Review

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CNS insulin signaling in the control of energy homeostasis and glucose metabolism – from embryo to old age

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Central nervous system (CNS) insulin signaling regulates energy and glucose homeostasis by acting on hypothalamic neurocircuits and higher brain circuits such as the dopaminergic system. However, overnutrition, obesity, and type 2 diabetes mellitus (T2DM) induce insulin resistance selectively in different regions of the brain, thereby impairing energy homeostasis and augmenting disease progression. Moreover, fetal hyperinsulinemia in response to maternal overnutrition, obesity, and diabetes disrupts hypothalamic neurocircuit development and predisposes to metabolic disorders later in life. In light of the current obesity and diabetes epidemic, we review the molecular basis of insulin action and resistance in the CNS, mechanisms which are causal to the development of these metabolic disorders, both in the neonate and in the adult.

Obesity, insulin, and the brain

Over the past decades the incidence of obesity has been steadily increasing towards epidemic proportions [1]. Although the impact of obesity as a disease is still not well accepted in public, the constant rise of obesity-associated comorbidities, such as T2DM, cardiovascular diseases, and cancer, demonstrate the necessity to define the molecular mechanisms underlying the onset, manifestation, and progression of obesity, as well as of its associated comorbidities, to ultimately combat this epidemic.

As one of the main consequences of obesity, T2DM is characterized by chronic hyperglycemia, and initially hyperinsulinemia, due to relative insulin resistance of insulin-target tissues. Under healthy conditions, insulin is secreted postprandially from pancreatic β cells in response to elevated blood glucose levels to regulate metabolic processes, such as peripheral glucose uptake, lipid synthesis, or inhibition of hepatic gluconeogenesis ([2] for review). However, in addition to the indisputable importance of insulin-mediated responses in peripheral target tissues, insulin signaling in the CNS is essential for the regulation of energy and glucose homeostasis, as well as for reproduction [3]. Moreover, insulin signaling in the brain has been implicated in mediating the counter-regulatory sympathoadrenal response to hypoglycemia, at least in part, by altering the sensitivity of glucose-sensing neurons in the hypothalamus [4,5]. Thus, in the extensive search for therapeutic approaches and preventative measures, the CNS has emerged as an important target in tackling the obesity epidemic. In this article we aim to summarize and review critically current advances in the field of central insulin signaling in the control of energy homeostasis, as well as the contribution of impaired or altered insulin action in the brain to the onset, development, and manifestation of obesity and its associated diseases.

Historical background

The notion that the CNS is a direct target of insulin action to regulate energy homeostasis emerged in the 1970s. Upon the finding that the insulin receptor (IR) is widely distributed throughout the brain, with marked regional variations in receptor density [6], pioneering work by Woods and Porte Jr established a role for central insulin in regulating feeding behavior and body weight in rats and monkeys. Chronic intracerebroventricular (icv) infusion of insulin in baboons resulted in a robust reduction of food intake that was associated with a decrease in body weight [7]. Moreover, icv insulin infusion in rats confirmed these findings and further suggested a possible link between brain insulin resistance and obesity because the effects of centrally applied insulin on energy homeostasis were blunted in obese, diabetic Zucker rats [8,9].

To date, the physiological role of insulin signaling in the CNS and its mode of action to regulate energy homeostasis and glucose metabolism remains a matter of debate. In rodents, insulin signaling in the CNS is critical for the suppression of hepatic glucose production (HGP). This aspect of central insulin depends upon phosphoinositide 3-kinase (PI3K)-mediated activation of ATP-dependent potassium (K_{ATP}) channels in the hypothalamus, specifically in orexigenic agouti-related peptide (AgRP) neurons, which in turn regulate efferent vagal innervation of the liver [10–13]. Some experiments indicate that a

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subsequent increase in hepatic interleukin-6 (IL-6) induces signal transducer and activator of transcription 3 (STAT3) phosphorylation, leading to inhibition of key gluconeogenic gene expression; in other words, glucose-6-phosphatase (*G6Pase*) and phosphoenolpyruvate carboxykinase (*Pepck*), and therefore to a decrease of net glucose output [11–15]. Nevertheless, central insulin-mediated suppression of HGP also depends upon IL-6-independent pathways because mice with hepatocyte-specific IL6 receptor α (IL6Rα) deficiency still show robust hepatic STAT3 phosphorylation that is associated with a reduction of *G6Pase* expression during euglycemic–hyperinsulinemic clamp studies [16]. Furthermore, central insulin signaling also regulates peripheral fat metabolism by modulating sympathetic innervation of white adipose tissue (WAT) to inhibit lipolysis, which reduces the availability of gluconeogenic substrates to the liver, additionally suppressing HGP [17,18].

However, studies in humans and dogs challenge the physiological relevance of CNS insulin action in the control of HGP. In contrast to rodents, insulin action in the brain fails to inhibit HGP in dogs, but instead suppresses net hepatic glucose output by decreasing glucose uptake and glycogen synthesis [19]. Moreover, insulin's direct effects on the liver are reported to be dominant in the suppression of net hepatic glucose output and are sufficient for normal glucose metabolism [20,21]. Nevertheless, although brain insulin action might have minor effects on HGP in healthy, lean individuals, the consistent findings from rodent studies suggest that additional or putatively shifted target sites for insulin action regulate energy homeostasis under pathological conditions such as those encountered in obesity and T2DM.

