Insulin action in the brain: Roles in energy and glucose homeostasis

G. T. Dodd | T. Tiganis

Metabolic Disease and Obesity Program, Monash Biomedicine Discovery Institute and Department of Biochemistry and Molecular Biology, Monash University, Melbourne, VIC, Australia

Correspondence
Garron T. Dodd, Metabolic Disease and Obesity Program, Monash Biomedicine Discovery Institute and Department of Biochemistry and Molecular Biology, Monash University, Melbourne, VIC, Australia. Email: garron.dodd@monash.edu

A growing body of evidence from research in rodents and humans has identified insulin as an important neuroregulatory peptide in the brain, where it coordinates diverse aspects of energy balance and peripheral glucose homeostasis. This review discusses where and how insulin interacts within the brain and evaluates the physiological and pathophysiological consequences of central insulin signalling in metabolism, obesity and type 2 diabetes.

KEYWORDS
AgRP, energy homeostasis, glucose homeostasis, hypothalamus, insulin, POMC

1 | INTRODUCTION

Insulin targets peripheral tissues, including skeletal muscle, adipose tissue, where it promotes glucose uptake from the blood, and the liver, where it inhibits gluconeogenesis and glycogenolysis and promotes glycogen synthesis to coordinately repress hepatic glucose production. In this way, insulin prevents postprandial hyperglycaemia and maintains euglycaemia. In addition to these peripheral targets, insulin also signals in the brain, although the physiological significance of this interaction has only recently started to emerge. Research over the past two decades has provided compelling evidence that central insulin signalling plays pivotal roles in many aspects of energy and glucose homeostasis (Figure 1). Given the epidemics of obesity and type 2 diabetes (T2D) in developed and increasingly developing countries, there is a pressing need to fully understand the mechanisms by which the body coordinates energy and glucose homeostasis. A key hallmark of obesity and T2D is insulin resistance, where defective insulin signalling downstream of the insulin receptor (IR) renders the peripheral target tissues of insulin insensitive to the action of insulin. What is becoming increasingly apparent is that neurones in the brain also become resistant to insulin; however the relative contributions of central insulin resistance to the development of obesity and T2D remain poorly understood.

This review discusses the role of insulin in the brain with respect to coordinating glucose metabolism with energy expenditure. In particular, we discuss our understanding of the locations and mechanisms by which insulin elicits its effects in the brain, its influence on glucose metabolism and energy expenditure, and the potential contributions of central insulin resistance to the development of obesity and the metabolic syndrome.

2 | INSULIN AND THE BRAIN

The integral role of the brain in glucose homeostasis was first described by Claude Bernard in 1855, where he found that puncturing the fourth ventricle resulted in marked glycosuria in dogs.1 Despite these early observations that inferred an important role for the central nervous system (CNS) in the control of glucose homeostasis, the discovery of insulin by Banting and Best in 1921 along with its ability to markedly reduce blood glucose overshadowed and diverted attention away from the role of the brain.2,3 Indeed, much of the research that followed the characterisation of insulin, particularly during the 1960s to 1980s, ultimately type-casted insulin so that it was understood to act solely through peripheral tissues to control glucose metabolism, ignoring any involvement of the brain.4 Despite the IR and its signalling intermediates being widely expressed within mammalian and human brains,5-9 this peripheral-centric view was reaffirmed by early observations indicating that the majority of glucose transport into neurones occurred independently of insulin; it is now known that, in a subset of hypothalamic neurones, glucose uptake may occur via the insulin-dependent glucose transporter GLUT4.10,11 Nonetheless, evidence emanating from decades of research now supports varied neuromodulatory roles for insulin signalling in the CNS.

3 | INSULIN TRANSPORT

The primary source of circulating insulin is from pancreatic β-cells.12 Insulin is secreted into the circulation in response to postprandial
rises in blood glucose and it readily accesses tissues throughout the body, including the brain. Early experiments described high concentrations of insulin in both animal and human brain homogenates. Because insulin is a 51-amino acid peptide, passive diffusion into the brain is limited by the blood-brain barrier (BBB). The precise mechanisms of how and where insulin enters the brain are unclear; however, emerging evidence suggests that the entry of insulin into the brain parenchyma can occur directly via the median eminence (ME) (Figure 2) or indirectly via the cerebrospinal fluid (CSF).

