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The role of insulin receptor signaling in the brain

Leona Plum[1,2], Markus Schubert[2] and Jens C. Brüning[1]

[1]Institute for Genetics and Center for Molecular Medicine Cologne (CMMC), Department of Mouse Genetics and Metabolism, University of Cologne, Weyertal 121, 50931 Cologne, Germany
[2]Second Department of Internal Medicine and CMMC, University of Cologne, Kerpener Straβe 62, 50937 Cologne, Germany

The insulin receptor (IR) is expressed in various regions of the developing and adult brain, and its functions have become the focus of recent research. Insulin enters the central nervous system (CNS) through the blood–brain barrier by receptor-mediated transport to regulate food intake, sympathetic activity and peripheral insulin action through the inhibition of hepatic gluconeogenesis and reproductive endocrinology. On a molecular level, some of the effects of insulin converge with those of the leptin signaling machinery at the point of activation of phosphatidylinositol 3-kinase (PI3K), resulting in the regulation of ATP-dependent potassium channels. Furthermore, insulin inhibits neuronal apoptosis via activation of protein kinase B *in vitro*, and it regulates phosphorylation of tau, metabolism of the amyloid precursor protein and clearance of β-amyloid from the brain *in vivo*. These findings indicate that neuronal IR signaling has a direct role in the link between energy homeostasis, reproduction and the development of neurodegenerative diseases.

Introduction

The actions of insulin are mediated via the insulin receptor (IR), which belongs to the family of tyrosine kinase receptors. Binding by insulin leads to rapid autophosphorylation of the receptor, followed by tyrosine phosphorylation of insulin receptor substrate (IRS) proteins, which induce the activation of downstream pathways such as the PI3K and the mitogen-activated protein kinase (MAPK) cascades (Figure 1 and reviewed in [1]). The signaling mechanism and the biological effects of insulin have been studied mainly in classical insulin target tissues, such as skeletal muscle, fat and liver, with respect to glucose uptake, regulation of cell proliferation, gene expression and the suppression of hepatic glucose production. Over the past few years, it has become clear that insulin also has profound effects in the CNS, where it regulates key processes such as energy homeostasis, reproductive endocrinology and neuronal survival. In this review, we focus on this rapidly expanding field by discussing recent insights into the molecular mechanisms underlying the processes regulated by insulin signaling.

Corresponding author: Brüning, J.C. (jens.bruening@uni-koeln.de).
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Expression of the IR in the CNS and source of cerebral insulin

In 1978, Havrankova *et al.* [2] localized the IR in the CNS for the first time by ligand autoradiography. The presence and localization of IRs in the CNS were subsequently confirmed by immunohistochemistry and autoradiography [3,4]. IRs are widely distributed in the brain with highest concentrations in the olfactory bulb, hypothalamus, cerebral cortex, cerebellum and hippocampus. Moreover, downstream effectors of insulin, such as IRS proteins and PI3K isoforms, show distinct patterns of expression in the CNS that partly overlap with expression of the IR [5].

To initiate signaling in the CNS, insulin has to reach its receptor, which is separated from the circulation by the blood–brain barrier. In the 1960s, Margolis *et al.* [6] showed that peripheral infusion of insulin leads to an increase in insulin levels in cerebrospinal fluid, suggesting that insulin can indeed cross the blood–brain barrier. These findings were later confirmed in studies indicating that less than 1% of the peripherally administered insulin reaches the CNS in rodents [7]. The amount of insulin passing the blood–brain barrier has been subsequently shown to vary strongly among different species [8].

The transport of insulin across the blood–brain barrier shows a saturable character [6,7]. Studies have led to the definition of several conditions that affect insulin transport across the blood–brain barrier; for example, fasting, obesity, aging and dexamethasone treatment decrease the transport of insulin into the CNS, whereas in some models of diabetes mellitus or during the neonatal period insulin transport into the CNS is increased [9]. Taken together, these findings indicate that insulin enters the CNS by crossing the blood–brain barrier through a receptor-mediated transport mechanism [10]; however, the molecular identity of this transport mechanism remains unclear [9].