Neuroanatomy of insulin action in the control of energy homeostasis

The arcuate nucleus of the hypothalamus (ARC)

The most intensively studied region of insulin action in the brain is the ARC. Owing to its location at the mediobasal hypothalamus adjacent to the third ventricle and the median eminence, a region in which the blood–brain barrier (BBB) is only weakly formed [22], the ARC is designed to integrate peripheral hormonal signals reflecting the energy status of the body. Thus, neurons in the ARC are ideally positioned to translate this information into appropriate neuronal responses that adapt energy homeostasis to the current needs of the body. Transduction of signals from the periphery into behavioral responses is orchestrated by two functionally antagonistic neuronal populations that reside in the ARC: the anorexigenic proopiomelanocortin (POMC)-expressing neurons and the orexigenic agouti-related peptide (AgRP) and neuropeptide Y (NPY)-coexpressing neurons [23,24]. In the postprandial state, the precursor protein POMC is processed into α-melanocyte-stimulating hormone (α-MSH), which is subsequently released from POMC-neuron synaptic endings to activate melanocortin 3 and 4 receptors (MC3/4R) on downstream neurons located mainly in the paraventricular hypothalamic nucleus (PVN) (Figure 1). Moreover, MCR-positive target neurons reside in the dorsomedial hypothalamus (DMH) and lateral hypothalamic area (LHA). MCR-activation decreases food intake, increases energy expenditure, and regulates glucose metabolism ([25] for review). By contrast, AgRP/NPY neurons are activated under fasted conditions to induce feeding, inhibit energy expenditure, and regulate glucose metabolism in multiple ways [23,24,26]. On one hand, AgRP acts as an inverse agonist of α-MSH on MC4Rs, thereby preventing α-MSH-mediated neuronal responses [27]. On the other hand, NPY mediates its orexigenic effects via several NPY receptor subtypes. Importantly, AgRP/NPY neurons can further directly hyperpolarize and thus inhibit POMC neurons by synaptic release of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) on POMC somata [28] (Figure 1).

Although acute ablation of both POMC and AgRP/NPY neurons in adult mice demonstrated their crucial role in the regulation of energy homeostasis and glucose metabolism [23,24], the relative contribution of insulin signaling in mediating these effects remains elusive. Surprisingly, specific deletion of the IR in either of these neuronal subtypes did not result in altered feeding behavior or energy expenditure [13]. However, insulin-mediated suppression of HGP was blunted in mice lacking IR specifically on AgRP neurons, demonstrating the role of central insulin in the regulation of glucose metabolism [13]. In addition, specific reconstitution of the IR in AgRP neurons of L1 mice (mice that exclusively express the IR in liver, pancreatic β cells, and several regions in the brain, but which exhibit a 90% reduction of IR expression in the ARC) further supported this finding. Here, HGP levels of L1 mice were restored to normal levels upon AgRP-specific reconstitution of the IR [29]. By contrast, POMC-specific deletion of IR had no effect on HGP [13]. However, specific reconstitution of the IR in POMC neurons of L1 mice further exacerbated their hepatic insulin resistance, but at the same time restored locomotor activity, suggesting a role for insulin signaling in POMC neurons in the regulation of locomotor activity and energy expenditure [29]. Together, these findings demonstrate that hypothalamic insulin signaling contributes to the control of glucose metabolism and energy expenditure in a cell type-specific manner.

At a cellular level, insulin regulates transcriptional events, leading to increased *Pomc* and decreased *Agrp* gene expression [30]. Insulin mediates phosphorylation of the forkhead transcription factor 1 (FOXO1) (Box 1), which functions as a transcriptional repressor of the *Pomc* gene and as a transcriptional activator of the *Agrp* gene (Figure 1). Thus, insulin-mediated phosphorylation and subsequent nuclear exclusion of FOXO1 de-inhibits the POMC promoter, thus allowing transcriptional activators (e.g., phosphorylated STAT3) to bind. At the same time, FOXO1-induced expression of *Agrp* in AgRP neurons is inhibited ([31] for review). Moreover, insulin hyperpolarizes both POMC and AgRP/NPY neurons by activating K_{ATP} channels in a PI3K-dependent manner [13,32,33]. This identical effect of insulin signaling on the neuronal activity of two functionally opposing populations, together with the mild effects seen in mice specifically lacking the IR on POMC or AgRP neurons ($POMC^{\Delta IR}$ and $AgRP^{\Delta IR}$), respectively, suggests that in lean animals signals other than insulin are more relevant to regulate food intake and energy homeostasis via their action on the melanocortin

