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REVIEW

Leptin Resistance and Obesity

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LEPTIN was discovered in 1994 in the genetically obese (*ob/ob*) mouse [1]. Leptin is one of the most important and powerful anorexigenic factors related to the brain-adipose axis [2]. It is synthesized in and released from adipose tissue, inhibits appetite, and enhances sympathetic activities, resulting in the reduction of body weight [3, 4]. The anorexigenic effects of leptin are predominantly mediated by both neuropeptide Y-containing neurons and pro-opiomelanocortin (POMC)-containing neurons in the arcuate nucleus of the hypothalamus [5–8]. Loss of leptin signaling causes severe obesity in humans [9, 10]. Leptin administration reduces appetite, and leads to a decrease in body weight in leptin deficient obese animals and humans [11, 12].

It is becoming clear that obese subjects are resistant to both endogenous and exogenous leptin. Circulating leptin concentration shows a strong, positive correlation with body mass index, percentage of body fat, and total body fat weight, and the level is significantly higher in obese people, independent of the distribution of adiposity in the body [13, 14]. However, leptin fails to inhibit feeding behavior in obese people. A clinical trial of leptin in obese subjects demonstrated that serum leptin concentrations 20–30 times higher than normal physiological concentrations were necessary for a significant reduction in body weight [15]. This finding provides support for the notion that the appetite-suppressing effects of leptin are markedly diminished in obese subjects. Leptin ineffectiveness is identified as leptin resistance. Therefore, improvement in leptin sensitivity is an important step in the treatment of obesity. Here, we summarize recent knowledge concerning the exact mechanisms underlying leptin resistance in animal models of obesity, and discuss possible interventions to overcome leptin resistance in obesity.

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Animal models of obesity with leptin resistance (Table 1)

Leptin resistance appears to involve two different mechanisms; central and peripheral resistance. Peripherally administered leptin fails to inhibit food intake in animal models with both central and peripheral leptin resistance. In contrast, centrally administered leptin inhibits food intake in animals with peripheral leptin resistance, but not in animals with central leptin resistance. The mechanisms by which leptin resistance occurs can be divided into three steps: the transport of leptin across the blood-brain barrier (BBB-peripheral), abnormalities of the leptin receptor (peripheral/central), and disturbances of post receptor signaling (central).

It is well known that a high fat diet causes obesity in humans. By analogy, leptin resistance is associated with diet-induced obesity (DIO) [16]. In rat and mouse models of DIO, both central (impaired leptin signal transduction) and peripheral (impaired ability to cross the blood-brain barrier) mechanisms are likely to contribute to the development of leptin resistance [17]. However, diet-induced obese AKR mice show only resistance to peripheral, but not to central administration of leptin [18]. There may be a strain difference in the mechanisms of the development of high fat-induced

Table 1. Proposed mechanism of leptin resistance in an animal model of obesity

	Peripheral resistance	Central resistance
1. Dietary		
High Fat Diet	(+)	(+)
n-3 PUFA	(+)	(–)
2. Genetic		
Diabetic (*db/db*) obese mouse	(–)	(+)
Zucker fatty (*fa/fa*) rat	(–)	(+)
NewZealand obese mouse	(+)	(–)
Osborne-Mendel rat	(+)	?
3. Others		
Aging	(+)	(+)
Hyperleptinemia	(–)	(+)
Continous central leptin infusion	?	(+)

leptin resistance.

Leptin gene expression in adipose tissue increases with age, and in spite of a high blood concentration of leptin, older rats become obese [19]. Therefore, it appears that age-related leptin resistance exists [19]. Aged F344 x BN rats have been reported to show both peripheral and central leptin resistance [20]. Impaired transport of leptin across the BBB of old CD-1 mice develops with obesity, and is reversible with even modest weight reduction [21]. Therefore, age-related leptin resistance may be reversible.

