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Review Article

Leptin agonists as a potential approach to the treatment of obesity

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Introduction

Obesity, a chronic and stigmatized disease in both children and adults, has reached epidemic proportions in industrialized and developing countries throughout the world (1-3). The basis underlying the spread of obesity, however, remains unclear. Heritability studies indicate that as much as 70% of the variability in human body weight may be related to genetic factors (4, 5). Although this contribution is significant, it is unlikely that changes in genetic background alone are responsible for the worldwide trend toward increased body weight. There is no doubt that lifestyles which have become less physically active, increasingly sedentary, and fueled by energy-dense diets, have enhanced the expression of a genetic background which may favor weight gain.

Obesity is defined as a significant increase (greater than 20%) in body weight above the ideal body weight, *i.e.*, a body weight which maximizes life expectancy (6). Actuarial tables have shown life expectancy to be reduced when body mass index (BMI), an indicator of adiposity (calculated as body mass in kilograms divided by the square of height in meters), is significantly increased above the ideal level (7, 8). The magnitude of the effect of obesity on life expectancy has been demonstrated by studies which show a BMI of 30 to be associated with a 1.5- to 2.0-fold increase in the risk of premature mortality when compared to a BMI of 20-25 (9). Obesity also increases morbidity and is associated with an elevated risk of diabetes mellitus, insulin resistance, dyslipidemia, hypertension, osteoarthritis, sleep apnea, cardiovascular disease and some cancers (10, 11).

Body weight is stabilized when a balance between energy intake and energy expenditure is achieved. A net excess in energy (positive energy balance), caused by either higher intake or lower expenditure, results in weight gain. In most individuals, however, only a small amount of positive energy balance occurs on a daily basis. Therefore, obesity is a slowly developing disease that results when energy intake exceeds energy expenditure for an extended period of time. Unfortunately, the weight loss required for its management is also achieved slowly.

It is clear that weight loss requires negative energy balance, *i.e.*, a state in which energy expenditure exceeds food intake. The etiology of obesity is multifactorial and arises from complex interactions among genetic, environmental and psychosocial factors. For this reason, it has proven to be very difficult, even with comprehensive multidisciplinary programs which combine diet, exercise and behavior modification, to sustain the negative energy balance required for obesity reduction. In recent years, much attention has been given to pharmacological intervention as an additional treatment option within this core therapy (12-14).

Regulation of body weight

As our understanding of the molecular mechanisms involved in the regulation of body weight increases, potential targets for pharmacological intervention in the treatment of obesity are being identified. Although most of these advances have come from studies utilizing spontaneous monogenic mutant or transgenic rodent models of obesity, the potential application of this information to the understanding and management of human obesity is becoming more evident (15, 16). Some of these efforts have, within a relatively short time, resulted in the identification of drugs, or drug targets, that are now in various phases of pharmaceutical development (13, 17-21).

Currently, the regulation of body weight is understood to involve a feedback system composed of four separate

elements: 1) afferent signals of peripheral and central origin; 2) a central controller in the brain; 3) neural and hormonal efferent signals; and 4) a controlled system that processes, absorbs and stores food energy (22). This model suggests a number of possible approaches for pharmacological intervention in the treatment of obesity and eating disorders. These include, but probably will not be limited to, the development of drugs that reduce food intake either by augmenting the suppressive effects of anorexigenic signals, or by suppressing the stimulatory effects of orexigenic signals; drugs that increase energy expenditure by stimulating thermogenesis; drugs that alter metabolism by either mobilizing fat or decreasing its synthesis; and drugs that affect nutrient partitioning, either at the preabsorptive or absorptive phase. Some of these pharmacological approaches have been tried, and their successes (and limitations) have been the subject of a number of recent reviews (12-14, 18).

The basis of obesity management is theoretically simple: achieving and sustaining energy balance. Because the underlying mechanisms regulating energy balance are much more complex in humans than in rodents, and not yet fully understood, reaching this goal in a climate where changing lifestyles make it even more elusive, is a significant challenge to healthcare professionals. Acknowledgement of the epidemic proportions of obesity, however, together with the unrealistic expectations of conventional approaches to its management, has generated an increasing awareness of the necessity to improve the quality and effectiveness of currently available treatments for this disease, and to explore new approaches.