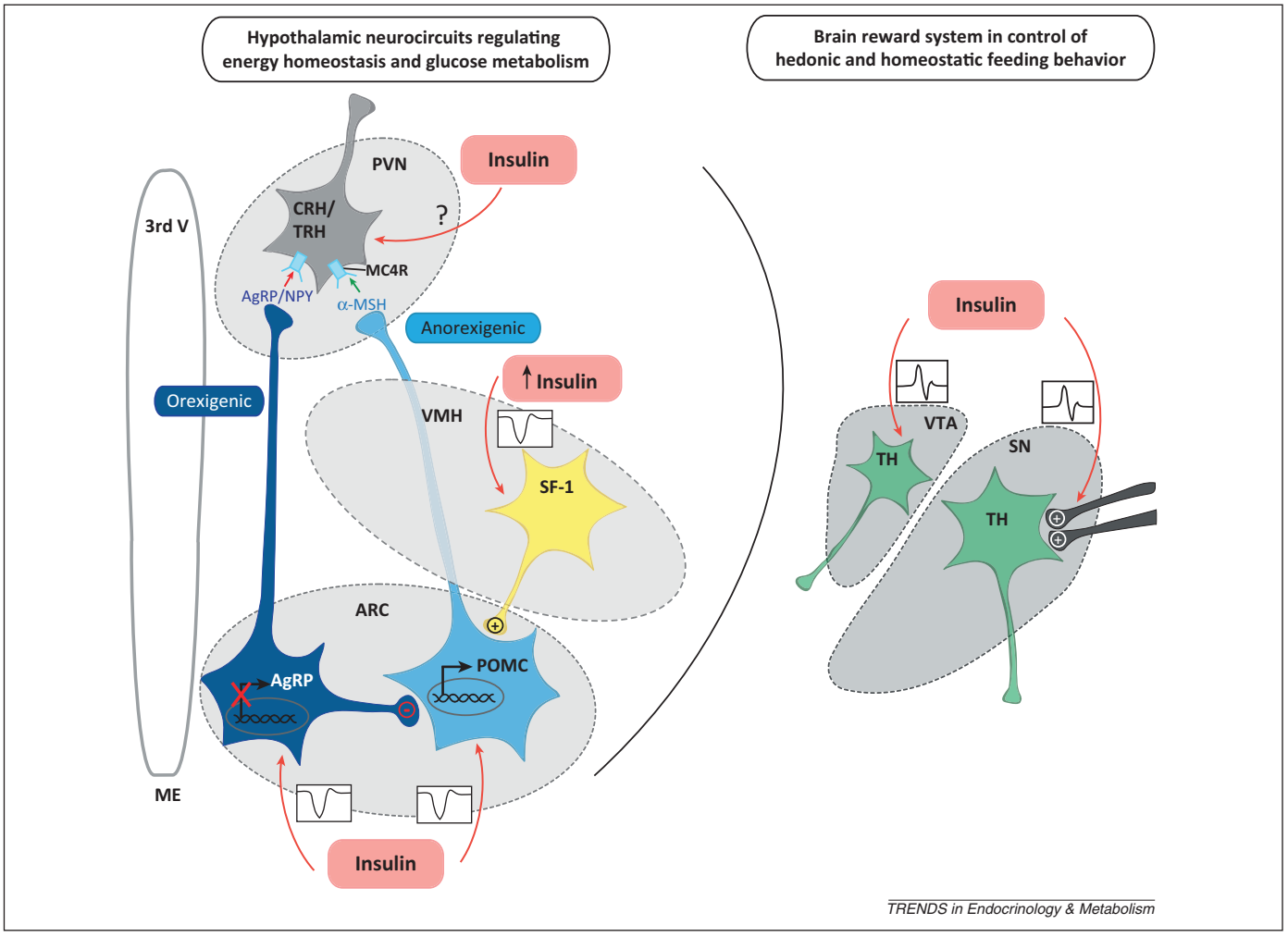

Figure 1. Insulin-responsive neurocircuits in control of energy homeostasis. Insulin directly acts on distinct neuronal populations to adapt energy homeostasis and glucose metabolism. In the ARC, the role of insulin signaling on neuronal activity seems to be complex because it hyperpolarizes both anorexigenic POMC neurons as well as orexigenic AgRP/NPY neurons. Nevertheless, by inducing *Pomc* transcription, and at the same time inhibiting *Agrp* expression, insulin increases the anorexigenic tone upon feeding. Moreover, AgRP neurons provide GABAergic input on POMC neurons and are thereby able to inhibit POMC neuronal activity directly. Neurons residing in the ARC project to the PVN (among other nuclei). Here, α-MSH (derived from the precursor protein POMC) and AgRP bind to MC4Rs to either induce or inhibit the anorexigenic response of CRH and/or TRH neurons, respectively. However, the direct role of insulin on neurons residing in the PVN is not known so far. Moreover, under conditions of hyperinsulinemia, insulin hyperpolarizes SF-1 neurons located in the VMH, which provide glutamatergic input on POMC neurons. In addition to acting on hypothalamic neurocircuits, insulin also depolarizes and thus activates TH neurons that reside in the VTA and SN, and further increases the excitatory input onto these neurons, thereby regulating hedonic as well as homeostatic feeding behavior. AgRP, agouti-related peptide; ARC, arcuate nucleus; CRH, corticotropin releasing hormone; GABA, γ-aminobutyric acid; MC4R, melanocortin 4 receptor; ME, median eminence; α-MSH, α-melanocortin stimulating hormone; NPY, neuropeptide Y; POMC, proopiomelanocortin; PVN, paraventricular nucleus; SF-1, steroidogenic factor 1; SN, substantia nigra; TH, tyrosine hydroxylase; TRH, thyrotropin releasing hormone; 3rd V, third ventricle; VMH, ventromedial nucleus of the hypothalamus; VTA, ventral tegmental area.