### 3.1 CNS entry via the median eminence

The brain is not entirely ensheathed by the BBB. Specialised regions named circumventricular organs lack the BBB but have fenestrated capillaries allowing for direct communication between the circulation and the brain parenchyma. The fenestrated capillary cells of the ME allow the passive and rapid extravasation of most blood-borne nutrients and metabolic hormones to the brain.

The importance of peripheral insulin access to circumventricular organs is inferred from quantitative autoradiography studies showing that peripheral administered [$^{125}$I]-insulin rapidly penetrates the circumventricular organs, most notably the ME. The ME is a circumventricular organ positioned directly below the mediobasal hypothalamus affording unique access for insulin to communicate with the energy and glucose-sensing neurons in the arcuate nucleus (ARC) of the hypothalamus. It is the neurones of the ARC that rapidly respond to a peripheral bolus of insulin; this effect is attenuated in insulin-resistant states such as T2D and obesity. Because this accumulation of insulin within the ARC occurs so rapidly, it likely involves access via the ME.

### 3.2 CNS entry via the CSF

For most studies, CSF has been used as a surrogate for brain ISF; however, recent studies show that CSF measured in the cerebral ventricles, lumbar spine or cisterna magna differs in composition from that of the brain ISF. By administering several different tracers into the CSF and tracing their movement over time, recent studies have redefined the relationship between the CSF and the brain ISF. CSF is produced by the choroid plexus in the ventricles and passes through the third and fourth ventricles into the cisterna magna, the subarachnoid space and eventually accesses the Virchow-Robin space. This para-arteriolar pathway brings the CSF into contact with the microvasculature, forming an interface facilitating the possible transport of insulin from the blood to the CSF. Insulin can enter the ISF either by passing through the astrocytes that line the Virchow-Robin space or interacting with tanycytes lining the walls of the ventricles. The precise point by which CSF insulin enters the ISF is unknown; however, it is important to note that transport of other metabolic hormones such as leptin to the rest of the brain interstitial fluid (ISF) is facilitated by tanycyte processes, a transport mechanism that is considered to be essential to the central metabolic actions of leptin. Peripheral insulin could therefore have a bi-phasic entry into the brain, via the ME and via the CSF; however, the relative contribution of either route remains unknown.
4 | INSULIN SIGNALLING IN THE CNS

4.1 | IR

Insulin signals both centrally and peripherally via the IR,5 a heterotetrameric receptor that comprises two extracellular ligand-binding α-subunits linked via disulphide bonds to two membrane-spanning β-subunits containing the cytoplasmic protein tyrosine kinase (PTK) domains, which signal upon ligand binding.5 The IR gene in humans is encoded by a single gene on chromosome 19 and is composed of 22 exons.34 Alternative splicing of the mature transcript yields two mature IR isoforms, designated isoforms A and B.35 The A isoform is expressed ubiquitously, especially in the brain,36 whereas the B isoform is expressed in liver, muscle, adipocytes and kidney. Isoform A lacks exon 11, which encodes a 12 amino-acid segment from the carboxy terminus of the ligand binding α subunit. The A isoform not only has a higher affinity for insulin, but also binds insulin-like growth factor (IGF)-II.36 The IR was first localised and quantified in the brain by Havrankova et al.13 and was shown to be expressed throughout all stages of development in the rodent brain. Subsequently, autoradiography studies have shown that [125I]-insulin binds to homogenates of the hypothalamus to a greater extent than the rest of the brain, along with significant binding being noted in the olfactory areas, limbic regions, neocortex, basolateral amygdala, cerebral cortex and choroid plexus, implying a broad neuromodulatory function within the brain.18 The IR is also widely distributed within the human brain37 and in situ hybridisation studies in rodents have shown that IR mRNA parallels insulin binding, with the highest IR expression occurring in the ARC.38

