A Tale of Two Hormones: Role of Leptin and Insulin in Hippocampal Synaptic Function

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Abstract: It is well documented that the endocrine hormones, leptin and insulin provide signals to specific hypothalamic brain regions to regulate energy balance. However, the past decade of research has not only revealed the widespread expression of insulin and leptin receptors in the CNS, but has also identified numerous additional functions of these hormones in the brain. In particular, there is growing evidence that these hormones markedly influence hippocampal excitatory synaptic transmission as well as hippocampal synaptic plasticity. More recent studies have also identified links between dysregulation of leptin and insulin systems and the development of neurodegenerative disorders such as Alzheimer's disease. Here we review the recent evidence supporting a role for these hormones in modulating hippocampal synaptic function in health and disease.

INTRODUCTION

The obese gene product, leptin is a 16 KDa protein hormone that is produced primarily by white adipose tissue and it circulates in the plasma at levels relative to body adiposity [1,2]. In addition to acting on a number of peripheral tissues, several lines of evidence indicate that leptin can enter the CNS *via* transport across the blood brain barrier. The hypothalamus was one of the first regions of the brain that was identified as a leptin-sensitive target. Indeed, it is now well documented that leptin plays a key role in a number of hypothalamic functions including the regulation of energy homeostasis [3], bone formation [4], reproduction [5] and the hypothalamic-pituitary-adrenal axis [6]. However, recent studies have demonstrated that leptin is a pleitropic cytokine that evokes wide-spread biological actions on numerous extra-hypothalamic brain regions, including hippocampus.

Insulin is a small 6 KDa protein that is made in large amounts by pancreatic beta cells. Following stimulation of beta cells, insulin is released into the circulation where it regulates the uptake of glucose. However, in a manner similar to leptin, insulin is also capable of crossing the blood brain barrier [7] and has been shown to influence numerous CNS functions, including hypothalamic driven feeding behaviour [8], excitatory and inhibitory synaptic transmission [9-11] as well as neuronal survival [12]. Impairments in the insulin signaling pathway and/or resistance to insulin are also thought to contribute to the development of neurodegenerative conditions such as Alzheimer's disease (AD) [13].

In this review we will highlight the putative role of leptin and insulin and their respective signaling cacades in synaptic function. In particular the influence of these hormones on hippocampal excitatory synaptic transmission and synaptic plasticity will be addressed. Moreover the role of these hormonal systems in CNS driven diseases will also be explored as several lines of evidence have implicated dysfunctional insulin and/or leptin receptor driven signaling in neurodegenerative processes.

LEPTIN AND EXPRESSION OF LEPTIN RECEPTORS IN THE BRAIN

In 1995, the leptin receptor (Ob-R) was cloned from mouse choroid plexus using expression cloning techniques [14]. Genetic mapping studies revealed that Ob-R is encoded by a gene located within the 5.1 cM interval of mouse chromosome 4 that comprises the *db* (diabetes) locus. The leptin receptor displays greatest homology to the class I cytokine receptor superfamily; a group of receptors that includes the interleukin 6 and granulocyte-colony stimulating factor receptors [15]. These receptors have characteristic extracellular regions that include WSXWS, four cysteine residues as well as numerous fibronectin type III domains [16]. Indeed, the extracellular region of Ob-R has four fibronectin domains and two cytokine receptor domains [17].

Six leptin receptor isoforms, generated by alternate splicing of the *db* gene, have been identified in rodents [18]. These isoforms, termed Ob-Ra to Ob-Rf have identical N-terminal extracellular domains, but have distinct intracellular C-terminal regions. All the isoforms, except Ob-Re, are membrane spanning receptors that contain a 34 amino acid transmembrane domain. As Ob-Re is not associated with the plasma membrane and it is a major site for leptin binding in the plasma, it is thought to act as a soluble receptor that enables transport of leptin within the plasma. The remaining isoforms fall into two distinct categories based on the length of the C-terminal domain: short forms of the receptor (Ob-Ra, c,d,f) with short intracellular domains, and a long form (Ob-Rb) of the receptor with a larger intracellular domain (302 residues) that contains various motifs that enable the initiation of various downstream signaling cascades.

