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Onset of leptin resistance shows temporal differences related to dose or pulsed treatment

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ABSTRACT

Leptin administration results in leptin resistance presenting a significant barrier to therapeutic use of leptin. Consequently, we examined two hypotheses. The first examined the relationship between leptin dose and development of physiological and biochemical signs of leptin resistance. We hypothesized lower doses of leptin would produce proportional reductions in body weight without the adverse leptin-induced leptin resistance. The second compared pulsed central leptin infusion to continuous leptin infusion. We hypothesized that pulsed infusion at specific times of the day would evoke favorable body weight reductions while tempering the development of leptin-induced leptin resistance. The first experiment examined leptin responsiveness, including food intake, body weight and hypothalamic STAT3 phosphorylation to increasing doses of viral gene delivery of leptin. Varying the dose proved inconsequential with respect to long-term therapy and demonstrated proportional development of leptin resistance. The second experiment examined leptin responsiveness to pulsed central leptin infusion, comparing pulsed versus constant infusion of 3 μg/day leptin or a 2 h morning versus a 2 h evening pulsed leptin infusion. Pulsed delivery of the supramaximal dose of 3 μg/day was not different than constant delivery. Morning pulsed infusion of the submaximal dose of 0.25 μg reduces food intake only over subsequent immediate meal period and was associated with body weight reductions, but results in cellular leptin resistance. Evening pulsed infusion did not decrease food intake but reduces body weight and maintains full leptin signaling. The positive benefit for pulsed delivery remains speculative, yet potentially may provide an alternative mode of leptin therapy.

1. Introduction

Leptin, product of the *ob* gene, is an adipocyte derived hormone exhibiting highly conserved homology between species (Zhang et al., 1994). The role of leptin in physiology is multi-faceted and has been expertly reviewed in several articles to mark the 20th anniversary of its discovery (Münzberg and Morrison, 2015; Park and Ahima, 2015; Sáinz et al., 2015); in brief, leptin is important in regulation of energy balance, reproduction, immune function, and bone metabolism among others (Mantzoros et al., 2011). Regulation of body weight through controlling food intake and energy expenditure occurs via binding of leptin to the long form of the leptin receptor (Fei et al., 1997). Expression of leptin receptors within the central nervous system (CNS) are highest in the hypothalamus (Hypo), (Elmquist et al., 1998; Scott et al., 2009) however other CNS nuclei express leptin receptors including hindbrain nuclei (Gautron and Elmquist, 2011). From the early evidence of leptin administration in murine models, there was a great hope in using leptin as a therapy to treat obesity in humans. However leptin therapy was shown to be ineffective at weight reduction in various randomized placebo controlled clinical trials (Heymsfield et al., 1999; Zelissen et al., 2005). Though leptin may not be an effective monotherapy for obesity, recently numerous studies have evaluated leptin in the treatment of diabetes type II (Mittendorfer et al., 2011; Moon et al., 2011), lipodystrophy (Chong et al., 2010; Diker-Cohen et al., 2015) and hypothalamic amenorrhea (Chou et al., 2011; Sienkiewicz et al., 2011) with promising

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results in selective outcome measures particularly in conditions where basal levels of leptin are low (Coppari and Bjorbaek, 2012). Additionally, efforts into sensitizing the effects of leptin have been undertaken (Roth et al., 2008), presumably such a strategy would enhance the effectiveness of leptin therapy where currently a leptin resistant state is present. Currently, there are numerous registered clinical trials ongoing investigating leptin or leptin in combination with complimentary therapeutic agents (Polyzos and Mantzoros, 2015). The continued investigation into leptin as a clinical treatment warrants a further understanding of the development of leptin resistance.

Levels of leptin are higher in obese rodents and humans and related to the observed adiposity (Maffei et al., 1995) thus common obesity exhibits a pattern of leptin resistance. The Fischer 344 x Brown Norway (F344BN) rat is a good model to study the effects of long-term leptin delivery due to the relative stability of body weight and adiposity as an adult (Altun et al., 2007), thus avoiding any potential development of leptin resistance in control animals over the course of lengthy experiments. Delivery of recombinant adeno-associated viral leptin (rAAV-leptin) into the third ventricle of F344BN rats produces a rapid decrease in body weight and food intake. The effect of rAAV-leptin however is not maintained and over time; there is an increase in weight gain despite continuous elevated central leptin levels (Matheny et al., 2011; Scarpace et al., 2003). In rats of the same strain following three months of high fat feeding, treatment with AAV-leptin produced no change in body mass or food intake compared to GFP treated controls (Wilsey et al., 2003). The high fat feeding elevates endogenous leptin thereby inducing leptin resistance (Knight et al., 2010), thus rendering the rats insensitive to rAAV-leptin gene delivery (Scarpace and Zhang, 2009).