cells. Indeed, a primary regulator of energy homeostasis is leptin, a hormone secreted by WAT in relation to overall fat content of the body. Insulin and leptin act synergistically via similar but distinct hypothalamic signaling pathways to mediate their full anorexigenic effect [34–36]. Of note, acute neuronal responses of POMC neurons to insulin or leptin occur in two different subpopulations [37]. In a recent study by Ren *et al.* the authors describe a novel alternative pathway in AgRP/NPY neurons that controls energy homeostasis in a FOXO1-dependent activation of the orphan G protein-coupled receptor 17 (GPR17). Here, GPR17 is activated under fasting conditions to modulate ion-channel activity that leads to increased orexigenic neuropeptide secretion, as well as to elevated glutamatergic input to AgRP/NPY neurons [38]. By contrast, under fed conditions, insulin- and leptin-mediated inactivation of FOXO1 reduces GPR17 expression to ultimately decrease food intake and HGP [38]. Despite these novel findings, identifying the insulin target sites in the CNS that are responsible for the anorexigenic response seen upon icv insulin infusion [7–9], and further deciphering the molecular mechanisms critical in mediating these effects under physiological and pathophysiological conditions, will be of great importance.

The ventromedial nucleus of the hypothalamus (VMH)

A less well studied but crucial insulin target site in the hypothalamus that is also involved in the regulation of energy homeostasis is the VMH. Direct insulin infusion into the VMH decreases food intake and body weight in rats [39]. Moreover, insulin-mediated activation of K_{ATP} channels hyperpolarizes a subpopulation of VMH neurons [32,40], which are clustered in the mediobasal part of the VMH, a region that provides glutamatergic projections to POMC neurons [41] (Figure 1). Steroidogenic factor 1 (SF-1) is a marker for VMH neurons (as well as for testes,

Box 1. Molecular basis of insulin action in the CNS

Similarly to insulin action in peripheral target tissues (i.e., liver, skeletal muscle, and WAT), insulin signaling in the brain mediates its plethora of effects mainly through the PI3K and MAPK/ERK signaling pathways.

Binding of insulin to its receptor leads to rapid autophosphorylation of the IR and thereby to further activation of its intrinsic kinase activity, resulting in tyrosine phosphorylation of IRS proteins. Unlike in the periphery, where IRS-1 is essential for signal-transduction, IRS-2 is the main mediator of insulin intracellular responses in the brain [69,70]. Apart from its relatively high expression in the hypothalamus, the crucial role of IRS-2-mediated signal transduction in the regulation of energy homeostasis was highlighted by the obese, insulin-resistant and glucose-intolerant phenotype of mice with a neuron-specific IRS-2 deletion, either throughout the brain or in specific neuronal populations [71,72]. Phosphorylated IRS-2 serves as a docking platform for proteins harboring a Src-homology (SH) domain, for example, the p85 regulatory subunit of PI3K. Subsequent phosphorylation of the membrane-bound phosphatidyl-inositol-4,5-bisphosphate (PIP2) to phosphatidyl-inositol-3,4,5-trisphosphate (PIP3) by the p110 catalytic subunit of PI3K results in a context-specific activation of several downstream molecules. On the one hand, PIP3 activates K_{ATP} channels, leading to potassium outflow and thus hyperpolarization and silencing of neurons [33,73]. On the other hand, PIP3 activates phosphoinositide-dependent kinase 1 (PDK1), which in turn phosphorylates and thus activates the kinase AKT to elicit downstream signaling events. AKT phosphorylates FOXO-1, which results in nuclear exclusion and thus inhibition of FOXO-1-mediated activation and/or repression of target genes [74–76]. Moreover, AKT phosphorylates the mammalian target of rapamycin (mTOR), a critical regulator of protein synthesis and inhibitor of autophagy [77,78]. The importance of neuronal PI3K-mediated signals in the regulation of food intake has been demonstrated by icv administration of specific PI3K inhibitors that blunted the anorexigenic effects of central insulin [79]. However, the role of IR-mediated activation of the MAPK/ERK-signaling in the regulation of energy homeostasis is less well defined so far. Apart from one *in vitro* study demonstrating that insulin-mediated inhibition of *Npy* and *Agrp* gene expression depends upon activation of the MAPK/ERK pathway [80], it is unknown to what extent insulin acts via this pathway to maintain energy homeostasis.