In both rodents and humans, the levels of leptin receptor mRNA and protein are particularly high in specific regions of the hypothalamus that are involved in regulating food intake and body weight, such as the ventromedial hypo-

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thalamus, arcuate nucleus and dorsomedial hypothalamus [19-22]. However, several studies have demonstrated that Ob-R expression is not confined to the hypothalamus. Indeed, high levels of leptin receptor mRNA and immunoreactivity have been detected in numerous extra-hypothalamic brain regions including amygdala, cerebellum, brainstem, substantia nigra and hippocampus [21-24]. In the human cerebellum, Ob-R mRNA is expressed at significantly higher levels than found in the hypothalamus [25]. In rodents Ob-R expression is also evident in cerebellar neurons [21, 26], with high levels of expression found during both embryonic and postnatal stages of development [21, 27, 28]. High levels of leptin receptor mRNA and immunoreactivity have been observed in hippocampal CA1/CA3 regions and in the dentate gyrus [22, 23, 29]. In primary hippocampal cultures, leptin receptors are expressed on the principle pyramidal neurons and on glial cells [30]. Furthermore, in a manner comparable to cerebellar granule cells, leptin receptor immunostaining is associated with axonal and somato-dendritic regions as well as points of synaptic contact [30].

TRANSPORT OF LEPTIN INTO THE BRAIN

The main route of leptin transport into the brain is *via* a saturable transport system [31]. Indeed, the short leptin receptor isoforms are highly expressed on brain microvessels and are able to bind and subsequently internalize leptin [32]. Recent studies have demonstrated that the leptin transport system can be regulated by a number of factors including triglycerides and epinephrine [7, 33]. Moreover impairments in the transport mechanism have been linked to the development of obesity, suggesting that resistance to leptin may occur at the level of the blood brain barrier [34]. Leptin is also thought to reach the brain *via* the cerebrospinal fluid (CSF) as high levels of ObRa have been detected on the main site for CSF production, the choroid plexus [35]. A number of studies have also shown that leptin mRNA and immunoreactivity are expressed widely in the brain [36, 37]. Thus the possibility that leptin is made and released locally in the CNS cannot be excluded.

INSULIN RECEPTOR EXPRESSION IN THE BRAIN

For many years it was generally thought that the peripherally derived hormone insulin was unable to enter the CNS and thus influence neuronal function. However, the first evidence that insulin may have central actions came from the identification of insulin receptor binding sites in the CNS [38-40]. Subsequent studies demonstrated the wide-spread expression of insulin receptors on neurons and glial cells in rat brain, with particularly high levels detected in the olfactory bulb, hippocampus, hypothalamus and cortex [39]. At the sub-cellular level, a combination of biochemical and immunocytochemical approaches have shown that insulin receptors are expressed at hippocampal synapses [41].

ORIGIN OF BRAIN INSULIN

Until around 10 years ago it was generally assumed that the CNS was insensitive to the circulating peripheral levels of insulin. However there is now considerable evidence for the existence of a saturable insulin transporter that enables peripherally-derived insulin to enter the brain *via* the blood brain barrier [42; for review]. The rate of insulin entry into the brain is influenced by many factors including fasting, refeeding as well as a number of pathological conditions [43]. Studies in neuronal tissue and cultures have detected the expression of insulin mRNA in a number of brain regions [44,45]. Moreover, insulin is reportedly secreted from neurons in a calcium-dependent manner when depolarized [46]. Thus together these finding indicate that insulin can enter the brain *via* a regulated transport mechanism but also that this hormone has the potential to be made and released from neurons.

