) elicits depressive-like behaviors in adult male rats by altering peripheral immune responses.

2. Materials and methods

2.1. Animal protocol

Adult male Sprague Dawley rats (CD strain, Harlan) weighing 225–250 g were individually housed with *ad libitum* access to food and water, in accordance with all guidelines and regulations of the WJB Dorn VA Animal Care and Use Committee. Animals were maintained in a temperature-controlled room, with a light/dark cycle of 12/12 h (lights on at 7:00 a.m.). Rats were acclimated to the animal facility for one week during which time baseline body weights and food intake measurements were obtained daily at 10:00 a.m.; these measures were performed daily for the duration of the study. On Days 1 through 17 rats received intraperitoneal (i.p.) injections of the Peg-LRA at a dose of 7 mg/kg daily at 5:00 p.m.; control rats received daily i.p. vehicle (sterile 0.4% $NaHCO_3$, pH = 8.0) injections. Non-pegylated and mono-pegylated rat superactive leptin antagonist (D23L/L39A/D40A/F41A) mutant was prepared according to the protocol used for preparation of non-pegylated and mono-pegylated mouse and human superactive leptin antagonists (D23L/L39A/D40A/F41A) mutant (Shpilman et al., 2011; Jamroz-Wisniewska et al., 2014). In vitro and in vivo activity of this dose of the Peg-LRA was demonstrated in Jamroz-Wisniewska et al. (2014). Behavioral testing was initiated on Day 10 of treatment. Rats were sacrificed on Day 17 following leptin administration as described below.

2.2. Endocrine analyses

All plasma endocrine analyses were performed in duplicate as described in our previous studies (Grillo et al., 2015; Grillo et al., 2014) Tail bleeds were performed 10 days following initiation of Peg-LRA or vehicle treatment under non-fasting conditions to isolate plasma for endocrine analysis. Blood glucose levels were measured by glucose oxidase method (Pointe Scientific, Inc., Canton, MI). Plasma triglycerides were determined using an enzymatic kit (modified Trinder) according to the manufacturer's instructions (Pointe Scientific, Inc., Canton, MI, USA). Plasma insulin levels were measured by enzyme-linked immunosorbent assay (ELISA, Millipore, Billerica, MA). Using plasma isolated at the time of sacrifice, plasma C-reactive protein levels were determined by ELISA (BD Biosciences, San Diego, CA). ELISA plates were analyzed according to the manufacturer's instructions using a Tecan SPECTRAFluor plate reader (Tecan U.S., Inc., Durham, NC). Plasma cytokine levels were measured using a Bio-Plex rat cytokine panel according to the manufacturer's instructions (Bio-Rad, Hercules, CA). Plates were analyzed using a Bio-Plex system coupled to Bio-Plex Manager software.

2.3. Open field test

On day 11 of the Peg-LRA treatment paradigm, Peg-LRA rats (N = 10) or Vehicle (N = 10) rats were placed in a 76 cm × 76 cm square, gray Plexiglas chamber with 45 cm walls and a floor grid for a five minute open field test. The test was conducted during the early light portion of the light/dark cycle and allows for the assessment of locomotor behaviors, indicated by distance traveled and velocity. Performance was recorded and analyzed using Ethovision 7, an automated system from Noldus Information Technology Inc. (Leesburg, VA).

2.4. Sucrose preference

On Day 12 of the Peg-LRA paradigm the sucrose preference test was performed as described previously (Grillo et al., 2011b; Grillo et al., 2014). Rats were exposed for 24 h to two identical bottles containing water and 1% sucrose solution. The next day the rats were water-deprived for 6 h (1:00 p.m.–7:00 p.m.) before testing their preference for sucrose (1%) or water in a three-hour two-bottle choice beginning at 7:00 p.m.

2.5. Forced swim test

The FST was modified from the test originally developed by Porsolt et al. (1978) as described in our previous studies (Grillo et al., 2011b). Testing for both days was done in the early light hours of the light/dark cycle. Animals were placed in a clear Plexiglas cylinder, 30 cm in diameter and 60 cm in height, filled with 35 cm of room temperature water (25 °C) in a separate room with no visual or audible stimuli. The first day consisted of a fifteen minute pretest (Day 14 of treatment), followed 24 h later by a five minute test (Day 15 of treatment). Animals were viewed and scored later by a scorer blind to the treatment group via video camera for three behaviors: immobility, climbing and swimming. Immobility behaviors were defined as little to no movement of the rat. Climbing was defined as vertical movement in contact with the side of the container. All other movements were defined as swimming. Behaviors were noted by the scorer every three seconds.

2.6. Immunoblot analysis of leptin signaling

A subset of Peg-LRA-treated rats (n = 5) and control rats (n = 5) were given an i.p. injection of leptin (5 mg/kg; National Hormone and Peptide Program, Torrance CA) sixty minutes prior to sacrifice. Adipose tissue, liver and spleens were collected to measure changes in fat mass and immune status as described below. The hypothalamus was also isolated for immunoblot analysis of leptin signaling. Briefly, 50 μg of total membrane fractions were separated by SDS/PAGE (10%), transferred to nitrocellulose (NC) membranes and blocked in TBS plus 10% nonfat dry milk. NC membranes were then incubated with primary antisera for the phosphorylated form of MAPK (Cell Signaling Technologies #9101) and the phosphorylated form of STAT3 (Cell Signaling