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Structure, production and signaling of leptin

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ABSTRACT

The cloning of leptin in 1994 was an important milestone in obesity research. In those days obesity was stigmatized as a condition caused by lack of character and self-control. Mutations in either leptin or its receptor were the first single gene mutations found to cause morbid obesity, and it is now appreciated that obesity is caused by a dysregulation of central neuronal circuits. From the first discovery of the leptin deficient *obese* mouse (*ob/ob*), to the cloning of leptin (*ob* aka *lep*) and leptin receptor (*db* aka *lepr*) genes, much has been learned about leptin and its action in the central nervous system. The initial high hopes that leptin would cure obesity were quickly dampened by the discovery that most obese humans have increased leptin levels and develop leptin resistance. Nevertheless, leptin target sites in the brain represent an excellent blueprint for distinct neuronal circuits that control energy homeostasis. A better understanding of the regulation and interconnection of these circuits will further guide and improve the development of safe and effective interventions to treat obesity. This review will highlight our current knowledge about the hormone leptin, its signaling pathways and its central actions to mediate distinct physiological functions.

1. Introduction

Even before leptin was cloned in 1994 [1], its presence was predicted based on *ob/ob* (leptin deficient) and *db/db* (leptin receptor deficient) mice. Douglas Coleman and colleagues performed parabiosis studies, where they joined the circulation of *ob/ob* and *db/db* mice. They concluded from these studies that *ob/ob* mice were missing a circulating factor that was plentiful in *db/db* mice. This circulating factor would cure obesity in *ob/ob* mice, while *db/db* mice were unresponsive to it [2].

It took over 40 years for the discovery of the gene that was thought to be responsible for the observed effect in *ob/ob* and *db/db* parabiosis studies; at a time when positional cloning was still in its infancy. The discovery of the hormone leptin by

Abbreviations: ob/ob mice, leptin deficient mice; *db/db* mice, leptin receptor deficient mice; LepRb, long form leptin receptor; ARC, arcuate nucleus; CNTF, ciliary neurotrophic factor; BBB, blood brain barrier; CVO, circumventricular organ; ME, median eminence; CSF, cerebrospinal fluid; JAK2, janus-kinase-2; Y985/1077/1138, tyrosine residues 985/1077/1138; SHP-2, src homology-2 domain protein; MAPK, mitogen-activated-protein-kinase; STAT3/5, signal-transducer-and-activator-of transcription-3/5; pSTAT3, phospho-STAT3; SOCS-3, suppressor-of-cytokine-signalig-3; PTP1B, phosphotyrosinephosphatase-1B; HFD, high-fat-diet; POMC, pro-opiomelanocortin; CART, cocaine-and-amphetamine-regulated-transcript; AgRP, agouti-related-protein; NPY, neuropeptide Y; IRS, insulin-receptor-substrates; PI3K, phosphoinositol-3-kinase; mTOR, mammalian-target-of-rapamycin (mTOR); AMPK, 5'-adenosine monophosphate-activated protein kinase; PVN, paraventricular nucleus; LHA, lateral hypothalamic area; MC4R, melanocortin-4-recepotors; GABA, γ-amino-butyric-acid; PB, parabrachial nucleus; NTS, nucleus of the solitary tract; GLP-1, glucagone-like-peptide-1; DA, dopamine; VTA, ventral tegmental area; NAc, nucleus accumbens; BAT, brown adipose tissue; UCP1, uncoupling protein-1; DMH/DHA, dorsomedial hypothalamus and dorsal hypothalamic area; POA, preoptic area; RPa, raphe pallidus; TRH, thyroid releasing hormone; STZ, streptozotocin.

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cloning was initially hailed as a cure for human obesity, and the production of recombinant leptin followed quickly after that [3]. As a proof of concept, daily injections of recombinant leptin fully corrected obesity and other associated neuroendocrine abnormalities in rare cases of leptin deficient humans and rodents [4,5]. However, for most obese patients leptin levels were high and correlated positively with their adiposity [6]. Also, leptin injections were ineffective to reduce body weight and food intake in obese mice compared to lean controls [7]; a condition now termed leptin resistance (for more detailed reading on this topic please see [8]). Thus, the vast majority of overweight and obese patients are unresponsive to leptin. Yet, despite its clinical ineffectiveness to treat general obesity, the importance of leptin signaling for the maintenance of normal energy homeostasis is undebated, and patients with leptin deficiency, chronically low leptin levels (lipodystrophy or anorexia) or insulin deficiency may benefit from leptin treatment [9].