FIGURE 2  Mechanisms of central insulin resistance. (A) In the lean state, insulin is transported to the arcuate nucleus (ARC)/hypothalamus across a fenestrated blood–brain barrier (BBB, dashed blue line).222 Insulin crosses the BBB and engages the insulin receptor (IR) on two opposing neuronal populations in the ARC known as pro-opiomelanocortin (POMC) and agouti-related peptide (AgRP)/neuropeptide Y (NPY) neurons.111 Insulin signals via phosphatidylinositol 3-kinase (PI3K) and the protein kinase AKT to regulate metabolic neuropeptide gene transcription and neuronal excitability.5,60,223 Insulin signalling in these neurones is propagated to the rest of the brain and peripheral tissues, such as white adipose tissue, brown adipose tissue and the liver to coordinate food intake, whole-body energy expenditure and glucose metabolism. (B) A key hallmark of diet-induced obesity is defective insulin signalling in the ARC, whereby AgRP and POMC neurones become insensitive to the action of insulin and are termed “insulin resistant”111. In diet-induced obesity (B1), the BBB is less permeable and the access of insulin to the ARC/hypothalamus is decreased (indicated by light grey shading) despite heightened plasma insulin levels.211-213,222 (B2) Increased inflammation and reactive gliosis in the ARC impair the access of insulin and the responsivity of the AgRP and POMC neurones to insulin.126,199 (B3) The enhanced expression of negative regulators of IR signalling, such as TCPTP, PTP1B and SOCS3 within the hypothalamus59,89,91,222 attenuate insulin signalling.5,59 The decreased insulin signalling diminishes the ability of insulin to repress food intake, increase whole-body energy expenditure, repress hepatic glucose production, promote glucose storage and influence lipid flux to maintain metabolic homeostasis. CSF, cerebrospinal fluid; ME, median eminence; PTP1B, protein tyrosine phosphatase 1B; TCPTP, t-cell like protein tyrosine phosphatase; SOCS3, suppressor of cytokine signalling 3
4.2 | Insulin-like growth factor 1 receptor

Insulin also binds and signals via the IGF-1 receptor (IGF-1R), albeit at a lower affinity than the IR.\(^{39}\) Similar to IR, the IGF1-R is expressed throughout the brain with abundant expression evident in the cortex, thalamus and hippocampus, and moderate expression in the cerebellum and hypothalamus.\(^{40}\) Moreover, the IR and IGF-1R can form homodimers and heterodimers,\(^{51}\) although IR homodimers exhibit a higher affinity for insulin than IR/IGF-1R heterodimers.\(^{59}\) The distribution of both brain, the IR exists predominantly as heterodimers, whereas approximately 50% of IGF-1 Rs form heterodimers.\(^{62}\) The distribution of IR and IGF-1R in the brain, using antisense oligonucleotides administered i.c.v., also enhances insulin signalling, attenuates body weight and adiposity, and improves glucose metabolism.\(^{70}\) Despite this, the role of PTBP1 within the brain may be cell-type dependent because PTBP1 deletion in steroidogenic factor 1 (SF1) neurones located in the ventromedial hypothalamus (VMH) enhances insulin signalling yet promotes adiposity and weight gain,\(^{71}\) whereas conditional PTBP1 deletion in pro-opiomelanocortin (POMC)-expressing neurones in the ARC has no effect on insulin signalling and glucose homeostasis.

4.3 | Canonical IR signalling pathways

The binding of insulin to the IR α-subunits induces conformational changes that induce intracellular IR autophosphorylation.\(^{53}\) A comprehensive review of the mechanisms of insulin signalling is provided elsewhere.\(^{54,44}\) In brief, IR autophosphorylation on Y1150/Y1151\(^{45}\) (Y1162/Y1163 on IR isoform B\(^{45,46}\) ) fully activates the PTK and results in the phosphorylation of additional IR sites, including Y960, Y1146, Y1316 and Y1322, as well as the phosphorylation of several cellular IR substrates, most notably the insulin receptor substrate (IRS) proteins. IRS-1 and IRS-2 are widely expressed in mammalian tissues and numerous studies have shown that IRS-1 and IRS-2 have both overlapping and distinct physiological functions.\(^{44}\) Both IRS-1 and IRS-2 null mice are glucose intolerant and insulin resistant;\(^{47-50}\) however, only IRS-2 null mice exhibit a decreased brain to body ratio,\(^{49}\) implying an important role of IRS-2 in brain development. IRS-2 is more abundantly expressed than IRS-1 within the brain and is particularly abundant in the hypothalamus.\(^{51}\) Brain-specific deletion of IRS-2 results in increased adiposity, hyperphagia, hypertension, hyperinsulinaemia and insulin resistance;\(^{52,53}\) and these effects are mediated, at least in part, by neurones expressing leptin receptors (LepRb).\(^{54}\) IRS-4 is also expressed within the hypothalamus;\(^{55}\) however, IRS-4 null mice exhibit only a mild metabolic phenotype.\(^{56}\) Despite this, a recent study found that IRS-4 may cooperate with IRS-2 in the hypothalamus and elicit synergistic effects on energy balance and glucose metabolism.\(^{57}\)

