Pharmacology, Biochemistry and Behavior 103 (2013) 573–581

Contents lists available at SciVerse ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Anorexic effects of intra-VTA leptin are similar in low-fat and high-fat-fed rats but attenuated in a subgroup of high-fat-fed obese rats

Adrie W. Bruijnzeel *, Xiaoli Qi, Lu W. Corrie

Department of Psychiatry, McKnight Brain Institute, University of Florida, 1149 Newell Dr., Gainesville, FL 32611, USA

ARTICLE INFO

Article history:
Received 4 May 2012
Received in revised form 12 October 2012
Accepted 22 October 2012
Available online 27 October 2012

Keywords:
Leptin
Cy3-leptin
Ventral tegmental area
Food intake
High-fat diet
Diet-induced obese
Rats

ABSTRACT

Leptin is an adiposity hormone that plays an important role in regulating food intake and energy homeostasis. This study investigated the effects of a high-fat (HF) and a low-fat, high-carbohydrate/sugar (LF) diet on leptin sensitivity in the ventral tegmental area (VTA) in rats. The animals were exposed to a HF or LF diet for 16 weeks. Then the effects of intra-VTA leptin (150 and 500 ng/side, unilateral dose) on food intake and body weights were investigated while the animals were maintained on the HF or LF diet. Long-term exposure to the HF or LF diet led to similar body weight gain in these groups. The HF-fed animals consumed a smaller amount of food by weight than the LF-fed animals but both groups consumed the same amount of calories. The bilateral administration of leptin into the VTA decreased food intake (72 h) and body weights (48 h) to a similar degree in the HF and LF-fed animals. When the HF-fed animals were ranked by body weight gain it was shown that the diet-induced obese rats (HF-fed DIO, upper quartile for weight gain) were less sensitive to the effects of leptin on food intake and body weights than the diet-resistant rats (HF-fed DR, lower quartile for weight gain). A control experiment with fluorescent Cy3-labeled leptin showed that leptin did not spread beyond the borders of the VTA. This study indicates that leptin sensitivity in the VTA is the same in animals that are exposed to a HF or LF diet. However, HF-fed DIO rats are less sensitive to the effects of leptin in the VTA than HF-fed DR rats. Leptin resistance in the VTA might contribute to overeating and weight gain when exposed to a HF diet.

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1. Introduction

Since the 1970s, there has been a gradual increase in the prevalence of overweight and obesity (Flegal et al., 1998; Popkin and Doak, 1998). It has been suggested that this is due to an increased availability of food, an increase in the consumption of refined carbohydrates and fats, and an increasingly sedentary lifestyle (Swinburn et al., 2011). Animal models have been developed to investigate the neuronal mechanisms that cause overeating and obesity in humans. Several studies have shown that high-fat (HF) diets lead to increased weight gain in rats and mice (El Haschimi et al., 2000; Woods et al., 2003). The increased weight gain is accompanied by an increase in fat mass, an increase in plasma insulin and leptin levels, and insulin and leptin resistance (El Haschimi et al., 2000; Woods et al., 2003). Not all rats that are fed a HF diet gain more weight than control animals (Omagari et al., 2008). HF-fed animals that gain more weight than animals that are fed standard laboratory chow are often referred to as diet-induced obese (DIO) or obesity prone and the HF-fed animals that do not gain an excessive amount of weight are referred to as diet-resistant (DR) or obesity resistant (Farley et al., 2003; Levin et al., 1997; Levin et al., 1989; Otukonyong et al., 2005).

A wide variety of neuropeptides and hormones have been implicated in the regulation of food intake (Coll et al., 2007). Leptin is one of the hormones that fulfills the criteria for adiposity signal (Schwartz et al., 2000). Leptin is considered an adiposity signal because plasma levels of leptin are proportional to body fat content and leptin enters the brain in proportion to plasma levels (Schwartz et al., 1996). Second, leptin receptors are expressed on neurons that regulate food intake (Baskin et al., 1999). Third, the administration of leptin into the lateral ventricles and specific brain sites such as the arcuate hypothalamic nucleus (Arc) and the ventral tegmental area (VTA) reduces food intake whereas a deficiency in leptin leads to an increase in food intake (Bruijnzeel et al., 2011; Hommel et al., 2006; Satoh et al., 1997; Seeley et al., 1996; Zhang et al., 1994).