spleen, adrenal glands, and the pituitary), and mice expressing the Cre recombinase under the control of the SF-1 promoter have thus been commonly used to genetically modify VMH neurons [40,42,43]. Only recently, Klöckener *et al.* showed that hyperinsulinemia-mediated silencing of SF-1 neurons in the VMH contributes to the development of obesity and impaired glucose metabolism. Interestingly, mice with specific deletion of the IR in SF-1 neurons (SF-$1^{\Delta IR}$) that were exposed to a high-fat diet (HFD) displayed a reduction in body weight, as well as improved glucose tolerance and insulin sensitivity, that could at least partially be attributed to a decrease in food intake [40]. Of note, this protection from diet-induced obesity was associated with an increased firing rate of POMC neurons in SF-$1^{\Delta IR}$ mice, most probably due to increased activity of glutamatergic projections from SF-1 neurons. However, another study reported that lentiviral knockdown of IR specifically in the VMH resulted in impaired glucose metabolism, as well as impaired pancreatic α- and β-cell function, in adult non-diabetic rats [44]. Although these differences in the metabolic outcome of VMH-specific IR-deficiency are most probably due to differences in study design (Cre–loxP mediated deletion of IR in SF-1 neurons starting during mouse development versus lentiviral knockdown of the IR in all cells of the VMH in adult rats; and HFD-induced diabetes and obesity in mice versus the use of non-diabetic rats), they demonstrate the need to dissect insulin-mediated regulation of energy homeostasis further in different regions of the brain to understand its contribution in the development of obesity and T2DM.

The brain reward system

Apart from acting on hypothalamic neurocircuits that directly regulate energy homeostasis, insulin also acts on dopaminergic midbrain neurons (DA neurons) of the brain reward system. Importantly, the desire to eat is not only driven by the current energy or nutritional status of the body, but also by the rewarding or hedonistic aspect of food ([45] for review). This reward-based eating behavior is thought to contribute significantly to the development and manifestation of obesity because signaling of these higher neuronal circuits can eventually override hypothalamic signaling. In dopaminergic neurons located in the ventral tegmental area (VTA) and substantia nigra (SN), the IR is coexpressed with tyrosine hydroxylase (TH), a key enzyme and marker for catecholaminergic neurons [46]. Insulin increases the firing rate of a subset of DA neurons and further promotes excitatory synaptic inputs onto these cells [47] (Figure 1). Moreover, icv injection of insulin modulates reward-related behavior; in other words it decreases acute sucrose intake [48] as well as conditioned place-preference for high-fat food [49]. Furthermore, IR-deficiency specifically in catecholaminergic neurons ($Th^{\Delta IR}$) increases sensitivity to low-concentration sucrose solution in comparison to control mice [47]. Similarly, direct administration of insulin into the VTA reduces hedonic feeding under sated conditions [50]. Interestingly, $Th^{\Delta IR}$-mice develop an obese phenotype that is associated with an increase in food intake [47], demonstrating a direct role for insulin signaling in catecholaminergic neurons in the regulation of food intake and energy homeostasis. However, the underlying molecular mechanisms resulting from insulin signaling on neurons involved in the brain-reward neuronal circuitry leading to altered hedonic, but also homeostatic feeding behavior, are far from being understood. Thus, future experiments will clearly be needed to decipher the exact neuronal populations, as well as insulin-mediated alterations in dopaminergic signaling, and the identification of downstream neurons responsible for the aforementioned effects on homeostatic and hedonic food intake.

Taken together, insulin does not only regulate feeding behavior by acting on hypothalamic nuclei, but also acts on other brain areas involved in this process, ultimately to adjust food intake and energy expenditure to the current needs of the body. However, if central insulin action (in combination with other factors) is able to regulate energy homeostasis in such a tightly controlled manner, the question arises as to why the incidence of obesity has been steadily increasing over the last decades.

Molecular basis of neuronal insulin resistance

The constant availability of highly palatable fat-rich foods, together with the trend towards a more sedentary lifestyle