The tyrosine-phorylated IRS proteins function as signalling nodes, recruiting adaptor proteins to transduce IR signalling and activate multiple downstream pathways, including the phosphatidylinositol 3 kinase (PI3K)/AKT and the mitogen-activated protein kinase pathways\(^{58}\) to coordinate neuromodulatory gene transcription and neuronal excitability (Figure 2) that influence energy and glucose homeostasis.\(^{59-61}\)

4.4 | Negative regulators of CNS insulin signalling

IR signalling is negatively regulated by protein tyrosine phosphatases (PTPs), most notably protein tyrosine phosphatase 1B (PTP1B) and T cell protein tyrosine phosphatase (TCPTP), which act to dephosphorylate the IR.\(^{46,59,62-65}\) Ptpn1\(^{-/-}\) mice that are globally deficient for PTP1B, or mice that lack PTP1B in muscle (Mck-Cre; Ptpn1\(^{-/-}\) ) or liver (Alb-Cre; Ptpn1\(^{-/-}\) ), exhibit enhanced IR phosphorylation and signalling in muscle and liver and improved whole-body glucose homeostasis.\(^{66-69}\) PTP1B knockdown in the brain, using antisense oligonucleotides administered i.c.v., also enhances insulin signalling, attenuates body weight and adiposity, and improves glucose metabolism.\(^{70}\) Another important negative regulator is suppressor of cytokine signalling 3 (SOCS3).\(^{72}\) In the brain, SOCS3 has mostly been studied in the context of LepRb signalling because leptin-induced signalling via Janus-activated kinase-2 (JAK-2)/signal transducer and activator of transcription 3 (STAT-3) drives Socs3 expression, which in turn negatively regulates JAK-2/STAT-3 signalling as part of a negative feedback loop.\(^{72,73}\) The elevated circulating leptin levels associated with obesity are thought to increase basal LepRb/STAT-3 signalling in ARC neurones,\(^{73,75,77-79}\) resulting in chronic SOCS3 expression and the development of cellular leptin resistance.\(^{75,77-79}\) The elevated SOCS3 also inhibits IR signalling either directly or by binding and promoting IRS-1 and IRS-2 ubiquitination and degradation.\(^{76,84,85}\) Socs3\(^{-/-}\) heterozygous mice, or mice with the conditional deletion of SOCS3 in neuronal and glial cells, or specifically in POMC neurones, are protected against the development of obesity-associated leptin and insulin resistance.\(^{80,86-88}\) Conversely SOCS3 overexpression in hypothalamic neurones leads to the development of glucose intolerance and systemic insulin resistance.\(^{83,89}\) The obesity-associated hyperleptinaemia and/or inflammation also promote the expression of PTBP1 and TCPTP, which dephosphorylate JAK2/STAT3 and/or the IR, thus contributing cellular leptin/insulin resistance (Figure 2b3).\(^{16,90-94}\)

5 | CNS TARGETS OF INSULIN

Although it is well established that insulin interacts with neurones of the brain, understanding the anatomical location and precise neuronal populations where this interaction occurs is vital to defining the role of insulin signalling in the brain.
5.1 Hypothalamic targets of insulin signalling

