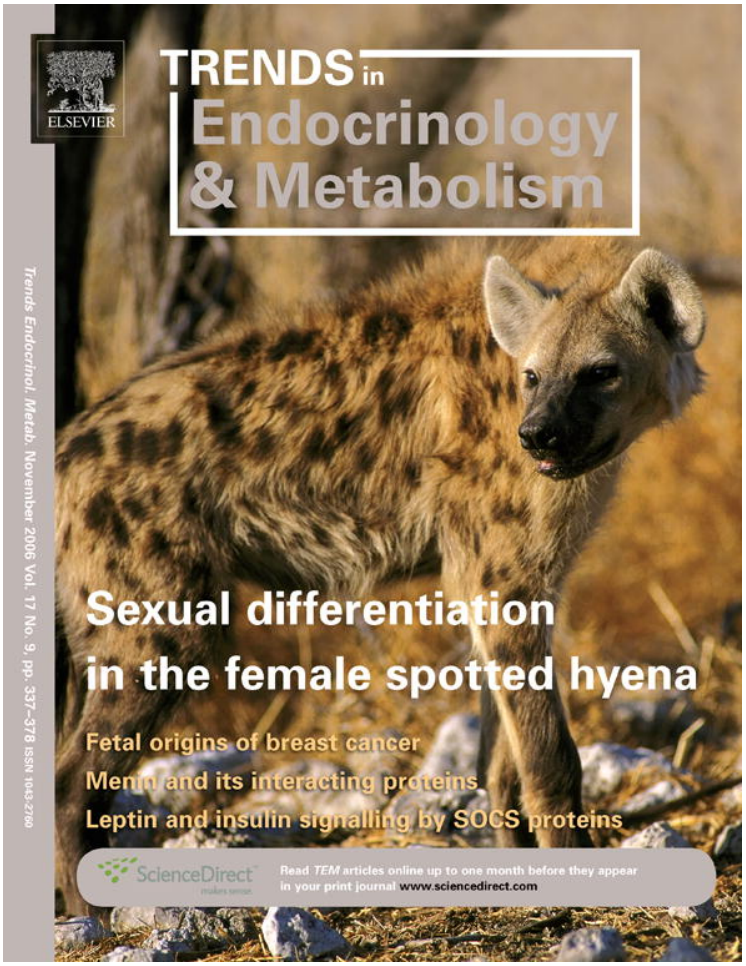

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Development of leptin antagonists and their potential use in experimental biology and medicine

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Leptin is a pleotropic hormone that acts both centrally and peripherally. Whereas leptin exhibits positive effects on several physiological functions, such as regulation of energy metabolism, reproductive function and immune responses, negative actions, such as enhancement of undesired immune responses in autoimmune diseases, tumorigenesis, elevated blood pressure and cardiovascular pathologies, have also been documented. The recent development of leptin muteins with antagonistic properties and other proteins that block leptin activity opens up new possibilities for their use in research and, eventually, therapy.