LEPTIN RECEPTOR SIGNAL TRANSDUCTION

The leptin receptor displays greatest homology with class I cytokine receptors and the signaling cascades activated downstream of leptin receptors are analogous to those activated by other members of this receptor superfamily. Thus, following leptin binding, janus tyrosine kinases (JAKs), in particular JAK2 associates with specific cytoplasmic domains of the leptin receptor, resulting in trans-phosphorylation of JAK2 and subsequent phosphorylation of tyrosine residues on the leptin receptor. This chain of events in turn leads to the recruitment and activation of various downstream signaling molecules, including the STAT (signal transducers and activators of transcription) family of transcription factors, insulin receptor substrate (IRS) proteins, phosphoinositide 3-kinase (PI 3-kinase) and adaptor protein associated with the Ras-Raf-MAPK (mitogen-activated protein kinase) signaling cascade.

INSULIN RECEPTOR SIGNALING

Insulin receptors are members of the tyrosine kinase receptor superfamily. The insulin receptor is a tetramer comprising of two extracellular α subunits and two β subunits that are located intracellularly. Insulin binding results in rapid autophosphorylation of the insulin receptor β subunits which in turn promotes recruitment of various SH2 and PTB domain-containing scaffold proteins, in particular the IRS proteins. Once recruited to the β subunit these proteins are phosphorylated on tyrosine residues which enables recruitment of various effector molecules. The main signaling pathways activated downstream of the phosphorylated IRS proteins are the Ras-Raf-MAPK and PI 3-kinase cascades.

CONVERGENCE OF LEPTIN AND INSULIN DRIVEN SIGNALING

Several lines of evidence indicate that leptin and insulin signaling networks converge at a number of levels. In hepatocytes leptin reduces insulin-induced gluconeogenesis and tyrosine phosphorylation of IRS-1 by insulin [47]. Moreover the effects of insulin on hepatic glucose metabolism are enhanced by leptin [48]. In an insulin-secreting cell line K_{ATP} channel activation by leptin is occluded by insulin [49]. In hypothalamic neurons the ability of leptin to activate K_{ATP} channels is mimicked by insulin [50] and the ability of both these hormones to stimulate K_{ATP} channel activation involves PI 3-kinase-driven actin filament disruption [51,52]. Actin filament disruption also plays a pivotal role in the activation of hippocampal BK channels by leptin and insulin however divergent signaling pathways couple these hormone receptors to changes in actin dynamics and subsequent channel activation [53-55].

LEPTIN AND SYNAPTIC PLASTICITY

There have been numerous reports implicating leptin in the regulation of hippocampal synaptic transmission and synaptic plasticity. In the mammalian CNS, excitatory synaptic transmission is predominantly mediated by the AMPA subtype of glutamate receptors. In contrast NMDA receptors contribute little to basal synaptic transmission but are required for the induction of certain forms of synaptic plasticity including hippocampal long-term potentiation (LTP) and long-term depression (LTD). Initially cellular studies by Shanley *et al.* (2001) [56] demonstrated that leptin selectively enhanced NMDA receptor-mediated synaptic transmission at hippocampal CA1 synapses. Moreover, exposure of acute hippocampal slices to leptin promoted the conversion of short term potentiation (STP) into LTP, indicating that this hormone has the ability to facilitate NMDA receptor-dependent LTP. Additional electrophysiological studies on *Xenopus* oocytes expressing recombinant NMDA channels showed that leptin receptor activation was required for facilitation of NMDA-evoked currents [56]. Moreover, using this approach Harvey *et al.* (2005) identified that changes in the trafficking of NMDA receptors is likely to underlie facilitation of NMDA receptor-mediated currents by leptin [57]. Recent studies also support a role for leptin in hippocampal synaptic plasticity as direct administration of leptin into the dentate gyrus enhances the level of hippocampal LTP [58]. Leptin-insensitive rodents with dysfunctional leptin receptors (*db/db* mice or Zucker *fa/fa* rats) exhibit impaired hippocampal LTP and LTD [59] and deficits in hippocampal-dependent memory tasks [59, 60]. More recent cellular studies have reported that leptin promotes rapid remodeling of hippocampal dendrites and increases the density of hippocampal synapses [61]; structural changes that are likely to play an important role in synaptic plasticity as numerous lines of evidence indicate that morphological changes in dendrites/synapses are pivotal for hippocampal LTP.