Functional in vivo imaging studies in both human and rodents all point toward an unequivocal role for insulin signalling in the hypothalamus (Figure 1). In humans, intranasal delivery of insulin modulates functional hypothalamic neuronal activity, an effect that is attenuated in obesity. In rodents, functional immunohistochemical studies indicate that several regions of the hypothalamus, including the ARC, VMH, dorsomedial hypothalamus (DMH) and paraventricular hypothalamus (PVH), show enhanced functional c-Fos immunoreactivity following peripheral or central insulin administration. A caveat of these functional studies is that they do not clarify whether insulin directly influences the response of c-Fos positive neurones. By immunohistochemistry, AKT Ser-473 phosphorylation is seen rapidly (<15 minutes) in response to insulin administration and is almost exclusively seen within the hypothalamus in two functionally antagonistic ARC neuronal populations; the anorectic POMC (α-melanocyte-stimulating hormone [MSH] precursor) and the orexigenic agouti-related peptide (AgRP)/neuropeptide Y (NPY)-neuropeptide expressing neurones (Figure 2). Genetic ablation or pharmacogenetic inhibition of AgRP neurones decreases food intake and body weight, whereas POMC neuronal activation promotes α-MSH release to agonise melanocortin-4 receptors (MC4Rs) on second-order neurones in regions of the brain such as the PVH, VMH and DMH. MC4R activation has been shown to decrease food intake, increase energy expenditure and regulate glucose homeostasis. AgRP/NPY neuronal activation promotes the release of AgRP and GABA that antagonise α-MSH/MC4R interactions and inhibit POMC neurones. Insulin inhibits AgRP/NPY neurones by activating PI3K, which subsequently activates ATP-sensitive potassium channels, resulting in membrane hyperpolarisation and decreased action-potential frequency. Insulin has long been considered to hyperpolarise and inhibit POMC neurones via the same mechanism described for AgRP neurones. Recent studies by Qiu et al. report that insulin depolarises and activates POMC neurones via the activation of transient receptor potential-5 channels, whereas Williams et al. report the existence of distinct insulin-responsive POMC populations throughout the hypothalamus. Such discrepancies could potentially be explained by the high synaptic plasticity of POMC neurones responding to insulin. Indeed, synaptic inputs to POMC neurones are re-modelled in response to feeding, fasting and diet-induced obesity.

Because the AgRP and POMC neurones in the ARC are positioned proximal to the ME, they are ideally positioned to be the first neuronal subsets that interact with peripheral insulin. From here, AgRP and POMC neurones transduce insulin signalling to the rest of the brain via their projections to nuclei such as the PVH, VMH, DMH, lateral hypothalamus (LH), amygdala, bed nucleus of stria terminalis, parabrachial nucleus and the dorsal vagal complex (encompassing the nucleus of the solitary tract), allowing insulin to influence a constellation of CNS processes. It is likely that many of the distinct actions of CNS insulin signalling in AgRP/POMC neurones (discussed later) are coordinated by projections to these distinct nuclei. For example, a recent neurocircuitry mapping study highlighted that discrete AgRP projections to the PVH coordinate feeding behaviour, whereas projections to the LH or bed nucleus of the stria terminalis (BNST) regulate peripheral insulin sensitivity.

Beyond the ARC, insulin has also been shown to act directly on SF1-neurones of the VMH. Conditional excision of the IR in SF-1 cells protects against diet-induced obesity and improves glucose homeostasis by enhancing glutamatergic signalling onto POMC neurones. In addition to neurones of the VMH, insulin has also been shown to act directly on melanin-concentrating-hormone (MCH) neurones in the LH. Interestingly, there are distinct insulin-sensitive subsets of MCH neurones within the LH, each responding electrophysiologically to insulin with either excitation, inhibition or nonresponsivity. The precise physiological functions of these distinct subsets remain unclear; however, conditional deletion of the IR in MCH neurones modulates locomotor activity and improves insulin sensitivity in diet-induced obese mice. In addition to neuronal insulin signalling, a recent study showed that insulin signals to ARC glial cells and regulates glucose transport into the ARC ISF, thereby influencing how ARC neurones sense glucose.

5.2 Extrahypothalamic targets of insulin signalling

Beyond the hypothalamus, dopaminergic midbrain neurones of the ventral tegmental area have been shown to be responsive to insulin. Conditional deletion of IR in tyrosine hydroxylase (the rate-limiting enzyme in catecholaminergic biosynthesis) expressing cells resulted in increased body weight, increased fat mass, hyperphagia and altered responses to cocaine under food-restricted conditions. Insulin signalling in dopaminergic neurones may therefore be critical for the integration of signals of food palatability/reward into the overall complex control of energy homeostasis.