Brain IRs in the control of energy homeostasis

Insulin and the adipocyte-derived hormone leptin have recently emerged as adiposity signals that exert modulatory functions at various sites in the CNS. The primary target of these hormones seems to be the hypothalamus, which comprises a tightly regulated and complex network of neuropeptides and neurotransmitters that influence parameters of energy homeostasis (reviewed in [11,12]). Because insulin is secreted acutely in response to an

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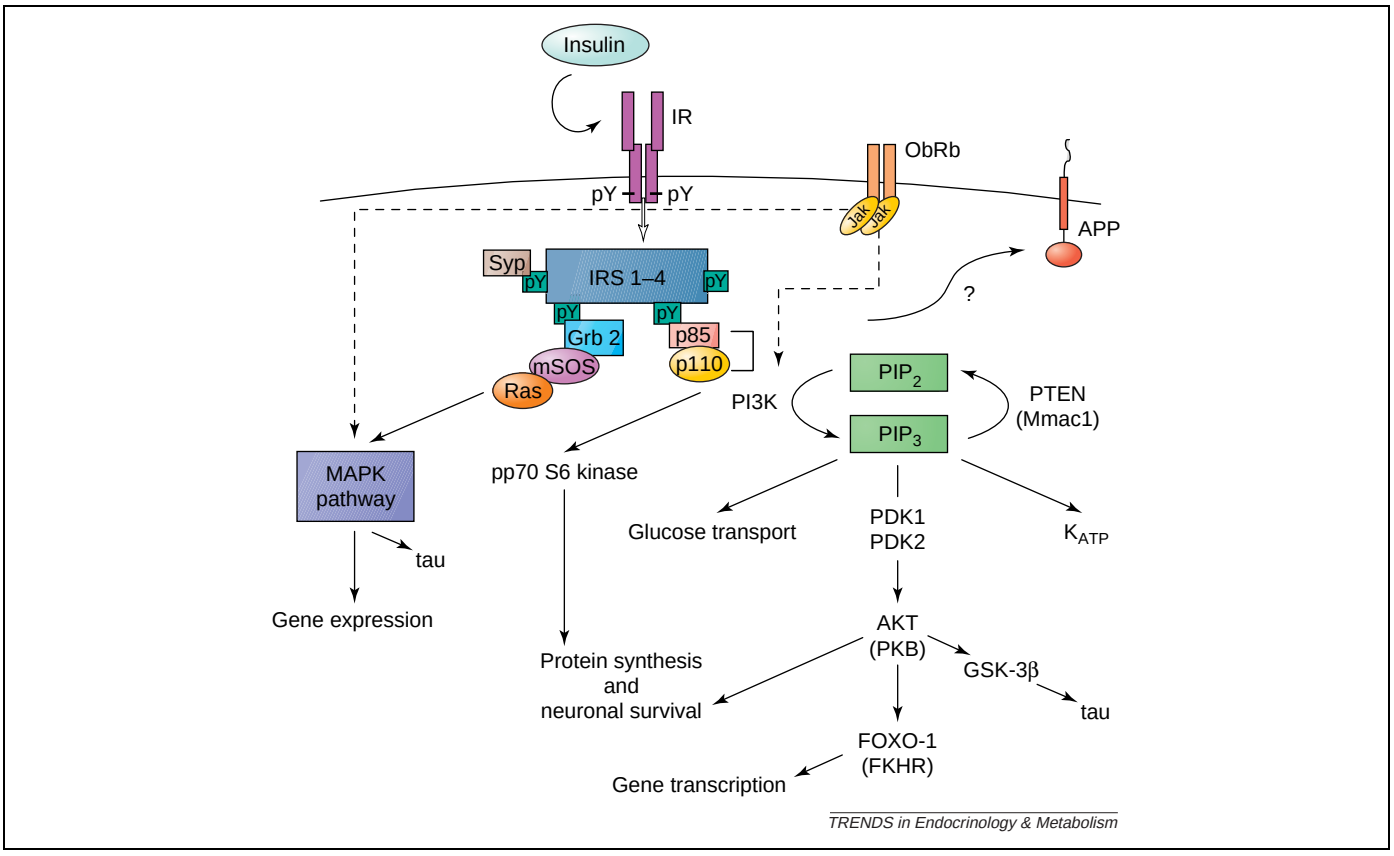