The past decade of research progress has continually expanded and refined the sites and mechanisms through which leptin acts to regulate energy homeostasis. Initial work demonstrated that leptin acts predominantly via the long form leptin receptor (LepRb) in the central nervous system, as deletion of LepRb from peripheral tissues does not affect energy homeostasis [10]. Attempts to understand leptin's effect on central feeding circuits initially highlighted the hypothalamic arcuate nucleus (ARC) [11], yet more recent work demonstrates that non-ARC LepRb populations importantly contribute to distinct physiologic aspects of leptin function [12]. This ever growing literature provides an improved, but still incomplete, picture of leptin function within the complex neural systems that control food intake and energy expenditure [13].

2. The leptin gene and peptide

Leptin (from the Greek word *leptos*, meaning "thin") is derived from the *lep* gene, located on chromosome 7, which transcribes a 167 amino acid peptide with a molecular weight of 16kD. The *lep* gene sequence is highly preserved across mammals, and leptin orthologs exist in amphibians, reptiles and fish with considerable divergence in primary amino acid sequences. The function of leptin is highly conserved in all mammalian and non-mammalian leptin due to the preservation of key second and tertiary structures allowing the formation of disulfide bridges [14]. Leptin belongs to the family of long-chain helical cytokines, which includes leukemia inhibitory factor, ciliary neurotrophic factor (CNTF) and human growth hormone, based on its crystal structure [15].

3. Leptin production

Leptin is produced and secreted predominantly from adipose tissue into the circulation. Circulating leptin levels positively reflect adipose tissue size, and communicate energy storage status to the brain [6,7]. Leptin expression and circulating levels show circadian fluctuations, and also change with nutritional state [16]. Fasting decreases circulating leptin levels, while feeding or obesity increases leptin levels [17]. Preventing the fasting induced fall of leptin reverses common physiological adaptations to fasting [17,18], highlighting the importance of leptin levels for energy homeostasis.

Leptin expression and secretion are regulated by many factors, e.g. inflammatory cytokines, glucocorticoids and insulin [19]. Also, sympathetic norepinephrine release and β-adrenergic receptor activation in adipose tissue are critical to decrease leptin gene expression in response to leptin injections [20] and are required for the reduction in circulating leptin levels during fasting [21]. Circulating leptin levels also reflect the physiological potency of leptin, such that *ob/ob* mice show pronounced responses to injected leptin, while hyperleptinemia results in diminished leptin response [22]. Indeed, hyperleptinemia is sufficient and necessary to induce leptin resistance, even though weight gain and hyperphagia are unaffected by the presence or absence of hyperleptinemia [23]. Overall there is compelling evidence that hyperleptinemia induces leptin resistance, but the importance of leptin resistance for whole body energy homeostasis and obesity development remains to be conclusively resolved [8].

4. Central leptin access

Leptin is too large to passively cross the blood brain barrier (BBB) and is instead transported across the BBB by a regulated, saturable transport system. Even though the molecular identity of this leptin transporter system is still unclear, it acts independent of LepRb [24]. While it is often implied that the ARC is outside the BBB, the existence of a functional BBB in the ARC is well established and indicated by a lack of fenestrated capillaries. Fenestrated capillaries are found in select brain regions in close proximity to the ventricular space, collectively termed circumventricular organs (CVO's). The median eminence (ME) is a CVO and as such clearly contains fenestrated capillaries. However, the border between the ME and ARC is lined by tanycytes, which are highly specialized glial cells connected via tight junctions. These tanycytes therefore shield the ARC from the circulation and the adjacent median eminence [25].

These and other data strongly indicate that the ARC is protected from the general circulation by the BBB and the ME/ARC tanycyte barrier, and that circulating signals cannot reach ARC neurons via passive diffusion. However, several lines of interesting data indicate that ARC neurons, particularly those in close proximity to the ME, are uniquely positioned to respond to circulating signals such as leptin,. First, fasting causes fenestrated capillaries to extend from the ME to proximal parts of the ARC, possibly allowing the diffusion of leptin to neurons at the ARC–ME border [26]. Second, tanycytes can transport leptin into the cerebrospinal fluid (CSF) from where leptin reaches LepRb target cells [27]. Third, the proximal ARC also connects to the perivascular space of the median eminence (Virchow–Robin space), allowing blood-derived substances to reach proximal ARC neurons by perivascular routes [28]. Finally, many ARC LepRb neurons send projections across the tanycyte barrier and into the ME and thereby gain direct access to circulating leptin levels [29]. While leptin likely reaches most central LepRb neurons via a saturable transport across the BBB or through