Introduction

Leptin is a 16 kDa hormone that is produced mainly by fat cells and secreted into the bloodstream; it was identified by positional cloning of the *ob* gene, which is responsible for the development of obesity in *ob*/*ob* mice [1]. The importance of leptin from body-fat deposits as a metabolic signal for many physiological functions has been documented in rodents, farm animals and humans, and >11 000 leptin-related papers have been published to date. The first observation regarding the use of recombinant leptin was that administration of leptin to leptin-deficient *ob*/*ob* mice leads to the rapid normalization of body weight [2]. However, since then, many direct, peripheral actions of leptin have been demonstrated and the expression of leptin receptors (LRs) in many organs and tissues, including hemopoietic precursors, immune cells, endothelium, smooth muscle, liver, bone, pancreas, gut and adipose tissue has been documented (reviewed in [3–5]). Initially, LRs were demonstrated in nuclei of the hypothalamus, which had been identified previously as crucial for energy homeostasis and regulation of food uptake. Inactivating mutations in the LR, as is the case in *db*/*db* mice, leads to extreme obesity [6]. Similar leptin and LR deficiencies have been identified in humans [7,8]. Under normal circumstances, the circulating concentration of leptin is proportional to body-fat mass [9]. Elevated leptin levels, which are recognized by the hypothalamus, activate a negative-feedback loop by reducing food intake and elevating energy expenditure [10]. Leptin appears to act as a metabolic switch in which lowered leptin concentrations during starvation down-modulate high-energy-demanding processes such as reproduction and immune responses. Interestingly, *ob*/*ob* and *db*/*db* mice also have impaired T-cell immunity and are resistant to the development of autoimmune diseases, including experimentally induced rheumatoid arthritis, multiple sclerosis and inflammatory bowel disease, and administration of leptin restores susceptibility to these chronic inflammatory diseases [11,12]. Leptin might affect several immune-response targets (summarized in Figure 2 of [11]). Effects of leptin on T-cell immunity have also been documented in humans and correlation studies in patients with rheumatoid arthritis, systemic lupus erythematosus and multiple sclerosis indicate that leptin has a role in autoimmune diseases. Leptin might also have a role in atherosclerosis: despite their elevated level of fat, *ob*/*ob* mice are resistant to experimentally induced thickening of the carotid artery. Again, leptin administration restores the normal physiological response [3,6,11,12]. Endogenously produced leptin is also involved in cardiac muscle function [13,14], and in the regulation of blood pressure and arterial hypertension [15]. That leptin is involved in reproduction was demonstrated a few years ago by us and others [16–18]. Leptin regulates several functions in pituitary cells [19], is involved in cognitive action [20] and, more recently, it has been reported that postnatal treatment of the offspring of calorie-restricted, pregnant rats with rat leptin blunts the increased adiposity and other undesirable effects that otherwise occur later in life [21,22]. There are correlations between several cancers and obesity, which is characterized by leptinemia, and leptin also has direct, growth-promoting and anti-apoptotic activities in several cancer-cell lines [23–26]. In conclusion, leptin has diverse and opposite effects, thus, the benefit of blocking leptin activity is real and timely.

Leptin is a pleiotropic hormone that is secreted mainly by adipose tissue, as well as by the placenta, gut and other tissues [3–5,27]. Thus, studying leptin action by classical replacement therapy is not feasible. Directly blocking the LRs that are responsible for transferring leptin through the blood–brain barrier, and its effects in the hypothalamus and the periphery is, therefore, a major, practical, pharmacological means of inhibiting leptin activity.

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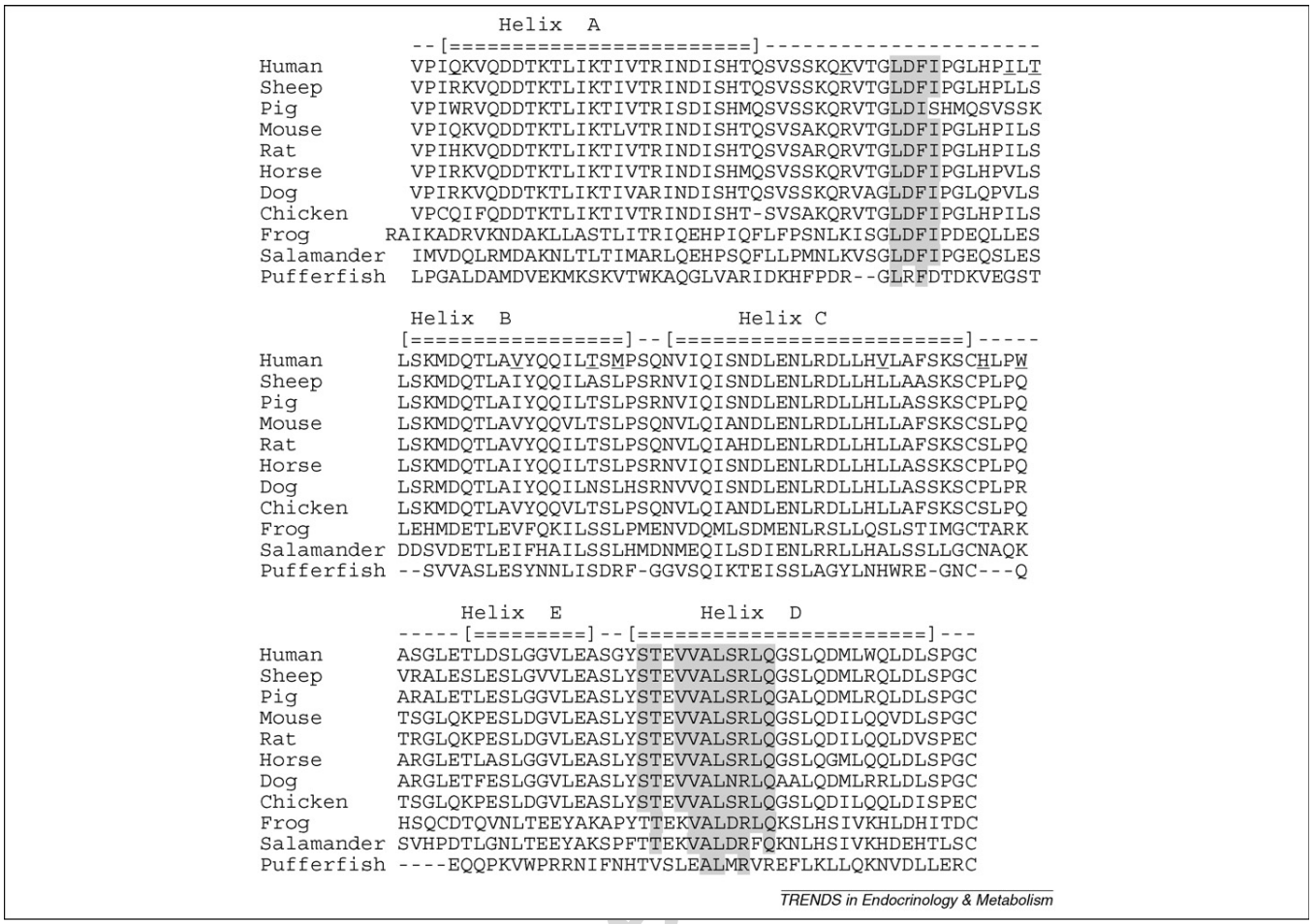