Leptin mediates some of its effects on metabolism and food intake via the phosphorylation of the transducer and activator of transcription 3 (STAT3) (Gao et al., 2004). Recent studies have used STAT3 phosphorylation as a marker to study leptin resistance (Matheny et al., 2011; Patterson et al., 2009). The administration of leptin into the third ventricle has been shown to induce STAT3 phosphorylation in the VTA and Arc and this effect is diminished in animals that have been exposed to a HF diet (Matheny et al., 2011). At this point, it is not known if long-term exposure to a HF diet would also affect the

* Corresponding author at: University of Florida, Department of Psychiatry, 1149 Newell Dr., P.O. Box 100256, Gainesville, FL 32611, USA. Tel.: +1 352 294 4931; fax: +1 352 392 8217.
E-mail address: awbruijn@ufl.edu (A.W. Bruijnzeel).

0091-3057/$ – see front matter. Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.pbb.2012.10.012

intra-VTA leptin-induced decrease in food intake and body weights. In addition, it is not known if the administration of leptin into the VTA affects food intake and body weights differently in HF-fed DIO and HF-fed DR rats. Therefore, the first aim of the present study was to investigate if long-term, 16 weeks, exposure to a HF diet affects the intra-VTA leptin-induced decrease in food intake and body weights in rats. The second aim was to investigate if leptin affects food intake and body weights differently in HF-fed DIO and DR rats.

2. Methods

2.1. Subjects

Male Sprague–Dawley rats (n = 42; Harlan labs, Prattville, AL) weighing 135–150 g (5–6 weeks of age) at the beginning of the experiment were used. Animals were single housed in a temperature- and humidity-controlled vivarium and maintained on a 12 h light–dark cycle (lights off at 9 AM). Food and water were available ad libitum in the home cages during all stages of the experiment. All subjects were treated in accordance with the National Institutes of Health guidelines regarding the principles of animal care. Animal facilities and experimental protocols were in accordance with the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) and approved by the University of Florida Institutional Animal Care and Use Committee.

2.2. Drugs

Rat recombinant leptin was purchased from Sigma-Aldrich (St. Louis, MO, USA) and fluorescent Cy3-labeled leptin (mouse) was purchased from Phoenix Pharmaceuticals (Belmont, CA, USA). Leptin was dissolved in 10 mM NaOH and after the leptin was dissolved the pH was titrated from about 2 to 7.3 with 10 mM NaOH. Distilled water was used to obtain the final leptin concentrations. Distilled water was also used for the control injections (zero-dose control). Cy3-leptin was dissolved in 0.1 M PBS with a pH of 7.9.

2.3. Surgical procedures

At the beginning of the intracranial surgeries, the rats were anesthetized with an isoflurane/oxygen vapor mixture (1–3% isoflurane) and placed in a Kopf stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) with the incisor bar set 3.3 mm below the interaural line (flat skull). The rats were prepared with 11 mm stainless steel 23 gauge cannulae above the VTA using flat skull coordinates according to Paxinos and Watson (1998) and a previous study by our research group (Bruijnzeel et al., 2011). Bilateral cannulae were implanted 2.5 mm above the VTA (anterior–posterior [AP] −5.3, medial lateral [ML] ± 1.0 mm, dorsal–ventral [DV] −5.2 from dura). At the end of the surgery, 11 mm removable 30 gauge wire stylets were inserted in the cannulae to maintain patency. The cannulae were permanently secured to the skull by using dental cement that was anchored with four skull screws.

2.4. Intracranial microinjections

Drugs (leptin and Cy3-leptin) were administered bilaterally into the VTA by using 30 gauge stainless steel injectors that extended 2.5 mm (length of injector tip was 13.5 mm) beyond the guide cannulae. The injection volume was 0.5 μl/side and the drug was infused over a 66 s period as described previously (Bruijnzeel et al., 2011; Yamada and Bruijnzeel, 2011). The rats were gently retrained by hand during the infusions. The infusion speed was regulated by a Harvard Apparatus syringe pump (model 975) and the pump was equipped with 10 μl syringes (Model 901 RN; Hamilton, Rena, NE, USA). The syringes were connected to the injectors with Tygon microbore PVC tubing (0.25 mm ID × 0.76 mm OD). The injectors were left in place for 30 s post-injection to allow diffusion from the injector tip. The dummy stylets, 11 mm, were re-inserted immediately after the injectors were removed.

