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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

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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.

Keywords

Leptin; Cy3-leptin; ventral tegmental area; food intake; high-fat diet; diet-induced obese; rats

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

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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 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 μ l/side and the drug was infused over a 66 second 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 μ l syringes (Model 901 RN; Hamilton, Rena, NE, USA). The syringes were connected to the injectors with Tygon microbore PVC tubing (0.25 mm ID $\times$ 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 reinserted immediately after the injectors were removed.

2.5 Histology

The brains were processed as previously described by our research group (Bruijnzeel et al., 2011; Marcinkiewicz 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 forty-micrometer 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%, 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%, 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 in the DIO (HF-fed upper quartile for weight gain, $n = 6$) and DR (HF-fed lower quartile for weight gain, $n = 6$) rats. We will refer to the HF-fed DIO rats as DIO rats and the HF-fed DR rats as DR rats. Food intake was recorded 1, 4, 24, 48, and 72 h after the leptin infusions. The body weights were recorded 24, 48, and 72 h after the leptin infusions. There were at least 96 h between the injections. At the end of the experiment, the rats were anesthetized, perfused, and the brains were removed to verify the anatomical localization of the injection sites. Histological verification indicated that all the injector tracts ended within or at the boundaries of the VTA and therefore all the rats were included in the statistical analyses (Figure 1A and B).

In order to investigate the spread of leptin, one rat was prepared with bilateral 11 mm cannula above the VTA (AP -5.3 , ML ± 1.0 mm, DV -5.2 from dura) and 0.5 μ l of Cy3-leptin was infused with an injector that extended 2.5 mm beyond the tip of the cannula. The rat received 562.5 μ g of Cy3-leptin (MW = 18 kDa, unilateral dose) which is equivalent to 500 μ g of leptin (MW = 16 kDa). Fifteen minutes after the infusion of Cy3-leptin, the rat was perfused and the brain was removed. A close inspection of the distribution of Cy3-leptin indicated that leptin spread over only a very small area and did not spread beyond the borders of the VTA (Figure 1C). Morton and co-workers also reported that a small volume of Cy3-leptin (0.5 μ l/side) does not spread beyond the borders of the VTA (Morton et al., 2009). Individual Cy3-positive cells can be identified in figure 1C. This is in line with a previous study that showed that Cy3-leptin binds to leptin receptors and then the ligand-receptor complexes are internalized (Lundin et al., 2000)

2.7 Statistical analyses

The effects of the diets (HF or LF) on body weights and food intake were analyzed with two way repeated-measures analyses of variance (ANOVA) with diet as the between-subjects factor and time as the within-subjects factor. Body weights were recorded daily and 7-day averages were used for the statistical analysis. The effects of leptin in the VTA on food intake and changes in body weights were analyzed with one-way repeated-measures ANOVA with the dose of leptin as the within-subjects factor. The effect of leptin on food intake and body weights in the DIO and DR rats at the various time-points were analyzed with one-way ANOVA's. Bonferroni post-hoc t-tests were conducted when the ANOVA revealed statistically significant effects. For all the experiments, the criterion for significance was set at 0.05. The statistical analyses were performed using IBM SPSS Statistics 19 for Windows software.

3. Results

There were no differences in body weights between the HF and LF-fed rats prior to switching to the HF or LF diets (Figure 2A; Diet: $F_{1,40}=0.42$, n.s.). The rats were exposed to the HF or LF diet for 16 weeks and during this period the animals in the HF and LF-fed groups gained the same amount of weight (Figure 2A; Diet: $F_{1,39}=0.84$, n.s.; Time: $F_{11,429}=24.43$, $P<0.0001$). The animals that were exposed to the HF diet consumed a smaller amount of food by weight than the animals that were exposed to the LF diet (Figure 2B; Diet: $F_{1,39}=69.37$, $P<0.0001$; Time: $F_{11,429}=24.43$, $P<0.0001$; Diet $\times$ Time: $F_{11,429}=2.53$, $P<0.004$). The animals in the HF-fed group consumed to same amount of calories as the animals in the LF-fed group (Figure 2C; Diet: $F_{1,39}=1.53$, n.s.; Time: $F_{11,429}=21.89$, $P<0.0001$).