Figure 1. Mammalian and non-mammalian leptin sequences. Areas in which mutations might lead to the creation of leptin antagonists are highlighted. The sequences are compiled from the NCBI databank (www.ncbi.nlm.nih.gov/Genbank/): human, NP_000221; sheep, Q28603; pig, NP_999005; mouse, NP_032519; rat, NP_037208; and horse, AAR88257, and from our unpublished results: chicken, AAC60368; dog, NP_001003070; frog, AAX77665; salamander, AAY68394; and pufferfish, NP_001027897.

Reagents that neutralize leptin activity might provide a research tool for studying leptin-related metabolic processes *in vitro* and *in vivo*. Such neutralization can be achieved with: (i) soluble LRs that bind free leptin in the circulation; (ii) leptin antagonists that bind to, but do not activate, LRs; and (iii) either specific anti-LR monoclonal antibodies (mAbs) that bind to the receptor and prevent the productive binding of leptin or anti-leptin antibodies (Abs). These reagents capable of blocking leptin activity are novel, potent research tools for elucidating the role of leptin in mammalian physiology and pathology. In addition, they have potential as drugs.

Blocking of free leptin by soluble LR

The extracellular domain (ECD) of human LR consists of 841 amino acids and is divided into several subdomains that include: (i) the signal peptide, amino acids 1–21; (ii) an N-terminal cytokine-receptor-homologous domain 1 (CRH1), amino acids 22–328; (iii) an immunoglobulin-like domain (IGD), amino acids 329–427; (iv) CRH2, amino acids 428–637; and (v) two, consecutive F3 domains, amino acids 636–841. CRH2 is the major leptin-binding domain (LBD) [28]. Human and chicken LBDs, which have been sub-cloned and expressed as recombinant proteins, have a 1:1 molar interaction with leptin [29,30]. However, these recombinant LBDs are not particularly effective at blocking leptin activity because their affinity for leptin is slightly lower than that of the intact LR (Table 1). Unfortunately, mutagenesis of chicken LBD has not resulted in muteins (proteins that result from mutations) with higher affinity [30]. The full-length recombinant ECD of LR was prepared first in insect Sf9 cells [31] and then in mammalian Cos7 cells [32]. Although the affinity of the full-size, membrane-embedded receptors was not compared directly with that of full-size ECDs and human and chicken LBDs, compilation of the data (Table 1) indicates that the full-size ECDs prepared in mammalian cells have higher affinity for leptin, which is comparable to that of the membrane-embedded receptor. However, the method used to measure the hormone–receptor interaction can also affect the results because the K_d or IC_{50} values measured using the radio-receptor assay (RRA) are mostly lower than those measured using surface plasmon resonance (SPR). The relatively low yields of the recombinant full-size LR and truncated ECDs mean that no data for their *in vivo* activity has been reported.