2.5. Histology

The brains were processed as previously described by our research group (Bruijnzeel et al., 2011; Marcinkiewcz et al., 2009). At the end of the experiment, the rats were deeply anesthetized with sodium pentobarbital (150 mg/kg, ip). The rats were then perfused via the ascending aorta with 100 ml of physiological saline followed by 150 ml of a 10% phosphate buffered formalin (4% formalin, w/v, Fisher Scientific) solution. The rats were perfused with the intracranial drug injectors in place in order to enhance the visibility of the injection tracts in the brain sections. Brains were postfixed for 24 h in phosphate buffered formalin and then stored in 0.1 M PBS until further processing. The rat that received Cy3-leptin in the VTA was perfused with 50 ml of physiological saline followed by 250 ml of freshly prepared ice-cold 4% paraformaldehyde in 0.1 M PBS. The brain was postfixed overnight in 4% paraformaldehyde in 0.1 M PBS.

Before the brains were sectioned with the cryostat, they were transferred to 30% sucrose in 0.1 M PBS for cryoprotection. The brains were kept in sucrose until they sank (~48 h) and then 40 μm coronal sections were cut at −25 °C on a Leica CM3050 S cryostat (Leica Microsystems GmbH, Wetzlar, Germany) and mounted on Superfrost Plus microscope slides. All the sections, with the exception of the Cy3-sections, were stained with cresyl violet. The locations of the guide cannulae and injections sites were verified with a Leica DM2500 light microscope and with reference to a stereotaxic atlas of the rat brain (Paxinos and Watson, 1998). Bright-field images were captured with a Leica DFC420 camera and the fluorescence images were captured with a Leica DFC345 FX camera. All images were processed with Leica LAS image analysis software and with Corel PaintShop Pro X3 (Ottawa, ON, Canada).

2.6. Experimental design

The first few days after arrival in the vivarium the rats were fed a standard rodent diet (7912 Teklad LM-485 Mouse/Rat, Harlan Laboratories, Indianapolis, IN, USA) and then they were switched to a purified HF or a purified low-fat, high-carbohydrate (LF) diet (HF, D12451; LF, D12450B; Research Diets, New Brunswick, NJ, USA). It should be noted that other research groups often refer to the low-fat high-carbohydrate diet (D12450B) as a low-fat diet (Gao et al., 2002; Lan et al., 2008; Posey et al., 2009). In the HF diet, 45% of the calories were derived from fat, 35% from carbohydrates (corn starch 21%, maltodextrin 29%, and sucrose 50% of total carbohydrates), and 20% from protein. In the LF diet, 10% of the calories were derived from fat, 70% from carbohydrates (corn starch 45%, maltodextrin 5%, and sucrose 50% of total carbohydrates), and 20% from protein. The energy content of the HF diet was 4.73 kcal/g and the energy content of the LF diet was 3.85 kcal/g. The rats received fresh food pellets daily and food intake was measured once a week immediately before the onset of the dark cycle (9 AM). The rats received the food pellets in a bowl at the bottom of the cage and for the food intake measurements all pieces of food were carefully collected. The cannulae were surgically implanted 12 weeks after the onset of the HF and LF diets and the leptin injections started 4 weeks later.

This experiment investigated the effects of leptin in the VTA (HF, n = 26; LF = 15) on food intake. Leptin (150, 500 ng VTA; unilateral doses) was administered bilaterally according to a Latin square design. Leptin was administered between 8 AM and 9 AM in a standard animals testing room shortly before the onset of the dark cycle. The food was removed immediately before the injections and the rats received new food when they were back in the animal housing room. There were a greater number of animals in the HF-fed group than in the LF-fed group in order to be able to investigate leptin sensitivity