Pharmacological approaches to obesity managemnt

Because of the significant health risks associated with obesity, the most important medical goal of weight loss is the reduction of comorbidities. There is now good evidence that a loss of 5-10% of body weight, if sustained, can result in a significant decrease in risk for cardiovascular disease (23), diabetes (24) and mortality (25) in obese individuals. Currently available therapies for the treatment of obesity, however, are at best palliative, that is, effective only while they are being used, and the ability to achieve long-term weight loss by behavior modification (diet and exercise) is limited. These realizations have resulted in intensified efforts to develop new pharmacological approaches to the treatment of obesity, most of which have arisen from what has been learned about the pathophysiology of obesity in rodent models. Because of the chronic nature of this disease, however, it is clear that the design of new antiobesity drugs must focus on formulations that: 1) are active when taken orally; 2) reduce subcutaneous and visceral body fat; 3) have few or no adverse side effects; 4) are inexpensive to produce; 5) have long half-lives; and 7) are nontoxic (26).

Although the effects of fenfluramine and phentermine, two Food and Drug Administration (FDA)-approved appetite suppressants, on weight loss were promising, their association with an increased incidence of valvular heart disease resulted in their withdrawal from clinical use in 1997 (27). This disappointing event has left only two drugs currently available for the long-term treatment of obesity in the United States. One of these, sibutramine, suppresses appetite by altering norepinephrine and 5-HT metabolism in the brain (28), and is the only FDA-approved drug that has been evaluated in long-term, prospective, randomized clinical trials (29). The other drug, orlistat, reduces fat absorption by inhibiting gastric, pancreatic and other gastrointestinal lipases (30). Data from four long-term clinical trials with orlistat have been published (31-34), and extensive information on its clinical effectiveness and side effects is available. The results of these studies, however, indicate that both of these drugs are of limited efficacy, and that the redundant physiological and molecular pathways that appropriately defend humans against negative energy balance will make it difficult, if not impossible, for any single pharmacological approach to manage obesity effectively.

Leptin: a potential approach to the management of human obesity

Leptin, the protein product of the *ob* gene, exerts its influence on food intake, energy expenditure, body weight and neuroendocrine function through actions on neuronal targets in the hypothalamus (35). The *ob* gene is expressed predominantly by white adipocytes, although leptin synthesis has also been demonstrated in the gastric epithelium and placental trophoblast (36, 37). Plasma leptin concentrations are positively correlated with body mass index, and are elevated in obesity (38) and decreased in anorexia nervosa (39). A similar correlation exists between serum leptin concentrations and *ob* mRNA levels in adipose tissue of obese individuals (40). In addition to its effects on energy balance, leptin has been shown to influence the regulation of FSH, LH, ACTH, cortisol and GH concentrations (41-43), to stimulate hematopoiesis (44), and to induce both proliferation of $CD4^+$ T-cells and cytokine biosynthesis (45). In humans, the *ob* gene is expressed almost exclusively in adipose tissue, and codes for a protein that is 84% homologous to mouse leptin (46).

Because leptin concentrations are high in the serum of most obese humans, but decrease with weight loss, human obesity has been characterized as a disease resulting from a state of leptin resistance (47). Furthermore, since obese humans have only modestly elevated cerebrospinal fluid levels of leptin, although their plasma concentrations may be fivefold higher than nonobese individuals, the rate-limiting factor contributing to leptin resistance in obese humans appears to be related to defective leptin transport into the CNS (48).

The *ob* gene is normal in most cases of human obesity (49). Frameshift mutations (50, 51) and polymorphism in the 5'-untranslated region of the human *ob* gene

(52), however, have been reported in rare cases of morbidly obese humans with low serum leptin concentrations. The possible linkage of extreme obesity to markers flanking the human *ob* gene has also been proposed (53, 54).

Theoretically, leptin resistance could result from mutations in the *db* gene (which codes for the leptin receptor OB-R), or in genes downstream of OB-R, such as genes for pro-opiomelanocortin (POMC) or the melanocortin 4 (MC4) receptor which have also been implicated in human obesity. In this regard, rare cases of morbidly obese individuals with mutations in the *db* gene have now been identified (55, 56), and mutations in the POMC gene (57) and MC4-R gene (58) have been demonstrated in a cohort of severely obese children. These recent findings suggest that administration of recombinant leptin, or leptin agonists or mimetics of even higher potency than leptin, may be possible approaches to the treatment of at least some forms of human obesity.