Other available research reagents in this group include recombinant human and mouse LR/Fc chimeric glycoproteins composed of the ECD of the respective receptor, tagged with Fc (upstream) and 6xHis (downstream), which

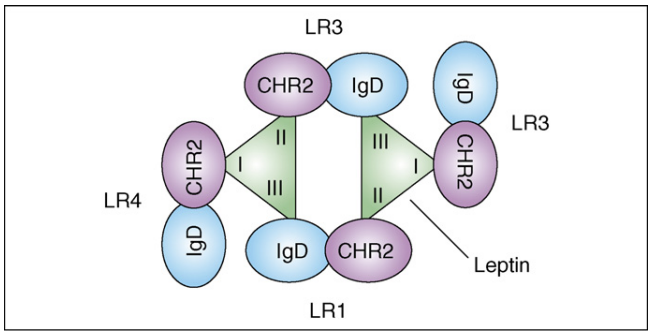

Figure 2. Model of leptin–LR (2:4) complex as suggested by Tavernier and coworkers [40]. The ECD of human LR consists of 841 amino acids divided into several subdomains, which include: signal peptide, amino acids 1–21; N-terminal CHR1 domain, amino acids 22–328; IGD, amino acids 329–427; CHR2, amino acids 428–637; and two, consecutive F3 domains, amino acids 636–841. CHR2 is the major LBD [28]. Binding site II of leptin (green) interacts with CHR2 (magenta) of one LR chain, binding site I interacts with the CHR2 domain of a second LR chain, and binding site III binds to the IGD (turquoise) of a third LR. Figure reproduced, with permission, from [41].

is produced by RandD Systems Inc. (www.RnDSystems.com). Interaction of these potent reagents with leptin has been studied by SPR (Table 1) [33], and these same reagents have been used recently by Matarese's group [34,35] to neutralize leptin in mice with experimental autoimmune encephalomyelitis (EAE). In these studies, they increased the percentage of regulatory T cells, ameliorated the clinical course and progression of the disease when given either before or after the onset of EAE, improved clinical scores, reduced disease relapses, inhibited proteolipid protein peptide-induced specific T-cell proliferation, and switched cytokine secretion to a Th2/regulatory profile [34,35].

Leptin muteins as antagonists

Mutagenesis of R128

The first attempt to use leptin antagonists to inhibit leptin action was reported several years ago with R128Q human leptin: this leptin mutein is an *in vivo* antagonist in C57Bl/6J mice and *ob*/*ob* mice, and lacks biological activity *in vitro* in BAF/3 cells transfected with a chimeric receptor composed of the ECD and transmembrane domain of the murine LR fused to the intracellular domain of the human βc receptor (a subunit that is common to several cytokine receptors) [36]. Because amino acids 123–130 (VVALSRLQ) are conserved in mammalian and non-mammalian leptins (Figure 1), we determined whether the R128Q mutation has the same effect in all leptins or whether it is species specific by comparing pure, recombinant, R128Q muteins of ovine and chicken leptin with similarly prepared human leptin and human R128Q mutein [37]. The mutation did not change the properties of binding to BAF/3 cells stably transfected with the long form of human LR compared with non-mutant human, ovine, and chicken leptins. However, in the same cells, the biological activity in a proliferation assay was reduced drastically: the R128Q mutein of human leptin lost its activity and became a weak antagonist, whereas the activities of ovine and chicken muteins were reduced 25-fold and 80-fold but did not result in antagonist activity. This indicates that mutagenesis of R128Q has species-specific effects [37].

Table 1. Comparison of K_d and IC_{50} values for interaction of leptin with full-length LR and fragments of the LR

LR	K_d (nM)	IC_{50} (nM)	Method	Refs
Human WT in Baf3-cell homogenate	0.83		RRA	[29]
Human WT in Baf3-cell homogenate		1.03	RRA	[29]
Human LR		5.93	RRA	[29]
Human LBD	15.3		SPR	[29]
Chicken LBD		1.02	RRA	[30]
Chicken LBD	23.2		SPR	[30]
Human WT in COS7 cells		~0.2	RRA	[32]
Human LR-ECD secreted by Cos7 cells[a]		~0.2	RRA	[32]
Mouse LR-R/Fc	0.50		SPR	[33]
Human LR-R/Fc	0.23		SPR	[33]
Human LR-ECD secreted by Sf9 cells[b]	9.5		SPR	[31]

Abbreviations: RRA, radio receptor assay; SPR, surface plasmon resonance; WT, wild type.
[a]Full-length ECD, engineered to be secreted.
[b]His × 6-tagged and FLAG-tagged ECD prepared in Sf9 cells using the baculovirus expression system. The FLAG epitope (DYKDDDDK) is a minimal epitope that is useful for tagging proteins to react with specific antibodies.

