The Neurophysiological and Neuroprotective Effects of Leptin

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Abstract

Leptin is a peptide hormone and growth factor. While it has become well known in the area of diabetes research and its effect on re-sensitising insulin signaling, its properties as a growth factor and its neuroprotective effects are less known. Leptin crosses the blood brain barrier and activates second messenger cell signaling pathways that are neuroprotective, activate cell repair, protect synapses and synaptic functions, normalise energy utilisation and mitochondrial dysfunction, and reduce chronic inflammation. This review summarises the impressive results of leptin in cell culture and in preclinical research of Alzheimer’s disease, Parkinson’s disease, ischemia and epilepsy. Leptin has shown good effect in protecting neurons from stressors, reducing apoptosis, and in preventing or reducing key pathological markers of these diseases in animal models. As leptin analogues are developed for the treatment of diabetes and the first drugs have been licensed to treat patients, the potential of testing such drugs in patients with neurodegenerative disorders has opened up. However, key questions have to be addressed such as leptin de-sensitisation in the brains of patients with chronic neurodegenerative disorders.

INTRODUCTION

Leptin is a 167 amino acid peptide that is encoded by the obese (ob) gene and was first identified in 1994 through its role in the hypothalamus in regulating food intake and body weight [1]. Spiegelman and Flier, 2001, [2]. The name leptin comes from the Greek word ‘leptos’ which translates to ‘thin’, as ob/ob mice lost body weight when they were given this hormone [2]. In addition to the adipose tissue, leptin is expressed in lymphoid tissues, placenta, ovaries, mammary epithelium and bone marrow [3]. Leptin binds to the leptin receptor (ObR), which was first isolated from the choroid plexus by expression cloning techniques [4]. ObRs are located in the central nervous system and peripheral tissues and show structural similarities with cytokine receptors [5]. Currently, six ObR isoforms (a-f) have been described, all with identical extracellular ligand binding domains at the amino-terminus. In this review, we describe the structure and function of leptin receptors and the physiological role of leptin signaling in the brain.

All isoforms bar the ObRe isoform are membrane-spanning receptors [6]. ObRa,c,d and f are short forms of the receptor and consist of 30-40 cytoplasmic residues, while the long ObRb receptor consist of 302 cytoplasmic residues [7].

LEPTIN CAN CROSS THE BLOOD BRAIN BARRIER

Most of the physiological actions of leptin including feeding and energy balance are due to ObRb as it can activate downstream signaling cascades more efficiently [7]. While short form receptors are involved in mediating the leptin transfer from periphery into the brain through the blood brain barrier (BBB), especially ObRa and ObRc isoforms that are capable of binding and internalising leptin, and are expressed on BBB microvessels and the choroid plexus [4,8,9].

Leptin is transported into the brain via two mechanisms:

A) a saturable transport system that involves receptor mediated transcytosis [10], and
B) an epinephrine and triglycerides [11-13]-regulated leptin transport and CSF-mediated mechanism.

A study testing how leptin crosses the BBB using radiolabeled (125I)leptin showed that leptin can cross the BBB via nasal application. In this study, serum and brain levels of radiolabeled leptin were measured 30min after treatment, and a significant increase was found [14].

LEPTIN SIGNALING IN THE HYPOTHALAMUS: REGULATION OF APPETITE

In the hypothalamus, leptin signaling regulates food intake and energy homeostasis. ObRb is expressed in the arcuate nucleus (ARC), dorsomedial nucleus (DMH), and the ventromedial nucleus (VMH) in the hypothalamus. In the ARC, the ObRb is found in the neuropeptide Y (NPY), agouti-related peptide (AgRP)
and pro-opiomelanocortin (POMC) expressing neurons. When leptin binds to the ObRb on POMC neurons, depolarisation and increased biosynthesis of α-melanocyte-stimulating hormone (α−MSH) occurs that in turn activates downstream melanocortin systems, which not only suppresses appetite but also increases energy expenditure [15,16]. Leptin acts on its receptors on NPY and POMC neurons and decreases release of the inhibitory neurotransmitter GABA. Thus, POMC neurons become free of inhibition and increase their firing rate leading to the production of α-MSH that is an inhibitor of appetite [17]. Overall, leptin signaling decreases expression of orexigenic peptides (NPY, AgRP) and increases the expression of anorexigenic peptides (α−MSH) in addition to the increased energy expenditure in the adipose tissue and skeletal muscle tissue [15, 16] (see figure 1).