Leptin administration has been shown to induce weight loss in all mammalian species tested to date (59). Although leptin therapy is expected to benefit obese humans whose disease is associated with leptin deficiency, its usefulness in individuals with hyperleptinemia is not yet known. Therefore, it will be clinically important in the management of these individuals to determine their level of resistance to exogenously administered leptin. In cases where leptin resistance is not complete, it is possible that administration of high doses of leptin may be sufficient to increase leptin sensitivity and induce weight loss. If leptin resistance is complete, however, as would be the case for individuals carrying *db* gene mutations (55, 56), it is clear that neither high doses of leptin, nor of leptin agonists or mimetics which act through OB-R to achieve their effects on energy homeostasis, will be useful.

Leptin influences energy homeostasis through activation of OB-R-expressing neurons in specific nuclei of the hypothalamus. It is now known that the post-receptor effects of leptin are mediated principally through its modulation of the synthesis and release of a number of neurotransmitters involved in food intake and energy expenditure. In rodents, leptin administration inhibits the expression of neuropeptide Y (NPY) (60, 61), agouti-related protein (AGRP) (62) and corticotropin-releasing hormone (CRH) (63), all of which stimulate appetite. Conversely, leptin has been shown to increase the expression of POMC (61, 64), the precursor of α-melanocyte-stimulating hormone (α-MSH), and the peptide product of cocaine- and amphetamine-regulated transcript (CART) (65), which are appetite suppressants. Leptin also stimulates thermogenesis by increasing sympathetic outflow to brown and white adipose tissue (66, 67).

The relationship of leptin to other hypothalamic neuropeptides, which include orexin, neurotensin, melanin-concentrating hormone (MCH) and cholecystokinin (CCK), has recently come under investigation (68-73).

Clinical trials wth leptin

The effectiveness of leptin treatment in rodent models of obesity suggested its possible application to human obesity. The results of two clinical trials with recombinant human leptin have been published. The data from phase I and phase II studies which examined the safety of leptin therapy in 73 obese subjects indicated that daily subcutaneous (s.c.) administration of recombinant human leptin induced modest, dose-related weight loss in most but not all subjects, with injection-site erythema the only adverse side effect reported (74). Glycemic control during the course of treatment, however, was unchanged.

In a second study, s.c. administration of recombinant human leptin to a leptin-deficient child with severe early-onset obesity suppressed food intake and induced significant weight loss (75). An initial improvement in gonadotropin responsiveness, however, could not be sustained, and the anticipated reduction in circulating plasma insulin levels never occurred. Anti-leptin antibodies were also detected. Although promising, the results of these preliminary trials demonstrate a distinct difference in leptin responsiveness between rodents and humans, and emphasize the need for caution when attempting to extrapolate the results of animal studies to human applications.

Although the findings of these initial trials in humans have generated a number of clinical questions regarding appropriateness of dose, route of delivery, patient compliance and potential side effects associated with prolonged leptin administration, they do suggest that there may be a role for leptin, more than likely in combination with other therapies, in the management of at least some forms of human obesity. Whether these initial results will be confirmed in larger phase III and phase IV clinical trials evaluating the therapeutic efficacy of exogenous leptin administration to obese and diabetic human subjects remains to be determined.

Leptin agonists and obesity

New insights into the physiology and pathophysiology of energy homeostasis have resulted in the identification of a number of potential targets for pharmacological approaches to the regulation of body weight in humans. Most interest has focused on NPY (76), the uncoupling proteins (77), the melanocortin system (78) and leptin (79). These studies have indicated that, because of the complexity and redundancy of the systems regulating energy balance, successful therapies for obesity will probably impact on both energy intake and energy expenditure. As required for the effective treatment of hypertension, in many instances combination therapy involving two or more drugs with different mechanisms of action may be needed for the successful management and reduction of human obesity.

