Long-term correction of type 1 and 2 diabetes by central leptin gene therapy independent of effects on appetite and energy expenditure

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ABSTRACT

Adipocyte-derived leptin is a hormone associated with the regulation of energy homeostasis, including glucose metabolism. Hyperleptinemia, induced by the consumption of energy-enriched diets, inhibits leptin transport across the blood–brain barrier, and thereby produces leptin insufficiency in the hypothalamus. As a result of sustained leptin insufficiency, the hypothalamic restraint on pancreatic insulin secretion is lost. Additionally, both glucose metabolism and energy expenditure are also diminished, and both type 1 and type 2 diabetes are induced. A replication-deficient recombinant adeno-associated virus vector engineered to encode the leptin gene (rAVV-LEP) has been used in models of diabetes as a novel therapeutic approach. After rAVV-LEP injection in ob/ob mice, hypothalamic leptin expression was increased, body weight was suppressed, and hyperinsulinemia was ameliorated. Additionally injection of rAVV-LEP into the hypothalamus suppressed the expression of orexigenic neuropeptide Y (NPY) and enhanced anorexigenic pro-opiomelanocortin (POMC) in the arcuate nucleus (ARC) in rats. It is proposed that central leptin gene therapy should be tested clinically to reduce the worldwide epidemic of obesity, diabetes, and shortened life span. In this article, the information has been assembled from published review articles on this topic.

Key words: Central leptin gene therapy, leptin insufficiency in the hypothalamus, type 1 and 2 diabetes

INTRODUCTION

Leptin, a 167 amino acid peptide, is the prototypical adipose tissue-derived hormone related to the regulation of energy homeostasis.[1] In the 1970s, Coleman postulated that the relentless hyperphagia and morbid obesity observed in obese ob/ob mice was due to the absence of a circulating satiety hormone from adipose tissue.[2] Circulating leptin levels increase in proportion to an increase in fat mass in the body.[3] Leptin exerts pleiotropic effects by binding to and activating its receptors in the hypothalamus and other tissues.[3] It is well documented that hyperleptinemia, caused by increased fat mass, disturbs various physiological functions, such as blood pressure, renal function, angiogenesis, wound healing, immune function, bone formation, and glucose–pancreatic insulin homeostasis.[3]

Leptin, a regulatory signal in glucose homeostasis, can directly suppress insulin release from β-cells and acts independently of peripheral and central targets that express the biologically active long form of the leptin receptor Ob-Rb.[4] A peripheral adipo-insular feedback loop was hypothesized to strictly regulate leptin and insulin secretion because leptin can suppress insulin release from β-cells. However, in vivo evidence has revealed an alternative route through which leptin can act on central targets, not involving a peripheral mechanism, to suppress insulin secretion from β-cells. It was later shown that the intraventricular infusion of leptin suppressed blood insulin levels and increased receptor sensitivity.[4]

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LEPTIN INSUFFICIENCY IN THE HYPOTHALAMUS

Hyperleptinemia, which is caused by increased adipose tissue, inhibits leptin transport across the blood–brain barrier (BBB), thereby inducing leptin insufficiency in the hypothalamus[6] [Figure 1]. Because of sustained leptin insufficiency, the hypothalamic restraint on pancreatic insulin secretion is lost, and both glucose metabolism and energy expenditure are diminished. By elucidating the mechanism of leptin insufficiency syndrome, a role for leptin in the hypothalamus has been revealed; leptin restrains rhythmic pancreatic insulin secretion while enhancing glucose metabolism and non-shivering thermogenic energy expenditure.[2]

Two mechanisms are hypothesized to reduce leptin transport across the BBB, where this is associated with hyperleptinemia.[6] The first mechanism is that target receptors become less responsive due to abnormal increases in rhythmic hormonal signaling. It is postulated that leptin receptors in the cerebral microvasculature are downregulated by increased pulsatile leptin secretion. The second line of defense is modulating leptin before it is transported across the BBB. Both the increased binding of leptin to C-reactive protein (CRP) and the modulation of blood-to-brain transport by metabolic variables in the peripheral circulation are included.

Leptin replacement significantly improves hyperinsulinemia and type 2 diabetes without affecting food intake and weight.[5] Because leptin replacement centrally abolished hyperinsulinemia in obese rodents, insulin hypersecretion by relieving the restraint on β-cells was confirmed to be a necessary response for enhancing the conversion of excess energy into fat.