A number of studies have examined the cell signaling cascades coupling leptin receptor activation to its effects on hippocampal synaptic plasticity. Inhibitors of PI 3-kinase and MAPK (ERK) prevent facilitation of NMDA receptor mediated responses by leptin thereby indicating that this process is mediated by PI 3-kinase and MAPK-dependent signaling [56]. In contrast, the rapid remodeling of dendrites by leptin requires the activation of NR2A-containing NMDA receptors, which is mediated by a MAPK, but not PI 3-kinase driven process [61]. It is known that NMDA receptors comprise an NR1 subunit and at least one copy of NR2A, 2B, 2C or D, with or without an NR3 subunit. Moreover several lines of evidence indicate not only that NR2 subunits regulate the receptor biophysical and pharmacological properties, but also that these subunits are differentially expressed and have distinct functions in the CNS. In view of this, it is feasible that leptin displays subunit-specificity in its ability to modulate NMDA receptor-dependent synaptic function and plasticity. In support of this possibility, leptin enhances NR2B-, but not NR2A-mediated responses in cerebellar granule cells [28]. It is also possible that distinct signaling cacades link leptin receptors to different combinations of NMDA receptor subunits. Indeed, leptin facilitation of NR2B-mediated responses in cerebellar granule cells involves a MAPK-, but not a PI 3-kinase-, dependent process [28].

In addition to influencing hippocampal LTP, a recent study demonstrated that leptin evokes a novel form of NMDA receptor-dependent LTD [62]. As leptin-induced LTD was observed following removal of extracellular Mg^{2+}, this suggests that this form of synaptic plasticity occurs under conditions of enhanced excitability. This form of LTD displays parallels to that evoked following low frequency stimulation (LFS) as it is NMDA receptor-dependent, has a postsynaptic locus of expression and is independent of mGluRs. However, the signaling pathways underlying leptin-induced LTD are distinct from LFS-induced LTD as it is negatively regulated by PI 3-kinase and serine/threonine phosphatases 1/2A [62]. In conclusion, leptin has the ability to influence different forms of hippocampal synaptic plasticity which in turn is likely to have important implications for the role of this hormone in neuronal development as well as learning and memory.

It is known that the circulating plasma levels of leptin are usually in the low nanomolar range (0.1-10nM) and are related to body adiposity. However during the first 2-3 weeks of postnatal development, before leptin plays a role in the regulation of food intake and body weight, the circulating levels of leptin are around 5-10 fold higher [63]. In most of the above studies examining the effects of leptin on hippocampal synaptic function, the concentrations of leptin utilized are well within the range likely to reach the hippocampus during early neuronal development and as such are likely to closely correlate with the physiological effects of this hormone at this stage of development. However, it is also likely that leptin plays a role in hippocampal synaptic function at later stages of development and in the ageing process. Indeed, the concentrations of leptin reaching this brain region may be derived not only from peripheral sources, but also from locally released leptin, as leptin mRNA and protein have been detected in the hippocampal formation.

INSULIN AND SYNAPTIC FUNCTION/PLASTICITY

In a manner similar to leptin, insulin has the ability to influence excitatory synaptic transmission and synaptic plasticity in the CNS. In hippocampal slices, brief application of low micromolar concentrations of insulin (concentrations that are only likely to occur during early stages of neuronal development; [44]), evokes a long-lasting depression (LTD) of excitatory synaptic transmission [64-66]. Like LTD induced by LFS and leptin, insulin-induced LTD is NMDA receptor-dependent, expressed postsynaptically and is independent of mGluR activation. Several reports have implicated a number of signaling pathways in insulin-induced LTD including PI 3-kinase and PKC [65, 66]. Moreover, recent studies have shown that an increased internalization of GluR2 AMPA receptor subunits [64,65], as well tyrosine phosphorylation of GluR2 are required for the synaptic depression induced by insulin [67].