Genetic animal models of obesity also show leptin resistance associated with different mechanisms. Both the genetically diabetic (*db/db*) mouse and the Zucker fatty (*fa/fa*) rat have genetic abnormalities in leptin receptors, developing central leptin resistance [22, 23]. In contrast, the transport of intravenous leptin across the BBB of the Koletsky rat is markedly reduced, indicating the existence of peripheral leptin resistance [24]. Intracerebroventricular administration of recombinant mouse leptin inhibits food intake, whereas no anorexigenic response to peripherally administered leptin is found in New Zealand (NZO) obese mice [25]. Therefore, NZO mice also show peripheral leptin resistance, in which leptin transport to the brain is thought to be disrupted.

Peripheral leptin resistance

Leptin is transported across the BBB, and it is suggested that short forms of the leptin receptor (Ob-Rb) may mediate its transport. There is a marked decrease in the leptin transport rate into the brain in rats lacking all leptin receptor isoforms [26], while diabetic (*db/db*) mice that lack only Ob-Rb, but have intact short-form receptor (Ob-Ra) show normal leptin transport rates into the brain [27]. However, neither NZO nor DIO mice with peripheral leptin resistance exhibit significant decreases in Ob-R gene expression in isolated cerebral microvessels, indicating no involvement of leptin receptor abnormalities in the leptin insensitivity of these models [28].

Circulating soluble leptin receptor (Ob-Re) levels are negatively correlated with body mass index, and are significantly lower in obese subjects [29]. Overexpression of soluble leptin receptor enhances the weight-reducing effect of leptin in genetically obese (*ob/ob*) mice [30]. These data indicate that soluble leptin receptor may be involved in the determination of leptin sensitivity, as soluble interleukin-6 receptor has been observed to enhance the anorexigenic effects of IL-6 [31]. This enhancement may be due to an increase in receptor binding or acceleration of the transport of its ligand across the BBB. Taken together, this suggests that the reduction of circulating Ob-Re in obese subjects contributes, at least in part, to the leptin resistance.

It has been reported that the ratio of cerebrospinal fluid leptin to serum leptin concentration is relatively decreased in obese patients, compared to that in normal subjects [32, 33]. These clinical observations indicate that the transport of leptin to the central nervous system is disturbed in obese patients, indicating the existence of peripheral leptin resistance in human obesity, and suggesting that the transport of leptin should be important for leptin action in the brain. The ratio of cerebrospinal fluid to serum leptin concentration is significantly higher in S5B/Pl rats, which are resistant to DIO but sensitive to exogenous leptin administration, than in Osborne-Mendel rats which are susceptible to DIO and relatively resistant to leptin [34]. The ability to transport leptin across the BBB determines the sensitivity to leptin.

Nutritional status appears to contribute to the development of peripheral leptin resistance. Fetal undernourished offspring with a neonatal leptin surge show an impaired response to acute peripheral leptin administration [35]. In both these mice and in offspring with normal intrauterine nutrition experiencing a premature

leptin surge caused by exogenous leptin administration, the transport of leptin into the brain appears to be disrupted, because those models are sensitive to central leptin administration. The mRNA expression of Ob-Ra is significantly reduced in these models, indicating the possible involvement of Ob-Ra in the disturbed leptin transport across the BBB caused by fetal undernutrition. Fasting significantly decreases the entry of leptin into the mouse brain, and refeeding reverses the reduced influx [36].

In contrast, triglyceride (TG) induces leptin resistance at the BBB [37]. Both starvation and DIO show an elevation of serum TG concentration, accompanied by a decrease in the transport of leptin across the BBB, whereas short-term fasting decreases serum TG concentration and increases the transport of leptin. It is of interest to note that treatment with gemfibrozil reverses both hypertriglyceridemia and impaired leptin transport. TG also contributes to the lipopolysaccharide-induced reduction of leptin transport across the BBB without inducing changes in Ob-Ra mRNA expression in isolated brain microvessels [38]. These data demonstrate the importance of circulating TG concentration in the development of peripheral leptin resistance.