The administration of leptin into the VTA decreased food intake by weight during the 0–1 h period (Figure 3A; Dose: $F_{2,78}=3.11$, $P<0.05$; Dose $\times$ Diet: $F_{2,78}=7.76$, $P<0.001$), the 0–4 h period (Dose: $F_{2,78}=26.52$, $P<0.0001$; Dose $\times$ Diet: $F_{2,78}=4.62$, $P<0.05$), the 0–24 h period (Figure 3B; Dose: $F_{2,78}=17.38$, $P<0.0001$), the 24–48 h period (Dose: $F_{2,78}=18.11$, $P<0.0001$), and the 48–72 h period (Dose: $F_{2,78}=3.83$, $P<0.05$). During the 0–1 h and the 0–4 h period there was a dose $\times$ diet interaction which suggests that the effect of leptin on food intake might be dependent on the diet. The posthoc test indicated that during the 0–1 h period food intake was lower in the LF-fed animals (vehicle) than in the HF-fed animals ($P<0.05$, vehicle). During the same time period, 150 ng ($P<0.01$) and 500 ng ($P<0.01$) of leptin decreased food intake in the HF-fed group but not in the LF-fed group. Leptin might not have decreased food intake in the LF-fed group because food intake in this group was relatively low during the 0–1 h period. When measured over an extended time period (24 h or more), the LF-fed animals consume more food by weight than the HF-fed animals. However, during the first few hours after the administration of vehicle the LF-fed animals consumed a smaller (0–1 h) or the same amount of food (0–4 h) as the HF-fed animals. It is not known why the food intake of the LF-fed vehicle (0 ng of leptin) animals was relatively low during the 0–1 h and the 0–4 h period.

There was an effect of diet (HF or LF) on food intake by weight during the 0–4 h period (Figure 3A; Diet: $F_{1,39}=8.05$, $P<0.007$), the 0–24 h period (Figure 3B; Diet: $F_{1,39}=38.11$, $P<0.0001$), the 24–48 h period (Diet: $F_{1,39}=38.98$, $P<0.0001$), and the 48–72 h period (Diet: $F_{1,39}=29.03$, $P<0.05$). Posthoc analyses indicated that during the 0–24, 24–48, and 48–72 h period the animals in the LF-fed vehicle group consumed more food than animals in the HF-fed vehicle group. This is in line with the observation that during weeks 1–12 (before the onset of the leptin injections) the animals in the LF-fed group consumed more food by weight than the animals in the HF-fed group. The administration of leptin decreased body weight gain during the 0–24 h period (Figure 4A; Dose: $F_{2,78}=12.48$, $P<0.0001$) and the

24–48 h period (Figure 4B; Dose: $F_{2,78}=8.75$, $P<0.0001$). The administration of leptin did not affect body weights during the 48–72 h period (data not shown). There was no effect of diet on leptin-induced weight loss.

The administration of leptin into the VTA decreased the number of calories consumed during the 0–1 h period (Table 1; Dose: $F_{2,78}=3.89$, $P<0.05$; Dose $\times$ Diet: $F_{2,78}=8.23$, $P<0.001$), the 0–4 h period (Dose: $F_{2,78}=26.96$, $P<0.0001$; Dose $\times$ Diet: $F_{2,78}=6.53$, $P<0.002$), the 0–24 h period (Dose: $F_{2,78}=17.54$, $P<0.0001$; Dose $\times$ Diet: $F_{2,78}=3.66$, $P<0.03$), the 24–48 h period (Dose: $F_{2,78}=17.86$, $P<0.0001$), and the 48–72 h period (Dose: $F_{2,78}=3.76$, $P<0.05$). In contrast to the effects of diet on food intake by weight, there was no effect of diet alone on the number of calories consumed. This is in line with the observation that before the onset of the leptin injections the LF-fed animals consumed a larger amount of food by weight than the HF-fed animals but they consumed the same amount of calories as the HF-fed animals (Figs. 2B–C).