Figure 1. Insulin receptor signal transduction with respect to neuronal function. Insulin exerts its pleiotropic biological effects by binding to and activating the membrane-bound insulin receptor (IR) tyrosine kinase, which consists of two α-subunits and two β-subunits forming an α2β2 heterotetramer. Upon insulin binding to the extracellular α-subunits, the receptor undergoes a conformational change, activating the tyrosine kinase activity of the β-subunits, resulting in receptor autophosphorylation and subsequently in the phosphorylation of intracellular insulin receptor substrate (IRS) proteins on tyrosine residues. These phosphorylation sites are located in domains that characterize them as binding sites for src-homology-2 (SH-2) domain-containing proteins such as the p85-regulatory subunit of phosphatidyl inositol 3-kinase (PI3K), the growth factor receptor binding protein 2 (Grb 2) and the protein tyrosine phosphatase (Syp). Binding of these proteins to tyrosine-phosphorylated IRS proteins results in their activation, initiating downstream signals such as the activation of the Ras-Raf-MAPK cascade or activation of serine/threonine kinases downstream of PIP_3. These signals finally result in the diverse biological effects of insulin signaling in the central nervous system, such as the inhibition of apoptosis, tau phosphorylation, the regulation of amyloid precursor protein (APP) secretion and the regulation of gene transcription (e.g. of hypothalamic neuropeptides). Abbreviations: FOXO-1, forkhead box protein O1A; Jak, janus kinase; MAPK, mitogen activated protein kinase; mSOS, son of sevenless; ObRb, leptin receptor; PDK, protein-dependent kinase; p110, catalytic subunit of PI3K; PIP_2, phosphatidylinositol 3,4-diphosphate; PIP_3, phosphatidylinositol 3,4,5-triphosphate; PTEN, phosphatase and tensin homolog; Raf, Raf proto-oncogene serine/threonine protein kinase; Ras, Ras small GTPase.

increase in blood glucose and because plasma insulin levels are also directly correlated with the degree of body adiposity [13–15], this hormone provides both short-term and long-term homeostatic signals.

Administration of insulin directly to the brain yields an anorexigenic effect (i.e. it inhibits food intake), resulting in a reduction in body weight [16–19]; by contrast, inhibition of insulin signaling in the brain has an orexigenic effect (i.e. it increases food intake), resulting in a gain in body weight associated with peripheral insulin resistance [16,17,20–23]. Much of the recent research has focused on the mechanisms underlying the anorexigenic effects of insulin. Administration of insulin to the third ventricle decreases expression of the orexigenic neuropeptide Y in the hypothalamic arcuate nucleus [24,25], which in turn results in an increase in expression of the anorexigenic peptide corticotropin-releasing hormone in second-order neurons of the paraventricular nucleus (Figure 2) [25,26].

Furthermore, intracerebroventricular delivery of insulin increases expression of the anorexigenic peptide α-melanocyte stimulating hormone (α-MSH) [27]. In particular, upregulation of α-MSH seems to mediate the anorexigenic effects of insulin, because application of a melanocortin antagonist prevents the insulin-induced reduction in food intake [27]. Taken together, these findings show that the anorexigenic effects of insulin in the CNS are associated with changes in the expression of hypothalamic neuropeptides. The exact interplay of these regulatory networks and their regulation by insulin has been the subject of numerous reviews [28–31].