Mutagenesis of binding site III

The lack of a clear model of the leptin–LR complex has been a major drawback for the rational design of leptin muteins with inhibitory properties. This situation changed with pioneering work from Tavernier and his group who documented that leptin binding to its receptor resembles the interaction between interleukin 6 (IL-6) and gp130 [38–40] (Figure 2) and suggested the existence of a novel, previously unidentified leptin binding site III that is composed of several amino acids [41], including Ser120 and Thr121. This site is responsible for the formation of active 2:2 or 2:4 leptin:LR complexes by interaction between leptin bound to the CRH2 domain of one LR and the IGD domain of another, as shown in Figure 2 [40,42]. Mutation to Ala of these residues in human and mouse leptin creates potent leptin antagonists [42]. To determine whether, in addition to the N-terminal portion of helix D, other parts of the leptin molecule also contribute to leptin site III, the structures of IL-6-receptor complexes (viral IL-6/gp130 and IL-6/IL-6 receptor α/gp130) [43,44] in which site III had first been identified were analyzed. Using the sensitive, bi-dimensional hydrophobic cluster analysis [45], amino acids 39–42 (LDFI) in the loop that connects helices A and B were identified as a putative sequence that contributes to leptin site III. Although mutation of some or all of these amino acids to Ala in human and ovine leptin did not change their binding properties, it abolished their biological activity and converted the muteins into potent antagonists [46]. Because the LDFI/S sequence is preserved in all known leptins (Figure 1), the corresponding muteins of mouse and rat leptin results in a large-scale production of potent antagonists [47]. Together, over ten muteins have been prepared to date, all of which are potent antagonists in various *in vitro* bioassays (Table 2). Although the accurate localization of LDFI in site III has been questioned recently, and an alternative explanation suggested [40], the L39A/D40A, F41A/I42A, L39A/D40A/F41A and L39A/D40A/F41A/I42A muteins act as typical antagonists; namely, they bind to LR with an affinity similar to that

Table 2. Some recombinant human, ovine, rat and mouse leptin muteins with attenuated agonist activity

Mutein	Effects *in vitro* and *in vivo*[a]	Antagonist activity[b]	Refs
Human R128Q	Baf/3 cell proliferation, weight gain in *ob/ob* and C57BL/6J mice	++	[36]
Human R128Q[c]	Baf/3 cell proliferation	+	[37]
Ovine R128Q[c]	Baf/3 cell proliferation	–	[37]
Chicken R128Q[c]	Baf/3 cell proliferation	–	[37]
Mouse R20N	Transactivation of luciferase reporter gene	–	[42]
Human L39A/D40A	Baf/3 cell proliferation, transactivation of luciferase reporter gene and ERK42/ERK44	+++	[46]
Ovine L39A/D40A	Baf/3 cell proliferation	+++	[46]
Human F41A/I42A	Baf/3 cell proliferation, transactivation of luciferase reporter gene and ERK42/ERK44	+++	[46]
Ovine F41A/I42A	Baf/3 cell proliferation		[46]
Human L39A/D40A/F41A	Baf/3 cell proliferation, transactivation of luciferase reporter gene and ERK42/ERK44	+++	[46]
Ovine L39A/D40A/F41A	Baf/3 cell proliferation		[46]
Rat L39A/D40A/F41A	Baf/3 cell proliferation	+++	[47]
Mouse L39A/D40A/F41A	Baf/3 cell proliferation	+++	[47]
Human L39A/D40A/F41A/I42A	Baf/3 cell proliferation, transactivation of luciferase reporter gene and ERK42/ERK44	+++	[46]
Ovine L39A/D40A/F41A/I42A	Baf/3 cell proliferation	+++	[46]
Mouse Q75S	Transactivation of luciferase reporter gene	–	[42]
Human S120A/T121A	Transactivation of luciferase reporter gene, weight gain in C57BL/6J mice	+++	[42]
Mouse S120A/T121A	Transactivation of luciferase reporter gene, weight gain in C57BL/6J mice	+++	[42]
Human S120A/T121A[d]	Baf/3 cell proliferation, transactivation of luciferase reporter gene and ERK42/ERK44	+++	[46]
Human L39A/D40A/F41A/I42A/S120A/T121A	Baf/3 cell proliferation, transactivation of luciferase reporter gene and ERK42/ERK44	+++	[46]