ROLE OF LEPTIN AS A REGULATOR OF APPETITE AND ENERGY EXPENDITURE

Leptin is a hormone that is related to the regulation of energy homeostasis, including glucose metabolism. In ob/ob mice, leptin-deficient mice, relentless hyperphagia, and morbid obesity are presented. Neuropeptide Y (NPY), the most potent appetite transducer, regulates appetite in the arcuate nucleus–paraventricular nucleus (ARC–PVN) of the hypothalamus.[7] NPY, Agouti-related peptide (AgRP), gamma-amino butyric acid (GABA), and alpha-1 receptors simultaneously repress anorexigenic melanocortin signaling in the ARC–PVN axis. Many afferent hormonal signals from the periphery can modulate NPYergic signaling. Primarily, anorexigenic leptin from adipocytes inhibits NPY expression, and orexigenic ghrelin from the stomach stimulates NPY expression. Leptin also inhibits the orexigenic effects of gastric ghrelin. Leptin directly decreases ghrelin release from oxyntic cells of the stomach and antagonizes the ghrelin-induced stimulation of NPY release in the ARC–PVN axis.[3]

In the hypothalamus, leptin increases the expression and release of two anorectic neuropeptides, pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART), while suppressing the synthesis and release of two orexigenic neuropeptides, NPY and AgRP.[8] Through protease cleavage, POMC produces a number of products, including alpha-melanocyte stimulating hormone (a-MSH). AgRP and a-MSH are the antagonist and agonist, respectively, of the melanocortin 4 receptor (MC4R). AgRP suppresses MC4R, increases feeding, and diminishes the response to leptin. However, a-MSH activates MC4R, reduces food intake, and increases energy expenditure.

A decrease in NPY or an increase in POMC by leptin in the hypothalamus not only suppresses food intake but also enhances sympathetic outflow to brown adipose tissue (BAT).[8] Leptin-induced stimulation in energy expenditure is hypothesized to be due to increased uncoupled respiration (thermogenesis) in BAT, which is indicated by markedly elevated uncoupling protein-1 (UCP-1) mRNA and protein levels. In general, sympathetic nervous system (SNS) outflow to BAT is inhibited by NPY.[9] Thus, the decrease in hypothalamic NPY induced by leptin may increase energy expenditure via enhanced sympathetic outflow to BAT.
To understand the molecular basis of leptin resistance, it is important to define the signaling cascades that mediate its effects in POMC and AgRP neurons. One of the hypothalamic signaling pathways mediated by the leptin receptor is the Janus tyrosine kinase 2 (JAK2)/signal transducer and activator of transcription protein 3 (STAT3) pathway. Briefly, when leptin binds to OB-Rb, tyrosine phosphorylation of the receptor is catalyzed by JAK2. When the leptin receptor is phosphorylated, cytosolic STAT3 is activated through subsequent phosphorylation by JAK2 and recruited. STAT3 binding to the POMC promoter increases POMC mRNA expression through the recruitment of histone acetylases. However, the expression of STAT3 in AgRP neurons decreases AgRP (and possibly NPY) expression by recruiting histone deacetylases. Leptin resistance in obese rodents is hypothesized to be due to impaired intracellular signaling downstream of leptin-Rb activation in the interactive appetite-regulating network (ARN) targets. However, further experiments showed that leptin inhibited food intake independent of STAT3 signaling and that varying degrees of hyperleptinemia were not associated with altered STAT3 signaling. Thus, it remains uncertain whether suppressed intracellular signaling downstream of leptin-Rb activation in ARN targets is a necessary component of leptin resistance in obese rodents. In addition to the JAK2/STAT3 pathway, there are other leptin-sensitive signaling pathways, such as phosphatidylinositol 3-kinase (PI3K) in POMC cells. PI3K, when activated, phosphorylates the membrane lipid phosphatidylinositol-4,5-bisphosphate (PIP2) to phosphatidylinositol-3,4,5-trisphosphate (PIP3). The accumulation of PIP3 recruits several kinases to the plasma membrane that induce the signaling cascade. PI3K-mediated phosphorylation of FOXO1 and the exclusion of FOXO1 in the nucleus cooperatively increase POMC mRNA expression. Leptin also induces PI3K activation in NPY/AgRP neurons, and stimulated PI3K signaling appears to decrease NPY and AgRP mRNA expression, although leptin can activate PI3K signaling not in AgRP/NPY neurons themselves, but through synaptic transmission.