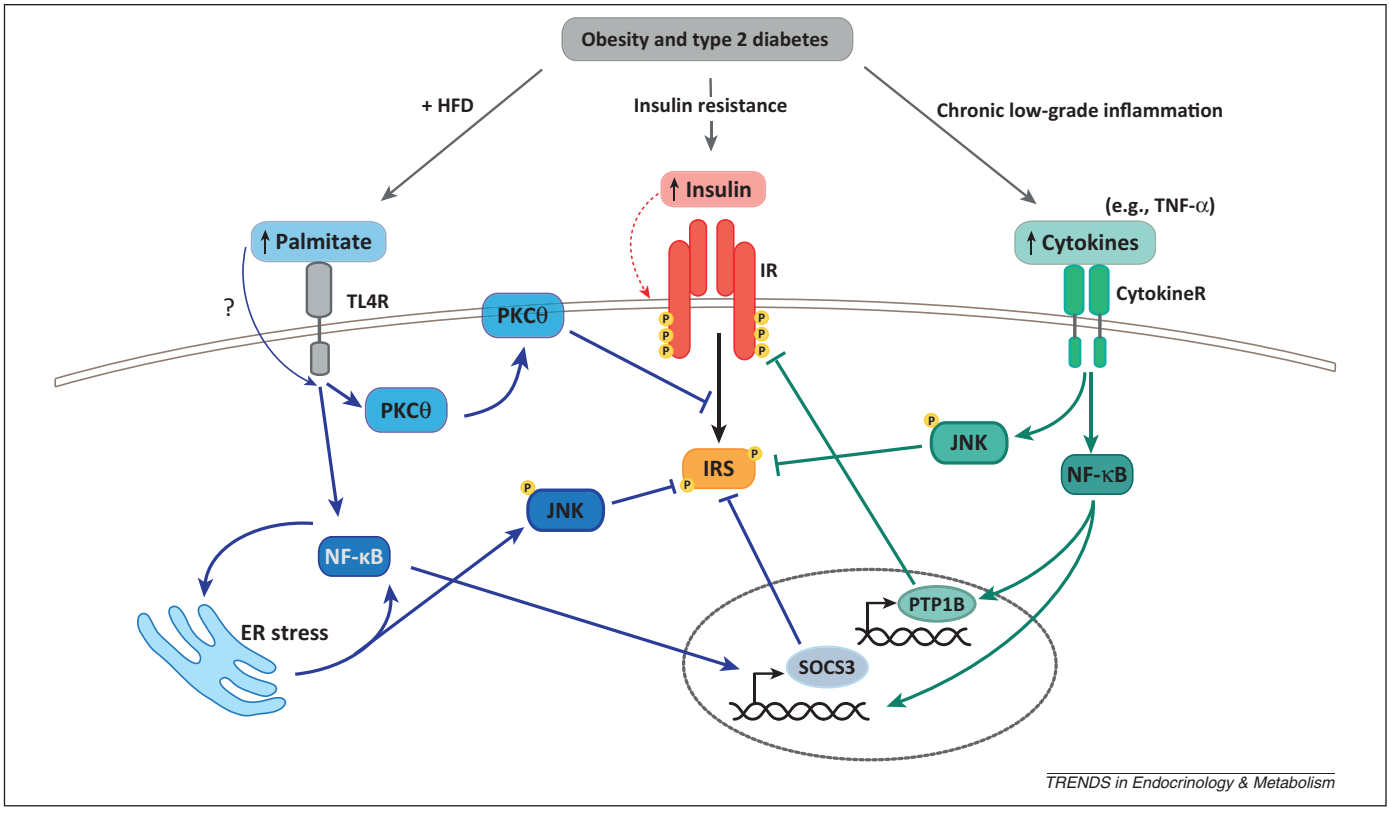

Figure 2. Molecular basis of neuronal insulin resistance. Obesity and type 2 diabetes mellitus (T2DM) are associated with elevated concentrations of insulin, saturated fatty acids (FAs, such as palmitate) and cytokines (such as TNF-α), both in circulation and locally in the hypothalamus, eventually leading to central insulin resistance. Chronic hyperinsulinemia desensitizes IRs, thereby reducing insulin-evoked signaling pathways via IRS proteins. Moreover, increased levels of palmitate induce intracellular signaling cascades, at least in part via TL4Rs, that further interfere with IR-mediated phosphorylation of IRS proteins. Palmitate activates PKCθ by inducing its translocation to the cell membrane where it directly interacts with the IR and IRS proteins. In addition, palmitate activates the transcription factor NF-κB, which induces the expression of one of the main negative regulators of insulin signaling, SOCS3. SOCS3 interferes with insulin-induced phosphorylation of the IR and its downstream molecules and further targets IRS proteins for proteasomal degradation. Additionally, NF-κB induces ER stress, which further accelerates NF-κB activity. ER stress results in JNK phosphorylation, which in turn leads to inhibitory phosphorylation events on IRS proteins. Similarly, increased hypothalamic levels of TNF-α also activate JNK and NF-κB, as well as their downstream signaling events. TNF-α further induces expression of PTP1B, at least in part via NF-κB, which dephosphorylates the IR and thereby inhibits insulin-mediated signaling events. CytokineR, cytokine receptor; ER, endoplasmic reticulum; HFD, high-fat diet; IR, insulin receptor; IRS, insulin receptor substrate; JNK, cJun N-terminal kinase; NF-κB, nuclear factor κ light-chain; p, phosphorylation; PKCθ, protein kinase Cθ; PTP1B, protein tyrosine phosphatase 1B; TL4R, toll-like receptor 4; enhancer of activated B-cells; TNF-α, tumor necrosis factor α; SOCS3, suppressor of cytokine signaling 3.