**LEPTIN SIGNALLING IN THE HIPPOCAMPUS – MEMORY MODULATION**

Leptin receptors are expressed in the areas CA1, CA3 and the dentate gyrus of the hippocampus. In hippocampal neurons, leptin receptors are located at both presynaptic and postsynaptic sites. Leptin regulates hippocampal excitability via synaptic and non-synaptic mechanisms. Firing activity of hippocampal neurons is regulated by leptin via modulation of BK potassium channels. Leptin can also facilitate synaptic plasticity. When perfusing hippocampal slices, leptin shifted short-term potentiation to long term potentiation (LTP) of synaptic transmission by enhancing Ca2+ influx through the NMDA receptor [18, 19]. Leptin also regulates AMPA glutamate receptor trafficking to the synapse [20]. As a growth factor, leptin increases synaptogenesis and neurogenesis in the dentate gyrus of adult mice by activating key cell signalling pathways. Leptin also has been shown to improve memory formation and retention when administered directly into the CA1 region in mice [21] (see figure 1).

**CELL SIGNALING PATHWAYS ACTIVATED BY LEPTIN**

A wide range of intracellular cell signalling pathways are regulated by leptin. They include key growth factor related pathways [22,23] such as the Janus-family tyrosine kinase-JAK/STAT pathway, MAP kinases, phosphatidylinositol 3-kinase (PI3k), protein kinase C (PKC) and a range of others (see figure 2-4). Importantly, anti-apoptotic signaling such as protein kinase B (PKB), and inflammation suppressor signaling (SOCS) is also activated by leptin [24, 25] (see figure 6).

The ObRb increases the activity of intracellular JAK2 kinases and ERK signalling, important growth factor signaling kinases. JAK2 activation leads to phosphorylation of Y985 and Y1138 of ObRb and Y705 of STAT3, after binding to pY1138. The activation of these kinases lead to gene transcription, and STAT3 negatively regulates ObRb signalling as a negative feedback loop to desensitise leptin signaling if the activity is too high. STAT3 also regulates the expression of cytokines via SOCS-3 [15](Figure 2 and 4).

The receptor itself is modulated by these kinases. The ObRb contains a JAK binding Box 1 motif, as well as four tyrosine residues. The activity of the receptor can be controlled by negative feedback signaling to induce de-sensitisation if the levels of leptin are too high[24] (See figure 3).

**THE ROLE OF LEPTIN IN CNS DEVELOPMENT**

Leptin plays a role in CNS development. High levels of leptin are found in the placenta, and synthesis of leptin and leptin receptors in foetal tissues indicates that leptin is involved in neuronal growth and development. Importantly, leptin is a neurotrophic growth factor that facilitates neurogenesis, neuronal migration, axon growth, and synapse formation as has been investigated in the development of hypothalamic circuits [26]. Leptin was shown...
Figure 2 An overview of the key cell signalling pathways regulated by leptin: Involvement of leptin has been shown in various second messenger pathways including, JNK (NH2-terminal c-Jun kinase), p38 (p38 MAP kinase), ERK (extracellular regulated kinase), SHP-2 (Src-like homology 2 (SH2) domain containing protein tyrosine phosphatase), PLC (phospholipase C), NO (nitric oxide), DGK-ζ (diacylglycerol kinase zeta), PGE2/PGF2 (prostaglandins E2/F2), PDE (phosphodiesterase), cAMP (cyclic AMP), SOCS-3 (suppressor of cytokine signaling 3), JAK (Janus-family tyrosine kinase), STAT (signal transducers and activators of transcription), PKB (protein kinase B, also known as Akt), PKC (protein kinase C), p70S6K (ribosomal p70 S6 kinase) and ROS (reactive oxygen species) by the leptin receptor (ObRb) [24,25].