Conversely, the cell surface expression of GluR1, but not GluR2, is elevated following exposure of hippocampal neurons to insulin [64, 68]. As an increase in the cell surface expression of GluR1 underlies the increase in synaptic strength associated with hippocampal LTP [69], this suggests that insulin also has the capacity to enhance the strength of

excitatory synaptic transmission *via* this process. Indeed, insulin facilitates the induction of LTP at lower stimulation frequencies than under control conditions [66]. Moreover insulin enhances NMDA receptor mediated currents in hippocampal neurons [70] and it promotes an increase in the cell surface density of NMDA receptors [11]; effects that are likely to promote the induction of hippocampal LTP. In contrast, rodents with streptozotocin-induced diabetes (hypoinsulinamemic conditions) display deficits in NMDA receptor-dependent signaling which may in turn reduce the likelihood of LTP induction [71]. Protein kinase C (PKC) is one potential mediator coupling insulin to NMDA receptors as selective PKC inhibitors prevent the potentiation of NMDA receptor activity by insulin [72]. Indeed, PKC plays a key role in modulating NMDA receptor trafficking and gating [73]. Recent studies have also demonstrated that as the scaffold protein, PSD-95 regulates the surface expression and channel gating properties of NMDA receptors [74]. As insulin increases the expression of PSD-95 in the hippocampal CA1 region, *via* a PI 3-kinase-dependent mechanism [75], another possible candidate mediating insulin coupling to NMDA receptors is PI 3-kinase. In support of this possibility, PI 3-kinase forms a complex with AMPA receptors and PI 3-kinase activity is required for AMPA receptor insertion during LTP [76].

Changes in the insulin receptor system have also been detected in the hippocampus of rodents following training in spatial memory tasks. Indeed, training was associated with an upregulation of insulin receptor mRNA, increased tyrosine phosphorylation of the insulin receptor as well as activation of the MAPK signaling cascade [77]. This not only provides further indirect evidence implicating the insulin system in cognitive functions such as learning and memory, but also suggests possible involvement of insulin-driven MAPK signaling in this process.

METABOLIC IMBALANCE AND NEURODEGENERATIVE DISEASE

A growing body of evidence supports the notion that metabolic imbalance is associated with the development of certain neurodegenerative disorders. Moreover, CNS-driven diseases such as Alzheimer's disease have recently been linked to dysregulation and/or CNS deficiencies in the leptin and insulin receptor systems [78,79]. Indeed, in Alzheimer's disease the circulating leptin levels are attenuated [79] and the CNS levels of insulin are reduced [78]. Furthermore, alterations in the levels of key components of both leptin and insulin signaling cascades have been detected in Alzheimer's disease brains [80], suggesting that impairments in leptin/insulin signaling play a role in the development of this neurodegenerative disease. In mouse models of Alzheimer's disease, administration of leptin significantly decreases the levels of amyloid β [81] and it improves performance in hippocampal-dependent memory tasks [82]. It is well documented that one hallmark of Alzheimer's disease is neurofibrillary tangles consisting of aggregates of hyperphosphorylated Tau. Recent studies have shown not only that insulin significantly reduces Tau phosphorylation *via* inhibition of GSK3β [83], but also that transgenic mice with ablation of neuronal insulin receptors display markedly increased levels of phosphorylated Tau [84]. However, these mice do not display deficits in glucose metabolism or spatial learning and memory, suggesting that lack of neuronal insulin receptors alone is insufficient to result in full-blown Alzheimer's disease pathology. This raises the interesting possibility that alterations in both insulin and leptin systems play a role in the development of this disease. However, little is known about how these two hormonal systems interact at the neuronal level and in turn how such interactions are deregulated or altered in neurodegenerative diseases.

CONCLUSIONS

Evidence is growing that the endocrine hormones, leptin and insulin, have multiple roles in the brain. Indeed, both hormones not only markedly influence hippocampal excitatory synaptic transmission but are also implicated in the cellular events underlying learning and memory. The discovery of alterations in the levels of these hormones in Alzheimer's disease, together with deficits in leptin/insulin signaling molecules has led to the suggestion that impairments and/or dysregulation of the leptin and insulin systems contribute to the development of neurodegenerative disorders associated with cognitive deficits. Thus, a greater understanding of the interplay between these hormones in neurons is required as it is likely that disturbances in both neuronal leptin and insulin systems play a role in the development of these diseases.

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