The intracellular mechanisms that mediate the effects of insulin and leptin in hypothalamic neurons are less well understood. Niswender *et al.* [32] have recently shown that selective inhibition of PI3K prevents both insulin- and leptin-induced anorexia, suggesting that PI3K is an important factor in mediating the intracellular effects of these two hormones (Figure 1) [33,34]. Activation of the PI3K pathway has been also recently linked to the regulation of ATP-dependent potassium (K_{ATP}) channels by insulin and leptin (Figures 1, 2). This connection is supported by findings that suggest that a PI3K-dependent pathway is involved in the activation of K_{ATP} channels by insulin and leptin in pancreatic β-cells and insulinoma cell lines [35,36]. Spanswick *et al.* [37,38] have demonstrated that both leptin and insulin activate hypothalamic K_{ATP} channels, resulting in hyperpolarization and inactivation

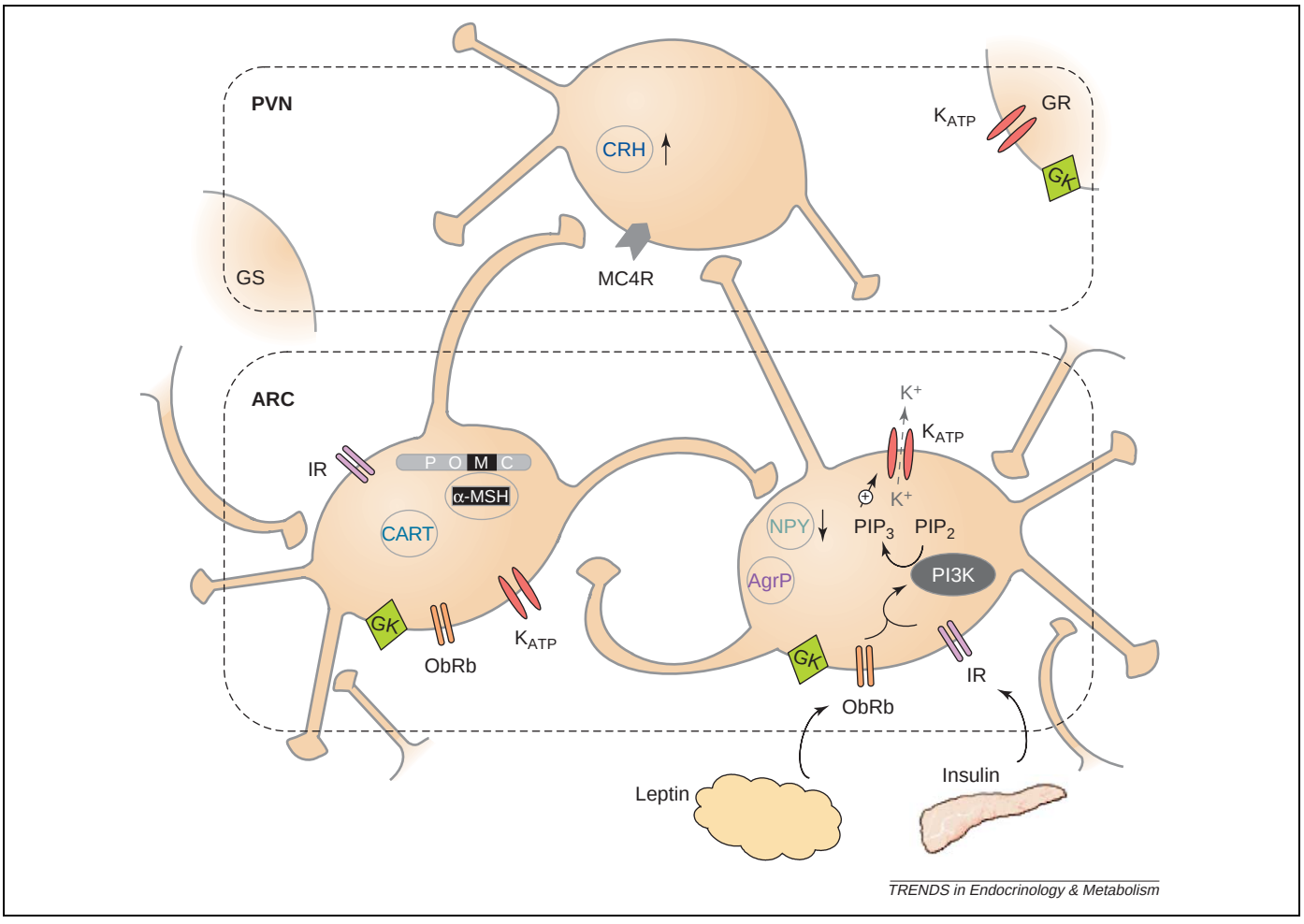

Figure 2. Mechanism of insulin action in the hypothalamus. Binding of insulin to its receptor, the IR, initiates a signal transduction cascade resulting in PI3K activation. PI3K phosphorylates phosphatidylinositol 3,4-diphosphate (PIP_2) to phosphatidylinositol 3,4,5-triphosphate (PIP_3), which opens K_{ATP} channels, thereby producing an outward flow of K^+ ions. This leads to hyperpolarization and reduced activity of the neuron. Moreover, signals mediated by the IR and the leptin receptor ObRb have been shown to regulate the expression of various neuropeptides in distinct neuronal populations in the hypothalamic arcuate nucleus (ARC), including downregulation of the orexigenic neuropeptide Y (NPY), which in turn results in increased expression of the anorexigenic corticotropin-releasing hormone (CRH) in second-order neurons of the paraventricular nucleus (PVN). Insulin also activates the melanocortin system, which comprises the anorexigenic hormone α-MSH, which is cleaved from pro-opiomelanocortin (POMC), and its receptors such as