Abbreviations: LUC, luciferase; ERK42/ERK44, extracellular-signal-regulated kinases (a member of family of mitogen-activated protein kinases).
[a]The details of the assays are given in the respective references.
[b]Strong, +++; medium, ++; weak, +; none, –.
[c]Prepared using the same method.
[d]Prepared as reported in [46] according to the sequence outlined in [42].

of non-mutant leptin, have no agonistic activity and act as antagonists [46,47].

The data compiled in Table 2 are from the laboratories in which the muteins were prepared, and other groups have published only limited data on the use of leptin antagonists *in vitro* or *in vivo*. Recently, Karmazyn and colleagues [13] have identified neonatal rat ventricular cardiomyocytes as a novel target for the direct hypertrophic effects of leptin and suggested that this hormone might be a biological link between obesity and cardiovascular pathologies. Treatment for 24 h with leptin (3.1 nM), angiotensin II (100 nM) or endothelin-1 (ET-1, 10 nM) significantly increases cell area, expression of the genes that encode myosin light chain-2 and α-skeletal actin, and leucine incorporation. The hypertrophic effects of all three agents are prevented by rat L39A/D40A/F41A leptin mutein (100 nM). Angiotensin II and ET-1 significantly increased the concentration of leptin in the culture medium by five-fold, which indicates endogenous leptin production. Furthermore, both angiotensin II and ET-1 increase expression of the gene that encodes the short form (LRa) and long form (LRb) of the LR by 180% and 200%, respectively, and these increases are abolished by rat L39A/D40A/F41A leptin antagonist [13]. The same antagonist (25 $\mu g\ d^{-1}$) given for seven days into the lateral ventricle, also prevents homeostatic down-regulation following high-fat-enhanced feeding in five-month-old rats [48]. The same group also report that acute central administration of leptin antagonist blocks leptin-induced hypothalamic phosphorylation of signal transducer and activator of transcription 3 (STAT3). Other recent studies report that luminal addition of leptin decreases active intestinal absorption of glutamine (by 76.6% with 10 nM leptin). This dose-dependent inhibition (IC_{50} ~0.10 nM) is almost completely blocked by luminal addition of a 10-fold excess of the ovine leptin L39A/D40A mutein [49]. It has also been documented recently that ovine leptin L39A/D40A/F41A mutein abolishes the effect of endogenous and exogenous leptin on α-casein expression in mammary-gland explants [50].

LPA-2 and LP-1 peptides

A different approach for inhibiting leptin activity has been reported by the group of Gonzalez who showed that high doses of peptides termed leptin peptide antagonists (LPAs) that correspond to amino acids 70–95 (LPA-2) and 3–34 (LPA-1) of human leptin (26 and 32 residues, respectively) [51,52] inhibit leptin activity *in vitro* and *in vivo*. [51–54]. Inhibitory effects include attenuation of leptin-dependent increases in the concentration of β3-integrin, IL-1, leukemia inhibitory factor, and their corresponding receptors in mouse endometrial cells [53], and reduction in the number of implantation sites and uterine horns in implanted embryos [52]. The same group has recently reported that LPA-2 attenuates leptin-induced growth in mouse mammary tumor cells [54], an effect that is more potent and more pronounced *in vivo* than *in vitro*. The mechanism of action of these peptides has yet to be clarified because their direct interaction with LR is unproven and binding experiments [51] show a non-typical dose-response curve that extends over eight orders of magnitude.

Blocking leptin action by anti-LR Abs and anti-leptin Abs

Using mAbs that interact with LR and prevent leptin binding and signaling, thereby acting as antagonists, is another way to block leptin activity. A few reports of this approach have been published to date. Most recently, a specific mAb (9F8) has been prepared that interacts with human LR [55]. This mAb has high affinity

(EC_{50} $\sim 10^{-11}$ M) for the LR and 50% of 9F8 is displaced from the LR at a 1:10 ratio of human leptin:9F8. The activity of 9F8 has also been tested *in vitro*, showing that at 8:1 weight:weight excess 9F8 attenuates leptin-induced luciferase reporter-gene activity and tumor necrosis factor α expression in leptin-activated monocytes, and partially attenuates the proliferation of peripheral blood mononuclear cells [55]. Both recombinant and chemically prepared Fab fragments of this mAb retain inhibitory activity [55].