Higher cortical regions have also been shown to be responsive to insulin in both humans and animals. For example, in humans, feeding-induced functional activation of the fusiform gyrus and prefrontal cortex is attenuated by insulin administration. Interestingly, the suppression of functional activity in the prefrontal cortices appears to be limited to lean but not obese individuals. The hippocampus, an area central to declarative memory formation, is also insulin sensitive in both rodents and humans. Beyond the hypothalamus, the hippocampus represents the only other significant brain region that signals via AKT in response to insulin administration. However, it is important to note that a lack of AKT signalling does not preclude insulin acting through alternate pathways. In addition, it is possible that the strength of downstream IR coupling could differ between brain regions, as seen with the cannabinoid 1 receptor. Moreover, it is possible that insulin-rich ISF may diffuse to different brain regions at different rates. Insulin administration in humans has been shown to improve higher cognitive function, memory formation and mood, an effect that could be facilitated by either the direct or indirect insulin engagement of such extrahypothalamic regions (Figure 1).
Interest in the actions of insulin in the brain was re-ignited when mice with a brain-specific knockout of the IR (Nestin-Cre; InsrΔfl/fl; NIRKO) were shown to be obese and hyperphagic and to develop whole-body insulin resistance with dyslipidaemia and hyperinsulinaemia.144 These studies were substantiated by others highlighting the importance of insulin signalling in the CNS in the coordination of energy and glucose homeostasis.145,146 Research over the past decade has revealed that central insulin signalling plays sentinel roles in coordinating feeding behaviour, energy expenditure, adiposity, peripheral insulin sensitivity and glucose homeostasis (Figure 1).

6.1 | Effects on feeding behaviour, adiposity and body weight

The first clues that insulin regulates food intake came from studies administering insulin into the ventricular system of the brain. Studies in rodents and non-human primates following i.c.v. delivery of insulin showed decreased in food intake and a significant reduction in body weight.82,147-152 Despite a plethora of studies recapitulating the hypothalamic effects of insulin in the CNS under many different conditions, a body of conflicting studies exists reporting little to no effect of insulin on food intake and body weight.149,153-155 The use of supraphysiological doses of exogenous insulin that possibly result in attenuated insulin sensitivity could underlie such discrepancies. Indeed, a recent study found that the hypophagia elicited by CNS administered insulin is compromised if mice are pre-administered insulin 2 days but not 7 days prior to the experiment.149 Furthermore, many studies only determined daily food intake as opposed to assessing feeding behaviour per se. Streptozotocin-treated mice, with ablated insulin secretion, show no alteration in cumulative food intake but exhibit a latency in feeding behaviour.90 Several human studies show that changing feeding behaviour (reduced meal size, enhanced frequency) as opposed to simply reducing daily food intake has a much more dramatic effect on weight loss and glucose homeostasis.156 Conversely, patients with uncontrolled T2D report feelings of excessive hunger, whereas healthy individuals show an attenuated valence towards images of food in response to exogenous insulin or heightened endogenous insulin levels.100,134,137 Insulin has also been shown to affect the capacity to smell in lean people, and this effect could regulate feeding behaviour in response to elevated postprandial insulin levels.157,158 Although complex, these studies highlight a potential role of insulin signalling in the CNS in the control of feeding behaviour; however, the neuronal populations mediating these responses remain unclear.

Substantial evidence points to insulin signalling in the ARC being instrumental for the effects of insulin in the CNS on energy homeostasis. For example, IR knockdown with antisense oligonucleotides in the ARC results in rapid onset hyperphagia and a marked increase in adiposity.145 Moreover, intranasal insulin delivery into the CNS of healthy patients improves whole-body insulin sensitivity, promotes weight loss and attenuates caloric intake via the activation of neurons within the hypothalamus.159

It is likely that these effects of hypothalamic insulin signalling on feeding, adiposity and body weight are mediated by AgRP and POMC neurons.111 However, studies attempting to delineate the specific contributions of IR signalling in AgRP, POMC, Sim1 (PVH) and Nkx2.1 (ARC, VMN) neurons of the hypothalamus to energy homeostasis have been underwhelming160,161 with conditional IR deletion in such neuronal subsets leading to no overt effects on feeding behaviour or body weight. One possible explanation for this is that IR deletion in utero is compensated by alternate pathways. A recent study by Loh et al.162 reported that the inducible deletion of IR from NPY expressing cells in adult mice increased body weight and adiposity, suggesting that insulin signalling in NPY neurons is important in the regulation of energy homeostasis. Similar inducible approaches may be required to explore the roles of AgRP and POMC IR signalling in energy homeostasis. It is worthwhile noting that conditional deletion of IRS-2 in the whole brain or specifically in POMC neurons promotes marked increases in adiposity.53