In order to investigate the relationship between body weight gain and leptin sensitivity, the HF-fed animals were ranked for weight gain and the effect of leptin on food intake was investigated. Animals in the top 25% for weight gain (HF-fed DIO) and the bottom 25% for weight gain (HF-fed DR) were used for this analysis. The animals were exposed to the HF diet for 16 weeks and during this period the DIO rats gained more weight than the DR rats (Figure 5A; Weight group: $F_{1,10}=122$, $P<0.0001$; Time: $F_{15,150}=6830.4$, $P<0.0001$; Weight group $\times$ Time: $F_{15,150}=27.8$, $P<0.0001$). The animals in the top 25% also consumed a larger amount of food per day (Figure 5B; Weight group: $F_{1,10}=18$, $P<0.002$; Time: $F_{11,110}=4.3$, $P<0.0001$). The 150 ng dose of leptin induced a greater decrease in food intake in the DR animals than in the DIO animals 24–48 h after the administration of leptin (DR -3.9 ± 1.3 g vs. DIO -0.6 ± 1.2 g), but this effect did not reach statistical significance (Figure 6A; Weight group: $F_{1,11}=3.05$, $P=0.11$, trend). The 150 ng dose of leptin had the same effect on food intake in the DR and DIO rats during the 0–1, 0–4, 0–24, and 48–72 h period (data not shown). The DR animals had a significantly greater reduction in food intake than DIO animals 24–48 h after the administration of 500 ng of leptin (Figure 6B; Weight group: $F_{1,11}=12.23$, $P<0.006$). This suggests that DR animals are more sensitive to the effects of leptin in the VTA than DIO animals. The administration of leptin into the VTA decreased body weight gain in the DIO and DR animals during the 0–24 h period (Figure 6C; Dose: $F_{2,20}=6.50$, $P<0.007$) and the 24–48 h period (Dose: $F_{2,20}=6.49$, $P<0.007$). Posthoc analysis indicated that 500 ng of leptin decreased body weight gain in the DIO and DR rats in the 24–48 h period. The administration of leptin into the VTA affected body weight gain differently in the DIO and DR animals during the 48–72 h period (Dose $\times$ Weight group: $F_{2,20}=5.02$, $P<0.017$). Posthoc analyses indicated that body weight gain was increased in the DIO rats treated with 500 ng of leptin compared to DIO rats treated with vehicle (0 ng of leptin) during the 48–72 h period. Because leptin decreased food intake during the 0–24 h and 24–48 h period, it is most likely that this is a compensatory increase in body weight. Posthoc analyses also indicated that weight gain was decreased in DR rats treated with 500 ng of leptin compared to DIO rats treated with the same dose of leptin during the 48–72 h period. This indicates that the administration of 500 ng of leptin into the VTA induces a more prolonged decrease in body weight gain in DR rats than in DIO rats. This is in line with the observation that 500 ng of leptin induced a greater decrease in food intake in the DR rats than in the DIO rats during the 24–48 h period (Figure 6A).

4. Discussion

The present study showed that the administration of leptin into the VTA led to a similar decrease in food intake and body weights in HF-fed and LF-fed rats. An additional analysis showed that HF-fed DIO rats were less sensitive to the effects of leptin on food intake and

body weights than HF-fed DR rats. This study suggests that leptin signaling in the VTA plays a role in the regulation of food intake and heightened leptin sensitivity in the VTA may protect against overeating and obesity.