**Leptin Gene Therapy as a New Therapeutic Approach to Diabetes**

New therapies that would enhance leptin transport across the BBB or reinstate leptin restraint on NPY signaling are expected for the treatment of type 1 and 2 diabetes. Central leptin gene therapy or pharmacological leptin mimetics are likely to improve the pathophysiological sequelae of diabetes and related ailments of metabolic syndrome. Replication-deficient recombinant adeno-associated virus (rAVV) is non-immunogenic and non-pathogenic. It can infect both dividing and non-dividing cells, and after integration into the host genome, it evokes transgenic expression for the lifetime of the cell. As a newer therapy for obesity and metabolic disorders, the rAVV vector engineered to encode the leptin gene (rAVV-LEP) has been used to validate gene therapy. In contrast to the intrahypothalamic injection of leptin, the systemic injection of rAVV-LEP may not be an appropriate strategy because ectopic leptin production is enhanced in peripheral tissues (skeletal muscle or liver) and the pleiotropic effects of leptin are exerted. For example, leptin influences immune functions, bone formation, and angiogenesis, and exerts effects on non-adipose tissues such as the kidney and pancreas. Furthermore, lipotoxicity is induced by the sustained effects of hyperleptinemia in non-adipocyte tissues. However, when a leptin gene was transferred into the hypothalamus or into selected hypothalamic sites, such as the medial preoptic area (MPOA), paraventricular hypothalamus (PVN), ventromedial hypothalamus (VMH), or ARC, ultradian insulin secretion from the pancreas was suppressed. It has also been suggested that after the intrahypothalamic injection of leptin, glucose uptake by some peripheral tissues is increased through the activation of the VMH-sympathetic nervous system.

**Effects of Central Leptin Gene Therapy on Appetite and Energy Expenditure**

Crosstalk between the hypothalamic NPY network and leptin is important for energy homeostasis. Enhanced NPY induces hyperphagia and decreases energy expenditure, obesity, dyslipidemia, glucose intolerance, insulin resistance, and hyperinsulinemia, which are all risk factors for type 2 diabetes. Repressing the NPY system with a stable increase in leptin availability in the hypothalamus inhibits age-related and high-fat-diet (HFD)-induced obesity, hyperinsulinemia, and diabetes. Body fat depletion drastically decreases adipocyte hormones, including leptin and adiponectin, as well as tumor necrosis factor-α (TNF-α), free fatty acids (FFAs), and pancreatic insulin.

After either intracerebroventricular injection or microinjection of rAVV-LEP into the ARC, VMN, or PVN of rats, the expression of the gene encoding NPY is attenuated, whereas the expression of the gene encoding POMC is enhanced in the ARC; however, expression of the gene encoding AgRP in the ARC is unaffected. Because NPY and AgRP are coexpressed in ARC neurons, it is hypothesized that leptin injected in the hypothalamus acts...
selectively. It has also been shown that central POMC gene therapy overcomes leptin resistance, decreases weight, and improves insulin sensitivity in obese Zucker and aged rats.\textsuperscript{[8]} Thus, the central melanocortin system may be a useful drug target for combating obesity.

Injection of rAVV-LEP also affects BAT and increases energy expenditure. A low dose of rAVV-LEP moderately suppressed weight gain in rodents without decreasing daily food consumption, but increased energy expenditure, which was reflected by enhanced UCP-1 mRNA in BAT.\textsuperscript{[16]} Furthermore, whereas microinjection of rAVV-LEP in the ARC, VMN, and PVN of rats decreased food intake, weight, and adiposity, and enhanced thermogenic energy expenditure, a similar injection in the MPOA did not diminish daily consumption, but increased thermogenic energy expenditure.\textsuperscript{[16]} This result may be explained by leptin expression in the MPOA being ineffective for decreasing NPY signaling, which affects appetite behavior.

**Amelioration of Type 1 Diabetes by a Stable Leptin Supply**

Leptin insufficiency in the hypothalamus produced by either leptinopenia or hyperleptinemia is related to the pathogenesis of diabetes.\textsuperscript{[5]} Increasing leptin levels in the hypothalamus by either leptin injection or leptin gene therapy normalizes glucose levels in the presence of insulin in wild-type rodents.\textsuperscript{[20]} Increasing leptin levels in the hypothalamus also induces euglycemia in the absence of circulating insulin, as shown in Akita mice and wild-type rodents pretreated with streptozotocin (STZ) to produce insulinitis.\textsuperscript{[20]} Centrally infused leptin does not control serum glucose by regulating the food intake or peripheral insulin levels in STZ-induced diabetic rats; it does so by regulating hepatic glucose production, peripheral glucose uptake, and energy expenditure in these rats.\textsuperscript{[21]} Through innervations from the hypothalamus, leptin-induced outflow is transmitted to upregulate glucose metabolism in the pancreas, liver, skeletal muscle, white adipose tissue (WAT), and BAT.\textsuperscript{[9]} For glucose homeostasis, optimal leptin signaling in the hypothalamus, which is independent of insulin secretion, is necessary.

It has been demonstrated that injection of rAVV-LEP increases leptin levels in the hypothalamus and prevents early mortality in the STZ-pretreated mice.\textsuperscript{[20]} Increased central leptin levels also gradually ameliorated hyperphagia to normalize intake by week 20 and maintained body weight at significantly lower values than the control range in the STZ-pretreated mice.\textsuperscript{[20]} The blood glucose levels in these mice began to decrease significantly by weeks 2–3 and were normalized by week 8, and euglycemia persisted during the remaining course of the experiment. These results show that central leptin gene therapy is effective for normalizing glucose levels for extended periods in the absence of insulin.