In NZO mice possessing peripheral leptin resistance, hypothalamic leptin receptor mRNA appears to be as abundant as in lean mice, and polymorphism of the leptin receptor gene plays only a minor role [39]. However, the ratio of n-3 polyunsaturated fatty acid (PUFA) against n-6 PUFA is significantly higher in NZO mice than in New Zealand black (NZB) controls due to the changes associated with lipid metabolism-related enzyme expression in adipose tissue [40]. Circulating n-3 PUFA levels are significantly higher in massively obese subjects [41]. Therefore, we examined the role of n-3 PUFA in the development of peripheral leptin resistance [41]. Only n-3 PUFA abolished the anorexigenic effect of leptin administered intraperitoneally while other fatty acids added to the diet failed to attenuate the leptin effects. However, intracerebroventricularly (i.c.v.) administered leptin significantly inhibited feeding behavior in animals fed n-3 PUFA. In this case, cerebrospinal leptin concentration was significantly decreased under conditions in which circulating leptin concentrations were similar to those in control animals, indicating that leptin transport into the brain was disrupted in those animals. This was additionally confirmed by leptin transport assay using human leptin in rats. These results indicated

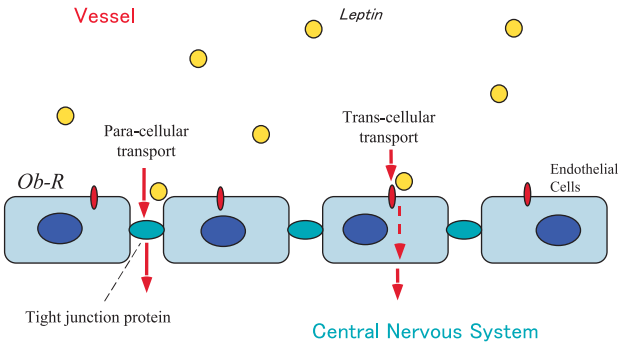

Fig. 1. The leptin transport system across the BBB in the hypothalamus.

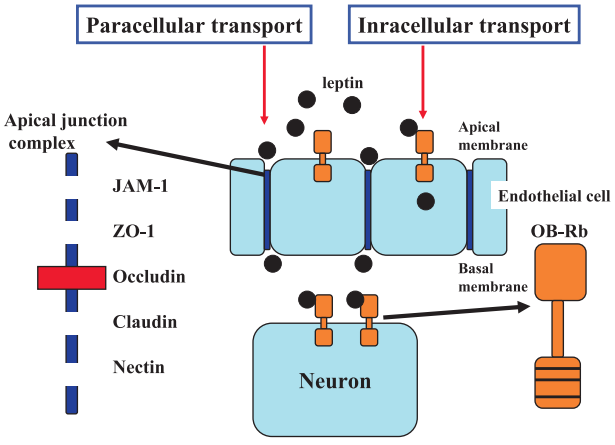

Fig. 2. Tight junction-related proteins in the hypothalamus.

that n-3 PUFA causes peripheral leptin resistance.

It is already known that neurotrophic factors are transported across the BBB via two possible pathways; transcellular and paracellular routes (Fig. 1) [42, 43]. However, the exact mechanism of leptin transport into the brain has not yet been clarified. To investigate the exact mechanism of peripheral leptin resistance induced by n-3 PUFA, we measured the expression of leptin receptors, intracellular signaling proteins associated with the leptin receptor, and hypothalamic tight junction proteins. Expression of leptin receptors and of intracellular signaling proteins was not changed. Among tight junction proteins, only hypothalamic expression of occludin was obviously increased by n-3 PUFA administration, while claudin-5, JAM-1 and ZO-1 were unaffected (Fig. 2). This observation was confirmed in *in vitro* isolated vascular fraction of rat hypothalamus in the presence of n-3 PUFA. Intraperitoneally administered leptin significantly inhibited food consumption in n-3 PUFA-fed rats given i.c.v. in-

jection of morpholino-oligonucleotide antisense against occludin. These data strongly indicate that occludin plays an important role in the induction of peripheral leptin resistance by n-3 PUFA. In addition, hypothalamic occludin expression was increased in mice fed a high fat diet, but not changed in (*db/db*) mice, the data indicating that hypothalamic occludin expression is able, at least in part, to explain the leptin resistance observed in DIO.