MC4R. Specific neurons – namely, glucose-responsive (GR) and glucose-sensitive (GS) neurons – seem to be capable of sensing changes in ambient glucose concentrations. Typical characteristics of glucose-responsive neurons are, for example, the expression of glucokinase (GK). Abbreviations: AgrP, agouti-related peptide; CART, cocaine and amphetamine regulated transcript.

of the respective glucose-responsive neurons. Thus, theses authors suggest that hypothalamic K_{ATP} channels might function as the molecular endpoint of the pathway after activation of the leptin receptor ObRb and the IR in hypothalamic neurons.

In summary, the hyperpolarization and inactivation of hypothalamic neurons as a consequence of the activation of K_{ATP} channels via PI3K might indeed provide a potential molecular mechanism for mediating the central effects of insulin and/or leptin on the modulation of glucose homeostasis, and might also affect the physiological regulation of food intake and body weight (Figure 2). Because leptin has been recently shown to modulate the firing rate and synaptic plasticity of neurons in the hypothalamic arcuate nucleus [39,40], it is tempting to speculate that insulin exerts similar effects.

Recent studies have indicated that insulin has an impact not only on the direct control of food intake and energy expenditure, but also on brain pathways associated with reward. Figlewicz *et al.* [41] have demonstrated that insulin, independently of its anorexigenic effects [42], directly activates neurons of the mesolimbic dopamine-mediated pathway that is implicated in the motivating, rewarding and reinforcing properties of natural stimuli such as food (reviewed in ref. [43]). Present studies are addressing whether the inhibitory influences of insulin and leptin on food intake can be overruled by palatability or by hedonic attributes of food that activate motivational pathways [44], which might be a key factor in the development of obesity and insulin resistance in western populations.