Figure 3 Phosphorylation sites on the ObRb receptor for the modulation of receptor signaling activity. ObRb contains a JAK (Janus-family tyrosine kinase)-binding Box 1 motif, as well as four tyrosine residues. On phosphorylation, these interact with SH2 domain containing proteins such as SOCS (suppressor of cytokine signalling), SHP-2 (Src-like homology 2 (SH2) domain containing protein tyrosine phosphatase) and STAT (signal transducers and activators of transcription) [24].

to promote the formation of neuronal connections, axon growth and synaptic plasticity in development [27]. Leptin was able to restore normal patterns of neuronal connectivity in neonates but not in adult mice [26]. In mice that lack leptin expression (ob/ob) or show impaired leptin receptor signaling (db/db), brain weight is much reduced. Postnatal administration of leptin normalised the levels of several key synaptic proteins, growth –associated proteins in the neocortex, striatum and hippocampus, as well as in brain weight in ob/ob mice. Deficiencies in brain myelin, reduced neuronal soma size and altered dendritic orientation has been reported in ob/ob mice.

During embryonic and early postnatal stages, mRNA expression of leptin receptor stays restricted to the ependymal cells of the third ventricle. Injecting leptin into the ventricle of
Neuronal leptin signaling. Leptin binding to ObRb increases the activity of intracellular JAK2 kinases and ERK signaling. JAK2 activation leads to phosphorylation of Y985 and Y1138 of ObRb and Y705 of STAT3, after binding to pY1138. STAT3, PI3-K and ERK activation leads to regulation of gene transcription. STAT3 pathway negatively regulates ObRb signaling in addition to regulating the expression of SOCS-3 (cytokine signaling). PI3-K is a classic growth factor kinase that is also activated by insulin signaling and can activate a range of downstream growth factor and anti-apoptotic signaling pathways (see Figure: 6)[5]. Red arrows = inhibition, black arrows = activation.

Possible effects of leptin in the brain that may protect against Alzheimer’s disease. Growth factors such as insulin and leptin have a role in neurogenesis, axon growth, synaptogenesis, dendritic morphology, development of oligodendroglial cells, neuron excitability, neuroprotection and regulation of beta amyloid levels [84]. Insulin signaling has been shown to be desensitised in AD [65]. Leptin receptor activated cell signaling may improve the impaired insulin growth factor cell signaling pathway, thereby normalising energy utilisation, mitochondrial activity and cell repair. Studies have shown that leptin inhibits the hyperphosphorylation of tau protein through PI3K and AKT activation, which inactivate GSK3β kinase at the Ser-9 phosphorylation site. Leptin also helps to reduce the formation of amyloid deposits by modulating the expression of secretases that produce β-amyloid (see [36]. Red arrows = inhibition, black arrows = activation.

Postnatal day 4 (P4) old mice, mRNA for SOCS3 in the cells lining the third ventricle was increased, showing that leptin signaling is functional and enhances gene expression. In P10 old mice, activation of STAT3 has been observed in the hypothalamus after peripheral leptin injection. However, not all brain regions show this increase in STAT3 activation at that age group, demonstrating developmental changes in leptin signaling in the brain [26, 27].

LEPTIN IN NEURODEGENERATIVE DISEASES

Alzheimer’s disease (AD)

AD is a multifactorial neurodegenerative disorder characterised by aggregation of β-amyloid protein to plaques...
and neurofibrillary tangles of hyperphosphorylated tau protein. Leptin lowers the levels of free fatty acids, cholesterol and lipoprotein in the blood, thereby lowering the risk factors for AD [28]. Leptin modulates the expression of the APOE gene. APOE is a transporter protein that binds lipids in the blood but also helps in the removal of β-amyloid [29,30]. Furthermore, leptin decreases the activity of beta secretase, the protease that cleaves APP and produces β-amyloid [31]. Activity of the tau-phosphorylating GSK3β kinase was also reduced by leptin via an AMPK in an e-signaling pathway [28, 32, 33] (see also figure 5). As leptin activates key growth signaling pathways such as AMPK and sirtuin 1 (SIRT1), this may be of crucial help in supporting cellular repair in AD [34]. Low levels of leptin facilitate β-amyloid synthesis and phosphorylation of tau, and the reduced activation of α-secretase, which prevents the formation of β-amyloid [35].