In the present study, the effects of a HF and LF diet on body weight gain and food intake were investigated. The HF and LF-fed animals gained the same amount of weight over a 16-week period. These two groups may have gained the same amount of weight because the HF-fed rats reduced their food intake to compensate for the higher energy density of the HF food. Omagari and colleagues also showed that Sprague-Dawley rats that are fed a HF diet (D12451, Research Diets) gain the same amount of weight as animals that are fed a LF diet (D12450B; Research Diets) (Omagari et al., 2008). It might be possible that HF and LF-fed animals gain the same amount of weight because both diets are relatively palatable. The HF diet has a high fat content (45 kcal%) and a relatively low sucrose content (17.5 kcal%). The LF diet has a low fat content (10 kcal%) but a relatively high sugar content (35 kcal%). The HF and the LF diets might be more palatable than standard nonpurified laboratory chow (mainly grains and no sugar or lard added). This is supported by the observation that rats consume a greater amount of HF and LF food (D12451 and D12450B; Research Diets) than of laboratory chow (McLaughlin et al., 2006; McLaughlin et al., 2003). Furthermore, Woods and colleagues showed that rats that were fed a sugar containing LF diet (Dyets, Inc., Bethlehem, PA, USA) gained more weight over time than rats that were fed standard laboratory chow (Woods et al., 2003). It should be noted that differences in food intake and body weight gain between animals that are fed a purified diet (HF or LF diet) or a standard nonpurified diet are not only due to differences in fat or sugar content. Factors such as the bioavailability of nutrients, types of fiber, and levels of plant chemicals (e.g., phytoestrogens) may also account for differences in food intake and weight gain between animals that are fed purified or nonpurified diets (Cederroth et al., 2007). Finally, the strain of rats might play a role in diet-induced weight gain. In the present study and in the study by Omagari and colleagues, Sprague-Dawley rats were used and weight gain was the same in the HF and LF-fed groups (Omagari et al., 2008). In contrast, Woods and colleagues reported that Long-Evans rats that are fed a HF diet (AIN93M, Dyets, Bethlehem, PA, USA) gain more weight over time than rats that are fed a LF diet (Dyets, Bethlehem) (Woods et al., 2003). In another study, Long-Evans rats were fed a HF (D12451, Research Diets) or LF (D12450B; Research Diets) diet for 11 weeks (Borowsky et al., 2002). At the end of the study period, the HF-fed animals weighed about 100 grams more than the LF-fed animals. Taken together, these findings suggest that it depends on the strain of rats and/or the control diet whether or not an effect of a HF diet on body gain is detected. It should be noted that in the present study fat pads were not collected and weighed. Previous research has shown that HF diets have a greater effect on the weight of the fat pads than on body weight (Woods et al., 2003). Therefore, although we did not detect an effect of the HF and LF diet on body weights, it cannot be ruled out that the HF-fed rats may have had larger fat pads than the LF-fed rats.

The present study showed that the administration of leptin into the VTA led to a similar decrease in food intake and body weights in the HF and LF-fed animals. The results of this study are in line with a previous study that showed that the administration of leptin into the VTA decreases food intake and body weights in female rats (Bruijnzeel et al., 2011). The present study suggests that a HF diet does not lead to a decreased sensitivity to leptin into the VTA. Some caution is warranted with the interpretation of the data. First, the LF diet contains less fat than the HF diet, but the LF diet contains more carbohydrates. Clinical studies suggest that carbohydrates play a role in weight gain and the consumption of carbohydrates stimulates the release of leptin (Jenkins et al., 1997; Mozaffarian et al., 2011; Rosell et al., 2006). Therefore, chronic exposure to the LF diet might also have affected leptin sensitivity.

The administration of leptin into the third ventricle increases the phosphorylation of STAT3 in the VTA and Arc and this effect is attenuated by pre-exposure to a HF diet (Matheny et al., 2011). Based on the aforementioned findings, it was concluded that exposure to a HF diet leads to leptin resistance in the VTA and Arc. This observation is not in agreement with our observation that a HF diet does not lead to leptin resistance in the VTA. There are several possible explanations for the discrepancy. First, different parameters were used to study leptin sensitivity. In the present study, the effect of leptin on food intake was assessed and Matheny and colleagues assessed the effect of leptin on STAT3 phosphorylation. It might be possible that chronic exposure to a HF diet differently affects leptin-induced STAT3 phosphorylation and leptin-induced changes in food intake. Second, the time-course for the effects of leptin on STAT3 phosphorylation and food intake are different. The administration of leptin leads to a rapid increase in STAT3 phosphorylation with a maximal response after 30 minutes. The effect of the HF diet on leptin-induced STAT3 phosphorylation was observed 1 h after the administration of leptin (Matheny et al., 2011; Vaisse et al., 1996). In contrast, the most pronounced effect of leptin on food intake was not observed during the first hour but during the first 24 hours. Third, in the study by Matheny and colleagues the HF diet contained 60 kcal% fat and the control diet was a standard laboratory chow that contained 17 kcal% fat. The HF diet in our study contained 45% fat and it cannot be ruled out that a higher fat content is required to induce leptin resistance in the VTA. Finally, in the present study leptin was administered into the VTA while Matheny and colleagues administered leptin into the third ventricle. The administration of leptin into the ventricles leads to the activation of leptin receptors in many different brain sites. It is most likely that the stimulation of leptin receptors in many different brain sites has a different effect on food intake and STAT3 phosphorylation than the activation of leptin receptors in one specific brain site.