**Amelioration of Type 2 Diabetes by a Stable Leptin Supply**

Leptin gene transfer by rAVV is effective for hyperinsulinemia and hyperglycemia.\textsuperscript{[9]} From the various paradigms, it has been demonstrated that leptin in the hypothalamus suppresses episodic insulin secretion and inhibits hyperinsulinemia in diet-induced obese rodents and leptin-deficient obese ob/ob mice.\textsuperscript{[20]} Increased hypothalamic leptin expression by rAVV-LEP injection in ob/ob mice suppressed body weight and adiposity and led to voluntarily decreased dark-phase food intake.\textsuperscript{[22]} Increasing hypothalamic leptin expression through rAVV-LEP also suppressed plasma levels of adiponectin, TNF-α, free fatty acids, and insulin, normalizing glucose levels in ob/ob mice.\textsuperscript{[22]} There is also a report that leptin transgene expression in the hypothalamus induced the apoptosis of adipocytes in rats.\textsuperscript{[16]} In wild-type mice, after injection with rAVV-LEP, the body weight and plasma levels of leptin and metabolic variables were suppressed to a lesser extent without decreasing food intake.\textsuperscript{[22]} Additionally, intracerebroventricular injection of rAVV-LEP in adult obese ob/ob mice displayed more than double the life span compared with control cohorts.\textsuperscript{[23]} These life-extending benefits were related to a drastic reduction in visceral fat, blood glucose, insulin levels, and anti-aging biomarkers. It was also reported that in wild-type and ob/ob mice, a single intracerebroventricular rAVV-LEP injection sustained the effects of suppressing food intake and body weight for the lifetime of the mice.\textsuperscript{[16]} Furthermore, the persistent efficacy of central leptin gene therapy in suppressing weight gain was also shown through all phases of reproduction, lactation, and post-lactation in dams.\textsuperscript{[24]}

In addition to ob/ob mice, an increase in hypothalamic leptin in extremely hyperglycemic HFD-consuming rodents reduced blood glucose levels and maintained euglycemia.\textsuperscript{[1]} Increased central leptin levels induce euglycemia by accelerating glucose metabolism in BAT, liver, skeletal muscles, and adipose tissue, independent of insulin involvement. Central leptin gene therapy in rats consuming an HFD also affected food intake, body weight, and energy expenditure. rAVV-LEP treatment in rats consuming an HFD led to reduced food intake and prevented the HFD-induced increase in weight and adiposity.\textsuperscript{[25]} UCP-1 mRNA expression in BAT was augmented by treatment with
rAVV-LEP in rats consuming an HFD, indicating increased thermogenic energy expenditure. By decreasing energy intake and increasing thermogenic energy expenditure, central leptin gene therapy efficiently blocked weight gain and increased adiposity and hyperinsulinemia in rats consuming an HFD. It has been reported that leptin transgene expression in the PVN normalizes weight and rapidly depletes adipose tissue in obese hyperleptinemic rats that continuously consumed an HFD. Thus, the leptin supply in the PVN alone can reverse dietary obesity.

In Koletsky rats with a leptin receptor (OB-Rb) gene mutation, after adenovirally inducing the expression of leptin receptors in the ARC, peripheral insulin sensitivity was improved via suppression of hepatic glucose production without changing insulin-stimulated glucose uptake or disposal. Furthermore, OB-Rb expression in the ARC through rAVV reduced food intake, and OB-Rb expression in all sites except for PVN increased energy expenditure, which was assessed through enhanced UCP-1 mRNA expression.

CONCLUSIONS

These studies show that a sustained increase in leptin availability in the hypothalamus alone can induce euglycemia and suppress body weight and adiposity. The fact that rAVV vectors are nonpathogenic and nonimmunogenic and can be used for the long-term expression of therapeutic genes suggests that central leptin gene therapy is a viable therapeutic strategy to control weight and prevent adiposity-related metabolic disorders. rAVV is expected to treat a wide spectrum of diseases, and several clinical trials have been conducted. For example, in patients with hemophilia B, after peripheral vein infusion of the codon-optimized human factor IX transgene, FIX transgene expression levels became sufficient to improve the bleeding phenotype. Currently, a clinical trial is being conducted to evaluate the safety and tolerability of gene therapy with an adeno-associated virus (AAV) bearing the glutamic acid decarboxylase (GAD) gene for Parkinson’s disease. Sustaining optimal sufficiency in leptin signaling solely in the hypothalamus is considered to be a novel strategy to treat obesity and metabolic syndrome. To decrease worldwide epidemic of obesity and diabetes, clinical tests should be conducted on central leptin gene therapy or novel long-acting leptin mimetics to facilitate their widespread use.

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