In addition, it has been demonstrated that epinephrine enhances leptin transport into the brain by working at the α_1-like adrenergic, luminal side, and epinephrine is effective only after peripheral, but not central administration [44]. However, adrenalectomy did not affect the entry of leptin into mouse brain [36]. These observations are interpreted to support the concept that hormonal stimulation modifies leptin transport into the brain.

Central leptin resistance

Leptin, released from adipose tissue, is transported across the BBB, especially in the arcuate nucleus of the hypothalamus where the BBB is poorly developed, and binds to its specific receptor, which belongs to the class I cytokine family [45]. The long form of the Ob-Rb is activated by formation of a homodimer. Genetic defects of Ob-Rb cause obesity in the genetically diabetic (*db/db*) mouse, Zucker fatty (*fa/fa*) rat and Koletsky rat [22, 23, 46]. Recently, it was demonstrated that human C-reactive protein (CRP) directly inhibits the binding of leptin to its receptor and blocks its ability to signal in cultured cells, and that infusion of human CRP into leptin deficient (*ob/ob*) mice blocked the effects of exogenously administered leptin upon satiety and weight reduction [47]. In addition, the actions of human leptin were completely blunted in mice that express a transgene encoding human CRP. Since circulating CRP concentrations are positively correlated with the degree of body adiposity, CRP may contribute to the development of leptin resistance in obese subjects.

Leptin receptor mRNA and protein expressions are diminished in the hypothalamus of aged Wistar rats [48]. Food-restriction in old rats results in lowered adiposity and recovered responsiveness to centrally administered leptin with an increase in hypothalamic leptin receptor expression, indicating that adipose tissue plays a key role in the development of leptin resistance associated with aging [49]. Central delivery of the adenovirus-assisted leptin gene causes complete unresponsive to additional i.c.v. infusion of leptin [50], and diminishes maximal leptin signaling capacity in the hypothalamus. This leptin-induced leptin resistance disturbs the regulation of energy homeostasis in response to high fat exposure, producing augmented energy consumption [51].

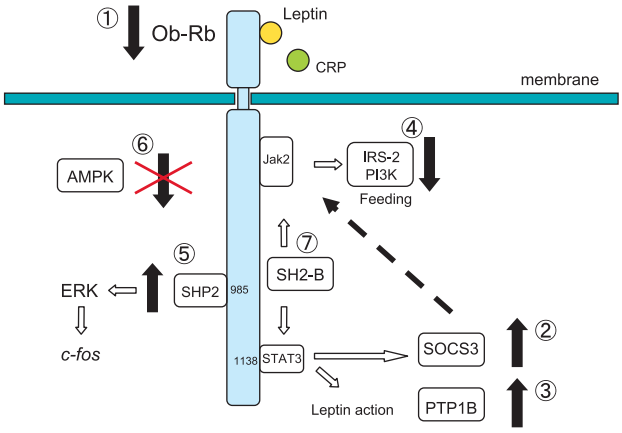

Fig. 3. A possible mechanism of central leptin resistance in animal models of obesity.

Hormonal status may be associated with central leptin resistance. Obesity accompanies hypergluco-corticoidism and adrenalectomy restores normal body weight in experimental rodents and conversely glucocorticoid administration induces central leptin resistance [52]. Withdrawal of glucocorticoid by means of adrenalectomy increases expression of the leptin receptor and its intra-cellular signaling molecule [53, 54], indicating an interaction between glucocorticoid and central leptin resistance. Another example is the relation of sex hormone to leptin. Premenstrual female subjects show higher leptin concentrations than body mass index-matched male subjects [55], and female rats are more sensitive to the anorexigenic effects of leptin, possibly due to increased leptin signaling in the arcuate nucleus [56].