In spite of the limited success of leptin in early clinical trials, the potential usefulness of leptin-like drugs which

utilize the same or similar pathways to mimic leptin's effects on energy balance has not gone unnoticed. The development of leptin-related synthetic peptide agonists and nonpeptide mimetics, which will be orally active, independent of a saturable transport system to cross the blood-brain barrier, and of even greater potency than leptin, remains a viable strategy. It is possible that such drugs may have efficacy not only in the treatment of obesity that is characterized by leptin deficiency, but also be beneficial to the larger population of obese individuals who are leptin-resistant.

Positional cloning of the mouse *ob* gene and its human homolog, and elaboration of the amino acid sequences of the protein products of these genes (80), initiated rapid and intensive interest in the biochemistry of the leptin molecule. The methodologies of solid-phase peptide synthesis and molecular biology, proven and powerful tools which have been used to produce protein and peptide molecules in large quantities and to elucidate their structure-function characteristics, have now been applied to the study of leptin. These efforts have generated a number of potential candidates for pharmaceutical development.

Protein chemists have long known that functional epitopes of a protein ligand are often much smaller than structural epitopes, and that the full activity of a protein is the product of its active site(s) and the conformational constraints conferred upon this site by structural epitopes within the sequence (81, 82). Thus, the initial task in the development of a peptide analog of a biologically active protein is to identify the domain within its primary structure which contains the active sequence(s). A number of laboratories have used solid-phase peptide synthesis to address this question for the leptin molecule, and the results of some of these studies are contradictory.

The first reports in this area described the effects of peptide fragments corresponding to the entire amino acid sequence of secreted rat leptin on body weight and thermogenesis (83, 84). In these studies, pools of 5 peptides, each 15 amino acids in length and overlapping at the *N*-terminus by 5 amino acids, were given intraperitoneally (i.p.) for 2 days to adult male Wistar rats. Only one pool, which contained five peptides encompassing the domain between amino acids 127 and 167, had any significant effects on body weight and rectal temperature. From these observations, the authors concluded that the active domain of rat leptin is at the *C*-terminus, between amino acid residues 127 and 167.

It is unfortunate that the experimental design of at least one of these studies did not include individual administration of each peptide in the active pool, thus permitting a more restricted localization of leptin-like activity. Even with this limitation, however, these studies were significant in that they were among the first to show that the entire leptin molecule was not required for at least partial expression of its effects on body weight and thermogenesis. Of equal importance, they also indicated that peripherally administered peptides could induce weight loss in rodents, an event known to be mediated by leptin action in the hypothalamus.

In another early report, three peptide fragments corresponding to selected sequences in human leptin were administered for two days into the lateral cerebral ventricle (i.c.v.) of adult Sprague-Dawley rats (85). The peptides used in this study were selected on the basis of predicted biological cleavage sites. These peptides included a cyclized analog of a linear peptide corresponding to amino acid residues 116-167 [which encompassed the region of rat leptin previously identified as having leptin-like activity (83, 84)], a peptide corresponding to amino acid residues 57-92, and a peptide toward the *N*-terminus of leptin corresponding to amino acid residues 22-56. Significant, although transient, dose-related inhibition of food intake was noted only in response to the *N*-terminal peptide (22-56), but the short duration of the study precluded any evaluation of the effect of the active peptide on body weight gain. Based on these results, these investigators suggest that, in contrast to rat leptin, the activity of human leptin is at the *N*-terminus of the molecule, between amino acid residues 22 and 56.

Although not discussed in these papers, a number of explanations may be given for the divergent results of these two studies. Although the *C*-terminal domains of rat and human leptin between amino acid residues 127 and 167 are highly homologous (80% identity; Table I), the few differences within this region may be sufficient to modify the activity of the peptides such that only one, a peptide corresponding to this domain of rat leptin, adopts a conformation in solution that is compatible with receptor binding and activation. Assessment of the activity of a synthetic peptide corresponding to amino acids 127-167 of human leptin would be needed to confirm this hypothesis.

A similar consequence could result from cyclization of the linear peptide, or the presence of additional amino acids at the *N*-terminus. Such modifications could induce the conversion of an active conformation (that corresponding to the domain between amino acid residues 127 and 167 of rat leptin) into an inactive conformation (that corresponding to the domain between amino acid residues 116 and 167 of human leptin). These observations emphasize the importance of conformational constraints to the stereospecificity of peptides in solution, a factor which contributes greatly to the challenges associated with the design and development of receptor agonists or antagonists.