In an additional analysis, we investigated the effects of intra-VTA leptin on food intake in the HF-fed animals that gained the largest (DIO) and least (DR) amount of weight over the 16-week HF period. The highest dose of leptin, 500 ng/side, induced a similar decrease in food intake in the HF and LF-fed animals during the 0–24 h period, but induced a greater decrease in food intake in the DR rats during the 24–48 h period. The increased effect of leptin on food intake in the DR rats was reflected in an increased effect of leptin on body weight gain during the 48–72 h period. During this period, body weight gain was decreased in DR rats compared to DIO rats. Taken together, these findings suggest that the DR rats are more sensitive to the effects of leptin on food intake and body weight gain than the DIO rats. It might be possible that the heightened leptin sensitivity in the DR rats plays a role in the relatively low intake of the HF food and the diminished weight gain during the 16-week period that the rats were fed the HF diet. The present finding would also suggest that an increased sensitivity to leptin could be a protective factor against overeating and obesity. It is interesting to note that a recent study showed that voluntary wheel running increases leptin sensitivity in the VTA and decreases the consumption of a HF food in rats (Scarpace et al., 2010). It was suggested that the heightened sensitivity to leptin in the VTA led to the decrease in the consumption of the HF food. This observation is in line with the present study, in which the DR rats were more sensitive to the effects of leptin in the VTA than the DIO rats.

The present study showed that DR rats are more sensitive to the anorexic effects of intra-VTA leptin than DIO rats but the underlying molecular mechanisms were not investigated. Studies by other research groups provide insight into the signaling molecules that may play a role in the decreased sensitivity to leptin in the DIO rats. Leptin mediates its effect on food intake by activating the leptin receptor which leads to the activation of the Janus kinase 2 (JAK2)/STAT3 pathway (Vaisse et al., 1996; Ziemiecki et al., 1994). One of the final steps in this pathway is the translocation of STAT3 into the nucleus where it leads to the

transcription of pro-opiomelanocortin (POMC) (Bates et al., 2003). The enzymatic cleavage of POMC leads to the formation of α -melanocyte stimulating hormone (α -MSH) which inhibits food intake by stimulating melanocortin-4 receptors (Adan et al., 2006; Bruijnzeel, 2009). The JAK2/STAT3 pathway is negatively regulated by protein tyrosine phosphatase 1B (PTP1B) and suppressor of cytokine signaling 3 (SOCS3) (Cheng et al., 2002; Mori et al., 2004). Experimental evidence indicates that SOCS3 inhibits this pathway by binding to the leptin receptor and PTP1B inhibits this pathway by dephosphorylating JAK2 (Cheng et al., 2002; Dunn et al., 2005). It might be possible that the DIO and DR rats have different levels of these signaling proteins. Although leptin levels were not measured in the present study, previous studies have reported that HF-fed DIO rats have higher plasma leptin levels than HF-fed DR rats (Levin and Dunn-Meynell, 2002; Levin and Keesey, 1998; Otukonyong et al., 2005). It might be possible that elevated leptin levels lead to changes in the signaling pathways in the VTA.