The intracellular signals involved in central leptin resistance are summarized in Fig. 3. The intracellular signal transduction of leptin is mediated predominantly through phosphorylation of the Janus kinase 2 (Jak2)—signal transducer and activator of transcription 3 (STAT3) pathway [57, 58]. Central administration of leptin partially restores STAT3 activation in animals with DIO, although STAT3 activation is de-

creased after peripheral administration of leptin [17]. STAT3 phosphorylation in the hypothalamic arcuate nucleus is selectively resistant in DIO mice, which are likely to show elevated expression of suppressor of cytokine signaling (SOCS)-3 [59]. Rats selectively bred to develop DIO have less leptin-induced immunoreactive phosphorylated STAT3 expression in the arcuate, ventromedial, and dorsomedial nuclei of the hypothalamus than those bred to be resistant to DIO [60].

The SOCS3 pathway is a leptin-inducible inhibitor of leptin signaling, and has been suggested as a possible mediator of central leptin resistance in obesity [61]. Chronic infusion of leptin into the third ventricle increases hypothalamic expression of SOCS3 mRNA and protein [62]. The level of SOCS3 is particularly increased in the arcuate nucleus of DIO mice [63]. Neural cell-specific SOCS3 deficient mice have been shown to enhance leptin sensitivity and resistance to DIO [64]. Heterozygotes for SOCS3 deficiency show weight loss and enhanced leptin receptor signaling in the hypothalamus in response to exogenous leptin administration, and are protected against the development of DIO [65]. Failure of leptin-induced phosphorylation of STAT3 by an increase in SOCS3 expression in the hypothalamus might explain central leptin resistance in rats with DIO.

In addition, another inhibitory molecule, protein tyrosine phosphatase (PTP)-1B, appears to be involved in the regulation of leptin receptor signaling [66]. PTP1B dephosphorylates the leptin receptor-associated kinase, Jak2, and mice deficient in PTP1B show leptin hypersensitivity [67]. Overexpression of PTP1B in a mouse hypothalamic cell line, GTI-7, has been demonstrated to result in a dose-dependent decrease in endogenous Jak2 and STAT3 tyrosine phosphorylation, and lead to a decrease in mRNA accumulation of SOCS3 [68]. Furthermore, leptin-deficient mice lacking PTP1B show an enhanced response to the effects of leptin [69]. These data suggest that PTP1B is an important molecule in the development of central leptin resistance, in addition to STAT3 and SOCS3.

Activation of insulin receptor substrate (IRS) mediates the activation of phosphatidylinositol 3-kinase (PI3K) [70]. Hypothalamus-specific IRS2 knockdown mice, in which IRS2 expression is markedly reduced in the arcuate nucleus, display obesity and leptin resistance [71], implying that IRS2 is important in leptin signal transduction in the arcuate nucleus. The PI3K-phosphodiesterase 3B-cyclic AMP pathway may be involved in the development of central leptin resistance [72]. The finding that central infusion of PI3K inhibitor blocks leptin-induced anorexia suggests the importance of this pathway [73].

Recent observations have accumulated to support the involvement of another intracellular signaling molecule in the development of leptin resistance. SHP2 is a positive regulator of mitogen-activated protein (MAP) kinase (ERK) at the leptin receptor [74, 75]. SHP2 down-regulates Jak2/STAT3 activation by leptin in the hypothalamus [76]. Inhibition of hypothalamic AMP-activated protein kinase (AMPK) is necessary for the anorexigenic effect of leptin, because constitutive expression of active AMPK blocks leptin-induced effects [77]. The recent finding that inhibition of α_2-AMPK activity by leptin was not observed in the paraventricular, arcuate, and medial hypothalamus of DIO mice indicates that defective responses of AMPK to leptin may contribute to resistance to leptin action on food intake and energy expenditure under conditions of DIO [78].