The disparity in the outcome of these studies may also be related to the different routes of peptide administration used by the investigators. The possibility that intravascular processing of peripherally administered peptides may be required for activity at the level of the hypothalamus warrents some consideration, since such processing is not likely to occur when peptides are introduced directly into cerebral ventricles. This is an important issue related to peripheral conversion/activation of leptin-related synthetic peptides and biological activity, and has not yet been addressed. It is also worthy of note

Table I: Amino acid residues 127-167 of rat and human leptin.

Rat	127	K P E S L D G V L E A S G Y S T E V	144
Human	127	T L D S L G G V L E A S L Y S T E V	144
Rat	145	V A L S R L Q G S L Q D I L Q Q L D L S P E C	167
Human	145	V A L S R L Q G S L Q D M L K Q L D L S P G C	167

Nonidentical residues are underlined.

that these early studies utilized nonobese rodents with normal levels of circulating endogenous leptin as test animals, rather than genetically obese animal models. Therefore, the efficacy of the peptide fragments used in these studies, in the presence of an obese phenotype and its related pathophysiologies, is unknown.

The first report describing antiobesity effects of leptin-related synthetic peptides in an obese animal model came from our laboratory in 1997 (86). The C57BL/6J *ob/ob* mouse was selected for this and follow-up studies because of its wide use in characterizing the effects of full-length leptin on the obesity syndrome (20, 30, 87-89), and the nature of the mutation responsible for its obesity (80). The *ob/ob* mouse is leptin-deficient. A single base mutation at codon 105 of the *ob* gene results in substitution of a premature stop codon in place of the codon for the arginine residue at this position. The consequence of this point mutation is excessive production of an mRNA species that encodes an inactive isoform of mouse leptin which is truncated at amino acid residue 105.

This information led us to suspect that the activity of mouse leptin may be localized toward its *C*-terminus, in domains distal to amino acid 105. To test this hypothesis, 6 peptide amides corresponding to amino acids 106-167 of mouse leptin, each 15 amino acids in length and overlapping at the *N*-terminus by 5 residues, were synthesized. The peptides were individually administered (i.p., 28 days) to female *ob/ob* mice, and their effects on food intake and body weight gain assessed. Only three of the peptides, encompassing the domain between amino acid residues 106 and 140, demonstrated significant antiobesity effects, although of a lesser magnitude than reported for leptin.

The reduced potency of the active peptides (approximately 30-fold lower on a molar basis than recombinant mouse leptin) suggested that full expression of leptin activity might require additional active sites toward the *N*-terminus of the molecule proximal to amino acid residue 106. To test this hypothesis, 14 peptides encompassing the entire sequence of secreted mouse leptin, each 15 amino acids long and overlapping at the *N*-terminus by 5 residues, were synthesized. Each peptide was individually administered (i.p., 7 days) to female *ob/ob* mice. As observed in our original study (86), only peptides corresponding to amino acid residues 106-120, 116-130 and 126-140 induced significant reductions in food intake and body weight gain (90). Thus, in contrast to the functional epitopes encompassed by amino acids 106-140, the *N*-terminus of mouse leptin appears to contain only structural epitopes. These epitopes may contribute to full activity of leptin by providing conformational constraints that optimize receptor binding and activation. It is interesting to note that the biological activity of one of these peptides, the leptin analog corresponding to amino acids 116-130 [LEP-(116-130)], has been confirmed by other laboratories, using *in vivo* and *in vitro* approaches, central and peripheral administration, and measuring a number of other biological endpoints known to be modulated by leptin action (91-95).

Initial studies to examine the mechanism of action of LEP-(116-130) generated some unsuspected and intriguing results (96). Utilizing cell lines expressing either the short or long isoform of OB-R, these studies indicated that the observed effects of LEP-(116-130) on food intake and body weight gain in *ob/ob* mice (86, 90) did not appear to be mediated by peptide binding and activation of OB-R. Additional support for a mechanism of action independent of OB-R was provided by the observed ability of LEP-(116-130) to reduce food intake, body weight gain and blood glucose levels in the *db/db* mouse, a hyperleptinemic rodent model of obesity that is genetically deficient in functional OB-R (97, 98).