The direct hypertrophic effects of leptin, as well as angiotensin II-induced and ET-1-induced gene expression of LRa and LRb, are prevented by antibodies to LRa and LRb (given at ~1 nM) [13]. The same group has recently examined the contribution of endogenous leptin to heart failure in a rat model of sustained, coronary artery ligation (CAL). Male Sprague-Dawley rats were subjected to either CAL or a sham procedure and injected intraperitoneally on alternate days with an LR antibody for four weeks. Injection of anti-LR mAb significantly increased body weight over the four-week period in both sham-operated and CAL animals, which confirms the efficacy of LR blocking. CAL-induced depression of cardiac function, indicated by several specific parameters, was not seen in animals treated with anti-LR mAb, and the two-fold increase in end diastolic pressure was abrogated by treatment with anti-LR mAb. The authors conclude that blocking LR improves post-infarction heart failure in rats [56].

In another example of anti-LR mAb activity, the proliferation of activated T cells that secrete endogenous leptin is sustained by an autocrine loop that is either completely or partially inhibited by a polyclonal anti-mouse LR (AF497) at a final concentration of 10 μg ml^{-1}. 1. However, because the leptin concentration was <500 pg ml^{-1} and no dose–response study was reported, the effectiveness of AF497 cannot be evaluated accurately [57]. Preventing binding of leptin with a neutralizing anti-rat LR mAb suppresses the growth of rat leukemic cells by inhibiting angiogenesis [58]. In rats treated with anti-rat LR mAb (0.5 μg every other day for three weeks), the growth of bone marrow leukemic cells is reduced by ~50%, with a concomitant decrease in angiogenesis, and survival is prolonged significantly. In these studies, the concentration of endogenous leptin is unchanged and the anti-LR Ab neither binds to the leukemic cells nor inhibits their growth *in-vitro*, but the proliferation of mononuclear cells is inhibited. Polyclonal anti-leptin Abs have also been used successfully to block leptin in EAE, similar to LR/Fc [34,35]. However the use of anti-leptin Ab *in vivo* might be problematic because they might enhance rather than block the activity of leptin and leptin antagonists by prolonging their half-life in circulation.

Perspectives for preparing effective LR blockers for *in vivo* studies

In view of the possible future pharmaceutical uses of recombinant leptin muteins, the general question of how to increase the biopotency of recombinant proteins *in vivo* needs to be explored (Box 1). The ability of a hormone to elicit a biological effect *in vivo* depends not only on the affinity for its receptor but also on the rate at which it is cleared from the circulation. Hormones with molecular masses similar to that of leptin, such as growth hormone (GH), prolactin and placental lactogen, are cleared primarily via the kidney and have a half-life of 8–30 min [59,60]. Kidney-mediated clearance depends mainly on molecular mass, and proteins >70–80 kDa are cleared much more slowly, so increasing the size of a protein will prolong its half-life *in vivo*. Increasing the size of the hormone without affecting its activity might be achieved either chemically, for example by attaching a polyethylene glycol (PEG) molecule (pegylation), or by preparing chimeric proteins with serum albumin from the respective species [61]. Chimeric mouse Fc/m–leptin and human Fc/h–leptin chimeras have also been reported that retain 10–20% of leptin activity *in vitro* while increasing the half-life in mice, from 18 min to 8.8 h and >10 h, respectively [62]. All chimeric proteins should be tested for immunogenicity. Pegylation increases the hydrodynamic volume of the protein and thereby slows its clearance. One of the most surprising results with respect to pegylated hGH is the finding that despite up to 500-fold lower activity *in-vitro*, it has a higher potency *in-vivo* because of the up to 25-fold increase in half-life in the circulation [63]. Thus, it was concluded that increasing the circulating half-life can compensate for deficits in receptor-binding affinity [63]. It should be noted that pegylation has already resulted in several drugs that have been approved and marketed, including peginterferon α 2a (Roche), pegfilgrastim (Amgen) and pegvisomant (Pfizer). One of the unanswered questions is whether the pegylated muteins cross the brain–blood barrier, or whether they act only peripherally. Hyperglycosylation might also improve the pharmacokinetics of recombinant proteins, and preparation of hyperglycosylated, biologically active erythropoietin and leptin in a mammalian system has been reported [64]. This approach was used successfully to prepare the erythropoietin analogue darbepoietin [65]. However, the main disadvantage of this methodology is that it cannot be used with proteins that are expressed in *E. coli*.