Another possible explanation for the difference in leptin sensitivity in the VTA between the DR and DIO rats might be a difference in leptin receptor levels. Leptin mediates its effects on food intake via the activation of the long form of the leptin receptor (Chua, Jr. et al., 1996; Tartaglia, 1997; Tartaglia et al., 1995). Previous research has shown that DIO rats are less sensitive to the anorexic effects of systemically administered leptin than DR rats and have lower levels of leptin receptor (long form) mRNA in the Arc (Levin et al., 2004). In addition, DIO rats have decreased ^{125}I -leptin binding in the Arc compared to DR rats (Irani et al., 2007). In contrast, ^{125}I -leptin binding is the same in the VTA of DIO and DR rats before and after long-term (7 weeks) exposure to a high energy diet (Irani et al., 2007). Therefore, it is unlikely that the differences in sensitivity to intra-VTA leptin between DIO and DR as reported in the present study are due to differences in leptin receptor levels in the VTA.

In conclusion, the present study suggests that leptin sensitivity in the VTA is the same in LF-fed and HF-fed rats. However, the HF-fed DIO rats were less sensitive to the effects of intra-VTA leptin than the HF-fed DR rats. This observation suggests that heightened leptin sensitivity in the VTA may protect against overeating and excessive weight gain in HF-fed animals.

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Highlights

- Decreased leptin sensitivity in the VTA of high-fat-fed obese rats
- Long-term exposure to a high-fat diet does not affect leptin sensitivity in the VTA
- Rats decrease food intake to compensate for an increased energy density of the diet

Fig. 1.

Histological overview of bilateral injection sites in the VTA. (A) Leptin was administered within or at the boundaries of the VTA (−5.20 to −5.60 anterior-posterior). The injection sites were evenly distributed over the levels depicted in this figure. The figures are copies from the Paxinos and Watson brain atlas (Paxinos and Watson, 1998). (B) The photomicrograph (1.5X objective) depicts a representative cresyl violet-stained brain section at the level of the VTA. The photomicrograph shows a cannula tract and injector tract that ends within the VTA. (C) The image depicts the spread of Cy3-leptin in the VTA (5X objective) and the insert depicts two Cy3-positive cells (40X objective). The scale bars at the bottom of the images correspond to 1000 μm (B), 200 μm (C), or 10 μm (C-insert). Abbreviations: PAG, periaqueductal gray; CC, corpus callosum; VTA, ventral tegmental area.

Fig. 2.

Effect of a high-fat diet and low-fat high-carbohydrate diet on body weight gain and food intake. (A) The rats in the HF ($n = 26$) and LF ($n = 15$) group gained the same amount of weight. The rats in the LF group consumed a larger amount of food by weight than the HF animals (B) and they consumed the same amount of calories (C). Asterisks (** $P < 0.01$) indicate a higher food intake by weight in the LF animals than in the HF animals. Data are expressed as means $\pm$ SEM. Abbreviations: HF, high-fat diet; LF, low-fat high-carbohydrate diet.

Fig. 3.

Effect of leptin in the VTA on food intake in rats after long-term exposure to a high-fat or low-fat high-carbohydrate diet. Food intake 0–1 h, 0–4 h (A), 0–24 h, 24–48 h, 48–72 h (B) after leptin infusion in the HF (n = 26) and LF (n = 15) group. Asterisks (* P<0.05, ** P<0.01) indicate a decrease in food intake compared to the corresponding vehicle group (0 ng of leptin). Pound signs (# P<0.05, ## P<0.01) indicate a lower (A) or higher (B) food intake in the LF group than in the HF group (LF vehicle vs. HF vehicle). Plus signs (++ P<0.01) indicate a decrease in food intake compared to the group treated with 150 ng of leptin. Leptin was infused bilaterally and the dose is expressed as the unilateral dose. Data

are expressed as means $\pm$ SEM. Abbreviations: HF, high-fat diet; LF, low-fat high-carbohydrate diet.

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Fig. 4.