Central insulin action and peripheral glucose metabolism

Similar to leptin, which has been shown to regulate hepatic glucose fluxes largely via its central receptors [45], insulin also modifies peripheral glucose metabolism through IRs localized in the hypothalamus. Obici *et al.* [46] have shown that central administration of insulin increases insulin sensitivity in peripheral tissues. This improvement in peripheral insulin action is associated with a reduction in hepatic glucose production, but not

with an increase in glucose uptake in skeletal muscle and adipose tissue. In addition, mice with targeted disruption of the IR specifically in the brain (termed NIRKO mice) do not suppress hepatic glucose production normally in response to a hyperinsulinemic clamp (S.J. Fisher *et al.*, unpublished).

The effect of centrally administered insulin on hepatic glucose production is not blunted by centrally administered melanocortin receptor antagonists, indicating that the reduction in hepatic glucose production is not mediated via insulin-induced activation of the melanocortin receptor system that is naturally activated by α-MSH [47]. Pharmacological inactivation of the main downstream targets of insulin signaling shows that inhibition of PI3K, but not inhibition of MAPK, reverses the reduction. Furthermore, intracerebroventricular administration of K_{ATP} blockers suppresses the effect of insulin on hepatic glucose production, revealing a possible novel site of the central effects of insulin on peripheral glucose production [21,46].

Conversely, chronic antagonism of melanocortin receptor 4 (MC4R) leads to a marked increase in food intake with subsequent abdominal adiposity and reduces both the reduction in hepatic glucose production and the uptake of glucose into peripheral tissues in response to hyperinsulinemia; by contrast, chronic intracerebroventricular administration of the MC4R agonist α-MSH results in the opposite effect [48]. Thus, the chronic bidirectional modulation of central melanocortin receptors has a role in regulating not only body weight and body composition, but also hepatic and peripheral insulin sensitivity.

Strong evidence indicates that the brain, in particular the hypothalamus, is important for controlling compensatory responses to hypoglycemia in addition to its regulation of energy metabolism (reviewed in [49]). These compensatory responses comprise glucagon release from the pancreas and sympathoadrenal activation, resulting in the secretion of epinephrine. In the ventromedial portion of the hypothalamus (VMH), specific neurons are influenced by glucose availability and affect the activity of the sympathetic nervous system [50]. Focal lesioning in this area abolishes the hormonal response to systemic hypoglycemia, suggesting that the VMH might be the region in the CNS that is responsible for activating counter-regulatory mechanisms [51]. Moreover, production of local neuroglycopenia in the VMH, despite systemic normoglycemia, has been shown to provoke the release of counter-regulatory hormones such as glucagon, cortisol and norepinephrine [51], whereas compensatory responses to systemic hypoglycemia are impaired when the VMH region is bilaterally perfused with glucose [52].

Notably, specific neurons in the lateral hypothalamus and the VMH seem to be capable of sensing changes in ambient glucose concentrations. Two different types of glucose-sensing neuron, namely glucose-responsive and glucose-sensitive neurons, have been identified [50]. If interstitial glucose levels rise, the firing rate of glucose-responsive neurons increases, whereas the firing rate of glucose-sensitive neurons decreases.

Although little is known about the sensing mechanism of glucose-sensitive neurons, glucose-responsive neurons seem to use K_{ATP} channels (reviewed in [53]). Even though explicit data on expression of the IR in glucose-responsive neurons are not available, indirect evidence indicates that the IR is present, because insulin has been shown to activate K_{ATP} channels in this group of neurons [37]. Notably, neurons expressing neuropeptide Y and pro-opiomelanocortin have been suggested to be important in glucose sensing because they show characteristics typical of glucose-responsive neurons, such as the expression of glucokinase (Figure 2). This observation provides a link between glucose sensing and feeding behavior at a cellular level and highlights the role of the IR in the brain in controlling the complex network that regulates the energy needs of the body. Consistent with these findings, NIRKO mice show a blunted sympathetic counter-regulatory response during hyperinsulinemic–hypoglycemic clamp studies [54].