in western societies, play an important role in the current obesity epidemic. Consumption of HFD for time periods as short as 72 h is sufficient to reduce hypothalamic insulin sensitivity, independent of changes in body weight and fat mass in rodents [51]. Moreover, elevated levels of saturated fatty acids (FAs), in the presence of normal caloric intake, not only increase body weight but also chronically reduce hypothalamic insulin sensitivity that is associated with hyperphagia even after dietary intervention [51]. At a molecular level, saturated FAs such as palmitate or stearate cross the BBB and accumulate in the hypothalamus, where they activate local inflammatory signaling cascades at least partially via toll-like receptor (TLR) 4 signaling, resulting in central insulin and leptin resistance [52] (Figure 2). On the one hand, palmitate mediates central insulin resistance by activating protein kinase Cθ (PKCθ). Subsequent translocation of PKCθ from the cytoplasm to the inner surface of the cell membrane is thought to interfere with the ability of insulin to activate PI3K by interacting directly with IR and insulin receptor substrate (IRS) proteins [53] (Box 1 and Figure 2). On the other hand, palmitate activates the transcription factor nuclear factor NF-κB (κ light-chain enhancer of activated B cells), which subsequently induces suppressor of cytokine signaling (SOCS) 3 expression [54]. As one of the main negative regulators of insulin signaling, SOCS3 interferes with insulin-induced phosphorylation of the IR and its downstream molecules, and further targets IRS proteins for proteasomal degradation [55] (Figure 2). Moreover, elevated NF-κB signaling triggers endoplasmic reticulum (ER) stress, a cellular response to overnutrition that is implicated in peripheral as well as central insulin resistance [56,57]. Under chronic HFD exposure, ER stress and NF-κB activity even enhance each other, leading to acceleration of obesity and T2DM disease progression [54]. Moreover, ER stress results in increased activity of c-jun N-terminal kinase (JNK) (Figure 2). JNK in turn mediates inhibitory phosphorylation events on IRS serine residue 307 (Ser307), and thereby blocks insulin-mediated IRS tyrosine phosphorylation [56,58]. Consistently, increased levels of phosphorylated Ser307 have been observed in different models of obesity [59,60]. Hence, JNK-mediated phosphorylation of IRS1 on Ser307 was proposed to be a critical contributor to insulin resistance [58]. However, recent findings question a role of Ser307 in the development and/or manifestation of insulin resistance because they demonstrate that phosphorylation of Ser307 is essential for the maintenance of insulin sensitivity [61]. Thus,

although JNK deficiency in the brain protects against diet-induced obesity (DIO) [62], and icv administration of JNK inhibitors restores hypothalamic insulin signaling under HFD conditions [63], the underlying molecular mechanisms of how elevated JNK signaling contributes to the development of insulin resistance remains controversial.

As seen by the complex and interconnected molecular responses to HFD consumption that result in impaired central insulin signaling, prolonged overnutrition can lead to drastic weight gain. Importantly, obesity is associated with chronic low-grade inflammation (Figure 2). Thus, during obesity progression, elevated levels of proinflammatory cytokines, such as tumor necrosis factor (TNF) α, exacerbate central insulin resistance by further activating NF-κB and JNK signaling, as well as the aforementioned respective downstream signaling events. Additionally, TNF-α induces expression of protein tyrosine phosphatase (PTP) 1B, at least in part by transactivation of NF-κB [64] (Figure 2). Elevated levels of PTP1B in the ARC, as seen after 20 weeks of HFD feeding, inhibit insulin-mediated anorexigenic effects by direct dephosphorylation of the IR [65,66]. Owing to the late increase of PTP1B levels during obesity progression, PTP1B is not considered to be involved in the onset, but rather in the continued deterioration of insulin action in the brain [64]. Of note, most of these molecular mechanisms responsible for central insulin resistance have also been implicated in the development and manifestation of central leptin resistance, well established to be involved in the onset and progression of obesity and T2DM ([35] for review).

Overall, acute over- and/or malnutrition mediates persistent changes in the CNS that result in impaired efficiency of peripheral hormones to help adapt to changes in energy homoeostasis, ultimately resulting in hormonal resistance, dysregulated energy homeostasis, and obesity. These findings demonstrate the inability of our bodies to cope with present day energy abundance, and highlight the importance of delineating the molecular signaling pathways involved in this (dys-) regulation of central energy homeostasis, to eventually find novel therapeutic approaches to combat the obesity epidemic.

The concept of selective insulin resistance in the CNS

To further complicate matters, not all insulin-evoked signaling pathways (Box 1), neuronal populations or hypothalamic nuclei, are uniformly affected by central insulin resistance. For example, whereas PI3K activity in hypothalami of obese Zucker rats is decreased in response to insulin, compared to lean controls, insulin-mediated activity of the other principal branch of IR-signaling – the mitogen activated protein kinase/extracellular signal-regulated kinase MAPK/ERK pathway – remains unaffected [67]. Moreover, due to the different locations of the individual hypothalamic nuclei relative to the median eminence and the third ventricle, their accessibility to periphery-borne signals differs. Thus, while obesity-associated hyperinsulinemia might lead to receptor desensitization of neurons located in the ARC, ultimately resulting in insulin resistance, insulin action on neurons and non-neuronal cell types of other hypothalamic or non-hypothalamic nuclei might still be maintained or even augmented under these conditions. This hypothesis is supported by the findings from Klöckener *et al.* who showed that deficiency of insulin signaling in SF-1 neurons of the VMH has no effect on the metabolic phenotype of mice under normal conditions. However, when challenged with a HFD, SF-$1^{\Delta IR}$-mice are protected from DIO. By contrast, in wild type control mice HFD-induced hyperinsulinemia inhibits SF-1 neurons, which results in decreased glutamatergic input on POMC neurons, and thus contributes to the development of selective insulin resistance in the ARC [40]. Consistently, the increased hypothalamic inflammatory response upon HFD consumption, as seen by increased levels of TNF-α and IL-1β, is exclusively detected in the ARC and LHA, but not in the VMH or DMH [63]. Moreover, HFD consumption increases PTP1B activity (one of the main negative regulators of insulin signaling) twofold in the ARC, to a lesser extent in the VMH, and not at all in the LHA [64]. These differential effects of obesity-induced hyperinsulinemia on distinct areas in the brain underline the general concept of selective hormone resistance that has been described to also occur in the periphery (for a perspective see [68]). Here, similar to selective insulin and leptin resistance in the brain, elevated levels of these hormones in response to obesity or T2DM do not impact to the same extent upon the same intracellular pathways and/or cell types of the different peripheral organs. Again, although they are ineffective in some cell types due to the development of hormone resistance, insulin and leptin might induce or even augment cellular responses in other cells, thereby further exacerbating disease progression. Thus, selective hormonal resistance, both in the periphery and the brain, represents a key contributor to the complex clinical outcomes of obese and diabetic patients.