Effect of leptin in the VTA on body weights in rats after long-term exposure to a high-fat or low-fat high-carbohydrate diet. Changes in body weights 0–24 h (A) and 24–48 h (B) after the infusion of leptin into the VTA in the HF ($n = 26$) and LF ($n = 15$) animals. Asterisks (* $P < 0.05$, ** $P < 0.01$) indicate a decrease in body weights compared to the vehicle group (0 ng of leptin). Leptin was infused bilaterally and the dose is expressed as the unilateral dose. Data are expressed as means $\pm$ SEM. Abbreviations: HF, high-fat diet; LF, low-fat high-carbohydrate diet.

Fig. 5. Body weight gain and food intake in diet-induced obese rats and diet-resistant rats. (A) Weight gain in DIO rats ($n = 6$) and DR rats ($n = 6$) and (B) food intake in the same rats. Asterisks (* $P < 0.05$, ** $P < 0.01$) indicate greater weight gain or higher food intake in the DIO rats than in the DR rats. Data are expressed as means $\pm$ SEM. Abbreviations: HF-DIO, high-fat diet-induced obese; HF-DR, high-fat diet-resistant.

Fig. 6.

Effect of leptin in the VTA on food intake and body weight gain in diet-induced obese and diet-resistant rats. Effects of 150 ng (A) and 500 ng (B) of leptin on food intake and 150 and 500 ng of leptin on body weights (C). Leptin was infused bilaterally and the dose is expressed as the unilateral dose. DIO rats $n = 6$ and DR rats $n = 6$. In figure 6B, asterisks (** $P < 0.01$) indicate that leptin induced a greater decrease in food intake in DR rats than in DIO rats. In figure 6C, asterisks (* $P < 0.05$, ** $P < 0.01$) indicate decreased weight gain (24–48 h) or increased weight gain (48–72 h) compared to corresponding control rats treated with vehicle. The plus sign (+ $P < 0.05$) indicates decreased body weight gain in the DR rats compared to the DIO rats treated with the same dose of leptin (48–72 h). Data are expressed as means $\pm$ SEM. Abbreviations: HF-DIO, high-fat diet-induced obese; HF-DR, high-fat diet-resistant.

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Table 1

Effect of leptin in the VTA on energy intake (kcal) in rats after long-term exposure to a high-fat or low-fat high-carbohydrate diet.

Dose	HF			LF			P-values
	Vehicle	150 ng	500 ng	Vehicle	150 ng	500 ng	
0-1 h	16.7 ± 1.4	10.5 ± 1.0**	11.5 ± 1.0**	9.8 ± 0.6##	11.3 ± 1.2	10.1 ± 0.9	P<0.05
0-4 h	42.2 ± 2.0	26.8 ± 1.0**	30.1 ± 1.4**	33.6 ± 1.3#	27.4 ± 1.8*	31.2 ± 1.1	P<0.0001
0-24 h	91.6 ± 3.2	76.8 ± 2.2**	71.1 ± 2.1**	85.9 ± 3.0	78.4 ± 2.3	78.7 ± 3.4*	P<0.0001
24-48 h	82.9 ± 2.3	77.4 ± 2.6	66.9 ± 2.2++**	80.7 ± 2.7	79.2 ± 2.5	72.0 ± 2.6*	P<0.0001
48-72 h	79.3 ± 2.5	82.7 ± 2.1	75.1 ± 3.0++	81.4 ± 3.0	81.1 ± 3.4	78.1 ± 1.9	P<0.05

Data are expressed as means ± SEM. HF-fed group (n = 26) and LF-fed group (n = 15).

Asterisks (* P<0.05, ** P<0.01) indicate a lower energy intake compared to the corresponding vehicle group (0 ng of leptin).

Pound signs (# P<0.05, ## P<0.01) indicate lower energy intake in the LF-fed group than in the HF-fed vehicle vs. HF-fed vehicle).

Plus sign (++) indicate a decrease in energy intake compared to the corresponding group treated with 150 ng of leptin. Leptin is infused bilaterally and the dose is expressed as the unilateral dose. P-values (ANOVA-analysis) indicate a leptin-induced decrease in energy intake.

Abbreviations: HF, high-fat diet; LF, low-fat high-carbohydrate diet.