Leptin also reduces neuronal apoptosis via activation of SIRT1 signaling. P53 signaling which induces apoptosis is reduced by acetylation [36] (see figure 6).

There have been reports on decrease of neuronal tau phosphorylation and beta amyloid accumulation/secretion, in cell cultures in vitro after leptin treatment [37,38]. Also, leptin injection improved cognitive performance and brain pathology in transgenic AD mouse model. Amyloid levels and tau phosphorylation was reduced after 8 weeks of treatment [29] (see figure 5).

**Leptin – a new treatment for AD?**

As described above, leptin facilitates cognitive processes as well as shows pleiotropic effects on the brain and is critical for brain repair and development as it increases neurogenesis, axonal growth and hippocampal synaptogenesis. It is therefore possible that impaired leptin signaling may be involved in the development of AD. In an epidemiological study, low levels of leptin correlated with the severity of Alzheimer’s disease in four independent studies with over 3000 patients. One study showed that people with plasma leptin levels in the lowest quartile were found to be four times more likely to develop AD than those in the highest quartile [34]. As circulating leptin levels were inversely correlated with AD severity, the disease modifying effects of leptin may benefit Alzheimer’s patients and improve cognition [34,38]. However, leptin is a signaling pathway that desensitises easily. In diabetes, leptin resistance is high, and the development of leptin analogues as a treatment has been questioned [39, 40]. In other diseases, leptin signaling also has been shown to desensitize [41]. A recent study demonstrates increased leptin levels in the CSF with reduced leptin receptor densities in the hippocampus [42]. Therefore, it will have to be evaluated if leptin signaling is still operational in the brains of AD patients before considering treatment with leptin agonists as an option [38,43,44].

**Parkinson’s disease (PD)**

PD is the second most common progressive neurodegenerative disease, mainly caused by the loss of dopaminergic neurons in the substantia nigra which leads to motor disturbances. Leptin may play an important role in the homeostatic regulation of the nigrostriatal pathway. Mice lacking leptin have reduced dopamine levels that result in diminished neurotransmission and motor activity [45]. Loss of dopaminergic neurons in the 6-hydroxydopamine (6-OHDA) model of PD is reversed by leptin administration [46]. Similar to leptin action in AD, leptin-mediated neuroprotection of dopaminergic cells involve the key signaling pathways involving JAK-STAT, MEK/ERK, and

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**Figure 6 Leptin reduces apoptotic signaling.** Like insulin and most other growth factors, leptin signaling reduces neuronal apoptosis through the activation of kinases such as PI3k and AMPK which then activate Akt/PKB and reduce apoptosis. An additional signaling pathway is the activation of sirtuin 1, which inhibits p53 by an acetylation process [34,36]. Red arrows = inhibition, black arrows = activation.
insulin signaling. This further activates downstream nuclear transcription factors including ERK1/2 and phospho-cAMP-response element binding protein (CREB). ObRb activation leads to leptin-induced ERK1/2 activation.

Leptin neuroprotection is blocked by an ObR receptor antagonist, and knocking down JAK2 or GRB2 prevented the leptin-induced ERK1/2 activation.

Importantly, leptin can increase the levels of brain-derived neurotrophic factor (BDNF) [46,47]. BDNF is a key synaptic growth factor that protects synapses from stress and neurodegenerative processes found in AD and PD [48,49]. BDNF also protects dopaminergic neurons and synaptic transmission, and levels are reduced in PD

[50]. BDNF binds to the TrkB receptor kinase and further activates PI-3-K and MAPK/ERK similar to leptin mediated signaling, including SH2 and GRB2 [51]. Activation of common signaling cascades by leptin and BDNF may induce a form of selective feedback by increasing BDNF expression thus in turn activating same signals. Leptin-induced increase of BDNF levels may be one of the main mechanisms mediating neuroprotection [7, 36].

Ischaemia

Studies in rodent models of cerebral ischaemia have demonstrated neuroprotective role of leptin via ERK1/2, AKT, NF-kb transcription and STAT3 signaling pathways [46,52,53]. Activation of the transcription factor NF-kb is associated with the induction of the anti-apoptotic Bcl-xl gene, a member of the BCL-2 family [54]. Thus, anti-apoptotic property of leptin in ischaemia can be explained by modification of the Bcl-xL/Bax ratio and the neuroprotective properties can be explained by the activation of ERK1/2 that phosphorylates Bad at Ser-112 and prevents its apoptotic activity [36].