Interestingly, leptin resistance may be selective and produce differential effects on various brain areas (Mark, 2013; Matheny et al., 2011; Münzberg et al., 2004). The ability of leptin administration to produce a leptin resistant state even in lean animals (Martin et al., 2000) generates a significant barrier to the use of leptin therapeutically, a condition termed leptin-induced leptin resistance. In light of these observations we set out to test two hypotheses. The first examined if there was a relationship between the levels of leptin and the development of both physiological and biochemical signs of resistance to leptin. We hypothesized that a lower dose of leptin would produce attenuated positive markers in relation to body weight and body composition but importantly not create the observed leptin-induced leptin resistance. The second compared pulsed leptin central infusion to continuous leptin infusion. We hypothesized that pulsed infusion at specific times of the day would evoke favorable body weight reductions while tempering the development of leptin-induced leptin resistance.

2. Materials and methods

2.1. Experimental animals

Six-month-old male F344BN rats were obtained from National Institute of Aging Colony. Animals were cared for in accordance with the principles of the Guide to the Care and Use of Experimental Animals and protocols were approved by the University of Florida Institutional Animal Care and Use Committee. Rats were singly-housed with a 12:12 h light-dark cycle (07:00–12:00 h) and maintained on chow (Diet 7912, 3.1 kcal/g; 17% kcal from fat, 25% kcal protein, Harlan Teklad; Madison, WI).

2.2. Experimental design

This study consists of two experiments; the first examining leptin responsiveness to increasing doses of viral gene delivery of leptin and the second examining leptin responsiveness to pulsed leptin icv infusion.

In the first experiment, rats were assigned to four treatment groups: Low-Dose Leptin (LD), Mid-Dose Leptin (MD), High-Dose Leptin (HD) or GFP-Control (Control) containing 10–12 animals per group. Rats were administered recombinant adeno-associated virus encoding either green fluorescent protein, or leptin by injection into the 3rd ventricle. Rats were provided *ad libitum* access to food and water, and food consumption and body weight were recorded daily. Body composition of animals was measured in 15 day intervals. Respiratory measures were conducted at experimental day 55. Half of rats were killed at day 60 for endpoint analysis while remaining rats continued to be monitored for long-term body weight effects for 4 additional months. Rats that were excluded from analysis displayed post-surgical complications.

The second experiment consisted of two parts, each with 6–7 rats per group. In the first, the amount of leptin delivered (3 μg) over a single day was held constant and the time of delivery was varied; either constant over the 24 h period (0.125 μg/h) or pulsed over a 4 h period (0.75 μg/h) prior to the dark cycle. The third group was the control in which ACSF was infused. Body weight and food were recorded daily except for days 7 and 8 in which food was recorded separately during the light phase and the dark phase. In the second part of this experiment, leptin delivery was pulsed controlling both the amount (0.25 μg) and rate of delivery (0.125 μg/h), but varied the timing, with a 2 h delivery prior to the light phase compared to a 2 h delivery prior to the dark phase.

2.3. rAAV-vector administration

The pTR(2)ObW construct encoding leptin transgene under a chicken β-actin promoter linked to CMV enhancer was packaged into recombinant adeno-associated virus (rAAV) serotype 1 using previously describe methods (Zolotukhin et al., 2002), with the exception that the AAV1 helper plasmid pKRAP1A was used. Dot blot titer of rAAV1-leptin was 5.86×10^{12} viral genomes (vg) per milliliter. UF11 rAAV1, encoding green fluorescent protein (GFP) was used for the control group. The rAAV1-GFP titer was calculated by dot blot at 5.90×10^{12} vg/ml. Stock rAAV1-leptin vector was used as the high-dose leptin treatment. 1:100 dilutions of rAAV1-leptin were made in artificial cerebrospinal fluid (aCSF) to obtain the MD and LD treatments. 3 μl of rAAV1 vector was delivered into the third ventricle of animals according to the following coordinates 1.3 mm anterior to Bregma, 0.0 mm from midline, depth of 9.6 mm ventral from surface of skull, at an angle of 20° (Paxinos and Watson, 2005). Coordinates were verified previously in separate rats using injection of bromothymol blue dye.

2.4. Central leptin infusion

Leptin infusion, either constant or programmable, was provided as follows. For constant delivery, osmotic minipumps (Durect Corporation) were implanted subcutaneously into the rat and leptin or vehicle was constantly infused into the lateral ventricle via an implanted cannula as described previously (Scarpace et al., 2007). Programmable or pulsatile leptin delivery was accomplished by using a subcutaneously implanted Imprecio programmable infusion pump (Durect Corporation) via a cannula implanted in the lateral ventricle. Infusion protocols consisting of flow rates, delivery and stop times were constructed using the Imprecio software and downloaded to the pump prior to implantation.