Box 1. How to convert LR-blocking reagents into efficient, peripherally acting drugs

Leptin antagonists

- Increase half-life *in vivo* either chemically (pegylation) or by species-specific chimeric proteins.
- Increase half-life *in vivo* by hyperglycosylation. This is unsuitable for proteins that are expressed in prokaryotic systems.
- Increasing competitive ability by increasing the affinity for LR. This can be done using either random or rational mutagenesis of sites I and II, with minimal possible changes to avoid undesired immunogenicity.
- Combining both approaches.

Anti-LR-neutralizing Abs

- Humanizing full-length LR or Fab fragments, prepared chemically or recombinantly.
- Increasing the half-life *in vivo* of Fab fragments by pegylation.
- Increasing the competitive ability by increasing the affinity for LR. This can be done by random mutagenesis, with minimal possible changes to avoid undesired immunogenicity.
- Preparing either nanobodies or muteins that do not cross the blood–brain barrier, thus restricting activity to the periphery.

An alternative, complementary approach is to increase the affinity of the antagonist for the receptor by either increasing k_{on} or, more likely for pegvisomant (see below), decreasing the k_{off} and, thus, prolonging receptor occupation. Theoretical thermodynamic considerations show that if antagonist and agonist have the same affinity, a 100-fold molar excess of antagonist will occupy 99% of the occupied receptors. Increasing antagonist affinity 100-fold achieves a similar result at ~1:1 molar ratio of antagonist:agonist. The best example of the feasibility of such an approach is pegvisomant, a human GH mutein that contains nine mutations; one mutation (G120R) converts the agonist into an antagonist, the other eight increase affinity for the receptor by 400-fold. Pegylation of this mutein combines both approaches, resulting in an effective treatment for acromegaly [66,67].

Another approach is the use of anti-LR mAbs, the main advantage of which is a high molecular mass, which ensures a long half-life in the circulation, and reasonable affinity for the receptor. However, these proteins are produced in mice, so they must be 'humanized' to eliminate their immunogenicity. Using human LR/Fc and Fc/LR chimeras as blockers seems to be less feasible because their affinity for leptin is no higher than that of membrane-embedded LR and, like the Abs, they might be immunogenic. Another recently reported possibility is the use of nanobodies that target the LR and block the ligand-induced conformational switch without interfering with the leptin–LR interaction. Nanobodies are a unique form of mAbs that are characterized by a single antigen-binding domain and are present in camels. Recombinant nanobodies are small (15 kDa), monomeric, bind the target with nM affinity, and are stable and easy to manipulate. Because nanobodies generally do not cross the blood–brain barrier, this type of antagonist might selectively inhibit the peripheral activity of leptin [68].

Conclusions

Leptin is a pleoptropic hormone and, as such, it has both wanted and unwanted effects. Present knowledge of the unwanted effects concern mainly autoimmune diseases, atherosclerosis and heart failure, but other pathologies such as cancer and high blood pressure might also be leptin dependent. Therefore, blocking leptin activity might provide future therapies, either alone or with other drugs. At present, two approaches seem to be most promising in achieving the therapeutic potential: potent, high-affinity leptin muteins that act as receptor antagonists and have extended persistence in the circulation *in vivo*, and humanized mAbs that block LR activity.

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Disclosure statement

Leptin muteins in amino acids 39–41 exhibiting antagonistic properties were developed in my laboratory in the Hebrew University of Jerusalem during a 3-year research grant received from the Israeli Science Foundation (grant no. 594/02), which terminated on September 2005. Production of these proteins was patented [US Patent Application No. 10/996 607 and PCT application that was filed by 26.11.2005 in the names of Yissum Co., Univ. Pierre et Marie Curie (Paris) and INRA (Paris), Title: 'Leptin antagonists', inventors: Arieh Gertler, Isabelle Callebaut and Jean Djiane]. Yissum Co. (http://www.yissum.co.il) is a Technology Transfer Company of the Hebrew University of Jerusalem, Israel. Production of some of these muteins is licensed to Protein Laboratories Rehovot (PLR) Ltd (http://www.plr-ltd.com), which is co-owned by Yissum Co. and myself, and I am CEO of this company.

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