The clinical significance of these observations may be great. Because most cases of human obesity are associated with hyperleptinemia and leptin resistance (35), drugs that use pathways independent of that of leptin to restore energy balance and reverse other metabolic dysfunctions associated with the obese syndrome may have the potential to be even more effective in the treatment of human obesity than recombinant leptin. Such drugs may also be helpful in those rare cases of human obesity arising from mutations in the *ob* or *db* gene.

An essential element in the design of biologically potent peptides is an understanding of the contribution of each amino acid within a peptide sequence to receptor recognition or to overall peptide conformation. As previously mentioned, amino acid residues which do not contribute to receptor binding often serve a structural role which enables the peptide to assume a conformation facilitating optimal interaction with receptor, or protects the active site(s) of the peptide from proteolysis. These amino acid residues can often be totally or partially deleted from a sequence with the remaining peptide fragment retaining full or partial activity (99). It is clear that isolation of the biological activity of a peptide to a small number of amino acid residues not only has biological advantages, *i.e.*, facilitates transport into the CNS, but also chemical and economic advantages related to the design and development of drugs for which the peptide may serve as a lead compound.

The activity of LEP-(116-130) has recently been localized to a restricted domain consisting of seven amino

acids at its *N*-terminus, between residues 116 and 122 (100). In addition to its leptin-like effects on food intake and body weight gain, OB3, a synthetic peptide amide corresponding to these residues, was shown to reduce blood glucose levels in both *ob/ob* and *db/db* mice. These findings suggest a potential benefit associated with glycemic control, which may have clinical significance in the treatment of both leptin-resistant and leptin-deficient models of human obesity, and of diabetes mellitus in the absence or presence of an obese phenotype. Replacement of the leucine residue at position 4 of OB3 with its D-isoform, [D-Leu-4]-OB3, significantly improved the potency of OB3 with respect to its anorexogenic activity, effects on weight loss and antihyperglycemic action (100). Most recently, we have shown that the antiglycemic effects of [D-Leu-4]-OB3 in *ob/ob* mice are independent of its effects on caloric intake (101).

Future perspectives

The recent upsurge of interest in the design and development of leptin agonists suggests that their application to the management of human obesity and its associated metabolic dysfunctions may not be left unexamined much longer. Before this can happen, however, a significant amount of work is needed to address questions related to potency, bioavailability, kinetics, half-life, clearance, toxicity and route of administration. Nonetheless, the development of leptin agonists remains a viable strategy.

Formulated to be orally available, such small-molecule therapeutics have the potential to be more potent agonists of OB-R than recombinant leptin, since access into the CNS would not be limited by saturable transport across the blood-brain barrier, a suggested locus of leptin resistance in humans (36). Also worthy of special note, the recently discovered activity of some of these agonists in *db/db* mice indicates that their mechanism of action involves the recruitment of signaling pathways that are different from leptin (96). The benefits of this divergence to the treatment of human obesity in which leptin resistance is the result of defective receptor signaling, or in cases resulting from leptin or leptin receptor deficiency, are obvious.

Conclusions

In the United States, an estimated 300,000 people die annually as a result of obesity, mostly from its effects in promoting diabetes, hypertension, cardiovascular disease and cancer (23). Therapies based on nutritional and behavioral modification have only partial and usually temporary benefits, and existing pharmaceutical interventions which target central serotonergic and adrenergic pathways, or inhibit fat absorption, are of limited efficacy. Because of the necessity for chronic therapy in the treatment of obesity, a past history of unsuspected toxicity associated with long-term use of some antiobesity drugs, and the potential of abuse for purely cosmetic reasons, it is clear that approval of future therapies will demand very high safety standards. Nevertheless, new insights into the dynamics of regulated energy balance, provided mostly by rodent models of obesity, have numerous implications for the understanding and management of human obesity. Given these recent advances, and the potential for the discovery of additional genes and/or regulatory pathways involved in energy balance, the development of new pharmacological approaches to the treatment of the obese patient seems inevitable.

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