In recent years, SH2-B, a Jak2-interacting protein, has been identified as a key regulator of leptin sensitivity [79]. SH2-B binds simultaneously to both Jak2 and IRS2, and promotes leptin-stimulated activation of the PI3K pathway in cultured cells [80]. Leptin-stimulated activation of Jak2 and phosphorylation of STAT3 and IRS2 are impaired in the hypothalamus of mice deficient in SH2-B, whereas expression of the long-form leptin receptor and SOCS3 are not changed. Deletion of SH2-B may severely impair leptin sensitivity in hypothalamic NPY/AgRP neurons [79]. Overexpression of SH2-B counteracted PTP1B-mediated inhibition of leptin signaling in cultured cells implying that SH2-B is indispensable in mediating the effects of leptin.

Leptin-induced anorexia is abolished by microinjection of adenovirus encoding a constitutively active nuclear mutant forkhead protein FoxO1 into the arcuate nucleus of rat hypothalamus, but the anorexigenic response to the MC4R agonist MT-II is unchanged in these rats [81]. Leptin signaling through Jak2-STAT3 inhibits AgRP expression by squelching FoxO1-dependent transcription of AgRP. These data indicate that loss of FoxO1 function is associated with increased sensitivity to leptin, but whether abnormalities of FoxO1 function involve leptin resistance in human and animal models is still unknown.

Many intracellular signaling molecules have been

reported to be involved in central leptin resistance. However, which molecule may play the most important role in leptin insensitivities remains to be clarified in order to develop therapy against central leptin resistance.

Therapeutic targets for leptin resistance

Here we discuss the possibility of clinical treatment of leptin resistance and obesity. In most obese subjects, the transport of leptin into the brain is disturbed, but it is possible that the brain itself shows the same sensitivity to leptin as in lean subjects. From the standpoint of peripheral leptin resistance, the development of longer and more permeable analogs, and the identification of an intrathecal delivery system for leptin are possible candidates for the treatment of leptin resistance [82]. The access of various drugs and bioactive substances to the nasal cavity is one useful way to effectively reach the brain [83]. Intranasal leptin administration causes longer suppression of appetite without a significant increase in circulating leptin concentrations in normal Wistar rats [84], suggesting that administration of leptin or analogs into the nasal cavity may be a possible route for leptin administration.

Caloric restriction is most important in the treatment of obesity, and changes in nutritional status can improve leptin resistance in DIO. Caloric restriction may improve serum TG profiles associated with leptin resistance as discussed above. Withdrawal of a high-calorie diet for only three days normalizes leptin-resistant DIO-prone mice to be sensitive to leptin [85], without a significant reduction of adiposity.

In addition, exercise is another important factor in the treatment of obesity. After a 12-week period of regular wheel exercise, Ob-Rb mRNA expression has been found to be decreased in the arcuate nucleus of normal male Wistar rats with reductions of abdominal fat pad weight and serum leptin concentrations [86]. Exercise for 8 weeks reversed leptin-induced phosphorylation of STAT3 and AMPK in rats given high-dose dexamethasone [87].

Administration of metformin restores leptin sensitivity in high fat-fed obese rats with leptin resistance [88]. Metformin treatment increased cerebrospinal fluid leptin concentrations in both standard and high fat diet-fed obese rats. These findings suggest the concept that improvement of the transport of leptin into the brain may correct leptin resistance in the rats with DIO after metformin administration. Thus, combined treatment with metformin and leptin may be useful in the treatment of obesity [88].

In addition, anorexigenic substances independent of the leptin pathway may be useful in the treatment of leptin-resistant obese subjects. Treatment with MTII, an agonist of the melanocortin 3/4 receptor, is another therapeutic approach to leptin resistance induced by diet, leptin exposure, and aging [50, 89, 90]. Ciliary neurotrophic factor reduces food intake by increasing STAT3 phosphorylation, and suppressing hypothalmic AMPK signaling in the arcuate nucleus of leptin resistant obese mice, being independent of leptin signaling [91]. This may therefore represent another possible therapeutic approach.

Summary

Since the discovery of leptin, there has been an accumulation of data concerning the mechanisms underlying leptin resistance. Central and/or peripheral mechanisms may be involved in the development of leptin resistance in various kinds of obese model animals. It is necessary to override the leptin resistance in order to use leptin clinically for the treatment of massively obese people. Further progress in this field is clearly necessary.

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