Regulation of reproduction

Clinical observations assessing the association of obesity with infertile conditions [55,56] have identified a potential link between reproductive and metabolic networks. NIRKO mice show mild diet-sensitive obesity and reduced fertility owing to hypothalamic dysregulation of luteinizing hormone [23]. Similarly, mice lacking IRS-2 in all tissues including the brain are obese and have small, anovulatory ovaries with reduced numbers of follicles as a consequence of impaired pituitary development and consecutive gonadotrophic insufficiency [57]. These observations indicate that the IR has an important functional role in the CNS with regard to the regulation of both energy homeostasis and reproductive endocrinology. This idea has been corroborated by recent results showing that both insulin and insulin-like growth factor-1 (IGF-1) have a stimulatory effect on the output of gonadotropin-releasing hormone in hypothalamic cells *in vitro* and *in vivo* [58,59].

The role of IR signaling in learning and memory

The role of insulin signaling in the regulation of glucose metabolism, learning and memory formation in the human and rodent brain is controversial. The conflicting information on insulin action in the CNS stems from difficulties in dissecting the direct actions of insulin from the effects resulting from hypoglycemia after peripheral administration of insulin (reviewed in [60]). Nevertheless, systemic infusion of insulin under euglycemic hyperinsulinemic conditions in healthy humans yields a significant improvement in verbal memory and selective attention [61]. Furthermore, intranasal administration of insulin induces a rapid increase in the insulin concentration in cerebrospinal fluid without affecting blood glucose levels [62,63]. This treatment has been associated with the facilitation of processing in working memory [64,65], which again suggests that insulin influences brain function directly and independently of changes in peripheral glucose.

Clinically, individuals affected with type 2 diabetes who are >64 years of age perform worse in learning tasks than

do age-matched non-diabetic subjects [66,67]. In younger individuals with diabetes, however, no evidence of learning and memory impairment has been detected [68]. Individuals suffering from Alzheimer's disease have lower cerebrospinal fluid and higher plasma insulin concentrations [69], which could indicate an impairment in insulin metabolism in the brain. In keeping with this, administration of insulin to individuals with Alzheimer's disease has been shown to result in an improvement in memory and performance [70].

By contrast, NIRKO mice show normal spatial learning in the Morris water maze task [71]. Indicating that IR signaling does not play a primary, substantial role in memory formation. However, deletion of the IR occurs in early development in NIRKO mice; thus, a lack of the IR might be compensated over time by other mechanisms, which could explain the unaltered learning and formation of memory in these mice. Taken together, animal studies have provided little direct experimental evidence to suggest that IR signaling has a substantial role in the regulation of memory.

The role of IR signaling in neurodegenerative diseases

The clinical association between diabetes mellitus and neurodegenerative disease is well established. Individuals suffering from Alzheimer's disease and Parkinson's disease show reduced expression of the IR in brain [72,73], raising issues of whether this phenomenon is a cause or a consequence of neurodegeneration, and whether neuronal insulin resistance indeed represents a risk factor for these disorders.

On a molecular level, several targets of the insulin signaling machinery with potential influence on the development of neurodegenerative diseases have been identified. For example, Hong *et al.* [74] have shown that glycogen synthase kinase-3 (GSK-3) can phosphorylate the microtubule-associated protein tau in cultured human neurons. In both cultured neurons and transgenic mice, overexpression of the active form GSK-3β results in increased phosphorylation of tau [75]. In addition, insulin and its related hormone IGF-1 reduce phosphorylation of tau protein by inhibiting the activity of GSK-3β [74].

Hyperphosphorylated tau is the principal component of paired helical filaments in neurofibrillary lesions associated with Alzheimer's disease [76]. Hyperphosphorylation reduces the affinity of tau for microtubules and is thought to be a crucial event in the pathogenesis of tauopathies (reviewed in [77]). Notably, NIRKO mice show a decrease in steady-state phosphorylation of protein kinase B (also known as AKT) and phosphorylation of GSK-3β, leading to hyperphosphorylation of tau [71]. In addition, IRS-2-deficient mice show tau hyperphosphorylation and develop intracellular deposits of hyperphosphorylated tau during aging [78]. These findings clearly indicate that IR and IGF-1 signaling have an essential role in regulating the phosphorylation of tau *in vivo*.