Concluding remarks

In summary, insulin signaling in the brain is a crucial regulator of energy homeostasis and glucose metabolism. Insulin acts on distinct neuronal populations within hypothalamic neurocircuits, as well as in non-hypothalamic areas that are classically not associated with the regulation of homeostatic feeding behavior, such as the dopaminergic system. However, numerous studies have demonstrated that the tight control of these neurocircuits becomes drastically diminished upon acute HFD exposure or in response to chronic overnutrition, as a result of selective insulin and leptin resistance, which further promotes disease progression. To find novel therapeutic approaches for the obesity epidemic it will be important to decipher insulin-mediated cellular and molecular changes in neurons located in other hypothalamic nuclei, such as the PVN, LHA or DMH, as well as in non-hypothalamic areas. Moreover, understanding the contribution of insulin signaling in non-neuronal cell-types such as glia cells in the regulation of energy homeostasis could further broaden our knowledge about potential therapeutic targets to restore central hormonal responses. Importantly, because insulin shares several signaling pathways with the adipocyte-derived hormone leptin, but also acts on distinct neuronal subpopulations, further delineating the different targets of these hormones will be valuable for future individualized therapeutic strategies. Finally, the notion that obesity does not only harm the

Box 2. Metabolic imprinting of hypothalamic neurocircuits

Overnutrition and obesity also increase the risk for the offspring to develop metabolic diseases. Human epidemiological and animal studies showed that abnormally elevated hormonal and nutrient levels, associated with obese and/or diabetic (i.e., T2DM and gestational diabetes) mothers, affect hypothalamic neurocircuit development, thereby predisposing the unborn child to developing metabolic disorders later in life [81,82], a phenomenon also known as 'metabolic imprinting' [83]. Development of hypothalamic neurocircuits consists of two major phases. First, the determination of neuronal cell number that includes neurogenesis, subsequent neuronal migration, and differentiation; and second, the formation of functional neurocircuits including the establishment of neuronal projections and synaptic connections [84]. Importantly, although both of these phases occur *in utero* in humans, hypothalamic neurocircuit development is not completed at birth, but instead continues until the third week of postnatal life in rodents [84–87]. Thus, because most studies are conducted in rodents, the two successive perinatal environments during gestation and lactation need to be considered when investigating metabolic imprinting.

Although maternal insulin cannot cross the placental barrier, maternal glucose is actively transported to the fetus, where it acts to stimulate insulin secretion early during fetal development, both in humans and rodents [88]. Under pathological conditions, maternal insulin-deficiency, as well as maternal hyperinsulinemia (a result of insulin resistance), leads to maternal hyperglycemia that in turn induces compensatory fetal and neonatal hyperinsulinemia [89], which are considered to be critical contributors to the perinatal programming for obesity and diabetes [90,91]. On a cellular level, fetal hyperinsulinemia increases POMC [92] and NPY [93] neuronal cell numbers (as well as of astrocytes [94]), possibly by increasing neurogenesis [95]. Additionally, abnormal insulin concentrations impair axonal formation of neurons residing in the ARC. Maternal hypo- and hyperinsulinemia decrease the fiber density of POMC and AgRP neurons that innervate the PVN, thereby further disrupting hypothalamic circuit organization in the offspring [92,96]. At a molecular level, perinatal hyperinsulinemia is associated with an increased ratio of hypothalamic orexigenic to anorexigenic neuropeptide expression, accompanied by upregulation of several inflammatory pathways, similar to what is seen in obese and/or diabetic adults [97–99].

Although the role of insulin signaling in the development of hypothalamic neurocircuits is only beginning to be unraveled, it has become clear that abnormal insulin action during development induces long-term effects on the metabolic phenotype in offspring from obese and diabetic mothers. It is therefore important to decipher insulin-mediated cell type-specific effects on the distinct phases of hypothalamic neurocircuit development and to distinguish them from leptin- and/or free FA-induced changes.

health of an individual, but also predisposes the unborn child to the development of metabolic disorders, underlines the urgency to intervene in the progression of obesity (Box 2). Therefore, investigating the basis of hypothalamic neurocircuit development under healthy, as well as hyperinsulinemic, conditions could lead to novel preventative measures, tackling the obesity epidemic at its roots.

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