Epilepsy

Epilepsy is a set of neurological disorders characterized by seizures and is associated with neuronal cell death. Research shows that leptin has both neuroprotective and anticonvulsant properties against seizures. Leptin receptors coupled to STAT3 activation has been found in the hippocampus, most susceptible brain area to seizure activity [53,55]. Leptin inhibits the firing of hippocampal neurons by activating large conductance calcium-activated potassium channels thus, may prevent aberrant firing that usually occurs during seizure activity [56]. Other studies found that high fat and low carbohydrate diet is anti-epileptogenic as such diet can increase leptin plasma levels in rats [57,58]. In leptin-deficient ob/ob mice, which are more prone to seizures, leptin protected hippocampal neurons against excitotoxicity [59].

Insulin signaling desensitisation - an underlying mechanism for the development of neurodegenerative disorders?

The protective and regenerative effects of leptin are impressive and demonstrate effective treatment of a range of symptoms pathological processes. This is not completely surprising as insulin and insulin-like growth factor 1 (IGF-1) signaling show very similar neuroprotective effects in preclinical and clinical trials. Insulin not only normalises blood sugar levels but also support neuronal growth and repair, utilising the same growth factor second messenger cell signaling pathways [22]. In human trials, insulin improves attention and facilitates memory formation [60]. Nasally applied insulin has been in clinical trials with MCI/AD patients and has shown good effects. Larger trials are currently ongoing [61].

Similarly, IGF-1 has shown neuroprotective effects in a range of studies [62, 63]. As a growth factor, IGF-1 also activates the growth factor cell signaling pathways that insulin and leptin activate [64] (Figure 5).

Insulin and IGF-1 signaling has been shown to be severely downregulated in the brains of AD [65-67] and PD patients [22]. Leptin is on of the peptide hormones that has the ability to compensate for the lack of insulin signaling, which is why a lot of research has been conducted on the physiological effects of leptin in diabetes [40,68]. There are other peptide hormones that also have been developed as treatments for diabetes. These are the incretin hormones, a family of growth factors that facilitate insulin release and glucose uptake, and therefore are of great interest to the diabetes researchers and clinicians. The incretin family includes Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). Both growth factors activate the same cell signaling pathways that insulin-IGF-1 and leptin activate [69,70]. In fact, several GLP-1 receptor agonists are on the market to treat diabetes [71], and clinical trials of such drugs are ongoing in patients with Alzheimer’s or Parkinson’s disease [72]. First promising results from a pilot clinical trial have been published [73, 74].

One can speculate if the main protective effects of these growth factors are via the re-sensitisation of insulin or via insulin independent effects. It is to be expected that the interaction between these growth factors are complex, as GLP-1 also increases the release of BDNF [75], similar to leptin.

FUTURE PERSPECTIVE AND CONCLUSION

In conclusion, this review discusses leptin signaling in different brain areas and sheds light on the involvement of leptin in neurodegenerative disorders. Leptin has neuroplastic and neurotrophic effects and may be useful for treating such diseases [37,76]. In addition, leptin contributes to the regulation of energy homeostasis, reward processing, neuroendocrine function, metabolism and brain development. Leptin replacement therapy has shown good physiological effects [38,77,78].

Clinical trials with leptin indicate that leptin is safe, at the proposed physiological doses and long-term use is indicated and no immunological reaction to leptin has been reported [79-81]. The leptin analogue Metreleptin has received approval as a treatment for lipodystrophy in Japan, and submissions to other countries for approval has been made [82]. Leptin may have an additional benefit as an insulin sensitiser but, further research is required to elucidate whether leptin analogues will be useful and if leptin has a major role in treating brain disorders [83]. Better understanding of the mechanisms mediating leptin’s neurodevelopmental actions and increased knowledge about the vulnerability of the brain to leptin level changes is needed.
Clinical trials will need to be conducted in patients with neurodegenerative disorders to establish efficacy and safety, and this relatively new area of research may open new avenues for understanding neuronal development and neurodegeneration [26].

REFERENCES