Previous studies have suggested that insulin and IGF-1 support neuronal survival *in vitro* and *in vivo*. In particular, insulin and IGF-1 strongly activate PKB to promote phosphorylation of the Bcl-2 antagonist of cell death (BAD) and its subsequent association with protein 14–3-3, which in turn releases Bcl-2 to inhibit apoptosis [79]. Conversely, the lack of neurodegeneration in both NIRKO and IRS-2-deficient mice shows that the IR and the IRS-2 branch of the IR and/or IGF-1 receptor (IGF1R) signaling pathway is not essential for neuronal survival *in vivo*, although alteration of this pathway causes hyperphosphorylation of tau. Whether these signaling pathways contribute significantly to neuronal survival under neuropathological conditions remains unclear.

IR- and IGF1R-mediated signals also regulate the secretion of amyloid precursor protein (APP). Notably, insulin resistance caused by diet-induced obesity results in marked increase in β-amyloid levels and age-dependent memory impairment in Tg2576 mice [80]. These mice express the Swedish double mutation of APP (K670N/M671L) and are a well-established animal model of Alzheimer's disease. Although Tg2576 mice show a marked increase in β-amyloid levels beginning at 2 months of age, and form extracellular plaques between 8 and 12 months of age, they suffer only very little neuronal loss [81]. The lack of neurodegeneration in these mice might be caused by activated survival signaling associated with a rise in PKB phosphorylation, which is due to an increase in expression of IGF-2, a potent ligand of the IR in brain [81]. These data suggest that IR and IGF1R signaling have a neuroprotective function under conditions of neurodegeneration.

Consistent with such a function, Carro *et al.* [82] have shown that IGF-1 has a protective effect on the development of amyloidosis in Tg2576 mice. Furthermore, insulin seems to be involved in clearing β-amyloid from the brain [82]. Insulin inhibits the breakdown of β-amyloid by competitively blocking insulin-degrading enzyme – one of the main proteases involved in degradation of β-amyloid [83,84]. These studies directly link IR and IGF1R signaling to important features of neurodegeneration.

Taken together, these findings show that insulin resistance influences tau phosphorylation and APP metabolism and thus might contribute to neuronal survival under neurodegenerative conditions.

Concluding remarks

Although the CNS has been classically considered to be an insulin-insensitive tissue, components of the insulin signaling pathway are widely distributed throughout the CNS and insulin has been shown to reach these components by crossing the blood–brain barrier. Recent advances in our knowledge of IR function in the CNS have yielded new insights into the role of IR signal transduction in the regulation of energy homeostasis, reproduction and neuronal survival (Box 1). Neuronal IRs are not only involved in the regulation of feeding and energy expenditure, but also have an impact on peripheral glucose metabolism. The molecular mechanisms underlying these processes have not been completely unraveled, but they seem to involve the activation of PI3K and K_{ATP} channels. Recent data have also highlighted the effects of insulin and IGF-1 on the molecular and cellular mechanisms underlying human neurodegeneration, such as tau phosphorylation and APP metabolism.

The availability of novel techniques to modulate the expression of genes encoding components of the insulin

Box 1. Physiological functions of IR signal transduction in the rodent brain

- Food intake
- Inhibition of hepatic gluconeogenesis
- Counter-regulation to hypoglycemia
- Reproduction
- Modulation of tau phosphorylation
- Metabolism of APP and β-amyloid clearance
- Neuronal survival
- Memory

signaling pathway in defined neuronal populations of mice should facilitate further insight into the exact site of insulin action in the regulation of these processes and the molecular mechanisms involved.

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