6.2 | Effects on energy expenditure

Feeding behaviour represents only one arm of the energy balance equation, with the other being energy expenditure. Whole-body energy expenditure can be accounted for by basal metabolic rate (55%-65%), physical activity (25%-35%) and adaptive thermogenesis (10%). Research aiming to define the hormonal influences and neurons coordinating energy expenditure has received much attention over the last 5 years, particularly with respect to understanding the phenomenon of adaptive thermogenesis.

Adaptive thermogenesis refers to the ability of the body to increase energy expenditure in response to thermal challenges (cold-induced thermogenesis) and/or nutritional state (diet/feeding-induced thermogenesis).163 The body does this by engaging brown adipose tissue (BAT; as expressed in the intrascapular regions in rodents and infants and possibly in different fat depots in adult humans) and by promoting the recruitment and activation of beige adipocytes in white adipose tissue (WAT; notably within the inguinal or interclavicular subcutaneous fat depots in rodents and human, respectively) as a process referred to as WAT browning.163-166 Unlike traditional white adipocytes that store energy, brown and beige adipocytes function to expend energy. In response to cold exposure or β-adrenergic stimulation, brown and beige adipocytes become activated and generate heat by expending energy.165 They do this, in part, by up-regulating the mitochondrial uncoupling protein 1, which acts to uncouple oxidative phosphorylation at the expense of ATP.168,169 Understanding the regulation of adaptive thermogenesis is of high therapeutic significance because the promotion of WAT browning and BAT activity protects against diet-induced obesity and T2D.169

The actions of insulin on adaptive thermogenesis were inferred as early as 1983 when studies in rats treated with diazoxide, a potent blocker of pancreatic β cell insulin secretion, demonstrated an attenuation of the thermic response to a carbohydrate meal.170 Subsequent studies found that insulin administration directly into various regions...
of the hypothalamus resulted in robust increases in body temperature and whole-body energy expenditure.\textsuperscript{23,171,172} Consistent with these observations, i.c.v. insulin administration increases sympathetic nervous system (SNS) activity, as assessed by recordings from nerve fibres,\textsuperscript{172,173} and intranasal delivery of insulin in humans enhances postprandial energy expenditure.\textsuperscript{174}

The neuronal populations by which insulin may influence adaptive thermogenesis remain unclear. The effects of conditional IR deletion in AgRP or POMC neurones on energy expenditure have not yet been directly characterised. However, Lin et al.\textsuperscript{175} found that restoration of insulin signalling specifically in POMC neurones in L1 mice (IR deficient mice expressing IR only in the liver and pancreatic β-cells) using a targeted knock-in approach increased energy expenditure and locomotor activity; however, BAT thermogenesis or WAT browning were not determined. Moreover, a recent study has shown that insulin and leptin can act together on ARC POMC neurones to promote SNS-dependent WAT browning and BAT activity\textsuperscript{60} and that enhancing this attenuates the development of diet-induced obesity. Therefore, in part, the effects of insulin on adaptive thermogenesis may be mediated by the POMC mediated control of WAT browning and BAT activity.

In addition to adaptive thermogenesis, physical activity accounts for a substantial proportion of total energy expenditure and can be ascribed to exercise and non-exercise activity-dependent thermogenesis (walking, sitting, standing and fidgeting, but excluding voluntary exercise, sleeping or eating).\textsuperscript{176} Interestingly, NIKKO mice and those lacking TCPTP in neuronal and glial cells in the brain show not only changes in energy, but also in ambulatory activity.\textsuperscript{91,144} Because no effects on activity are seen in mice lacking IR or the IR phosphatase TCPTP in POMC neurones alone,\textsuperscript{60,161} these effects on activity are likely mediated by other neurones and/or other brain regions such the cerebellum where the IR is abundant.\textsuperscript{177}

### 6.3 | Effects on peripheral glucose metabolism

A substantial body of data has defined an important role for insulin signalling in the CNS in the control of peripheral glucose metabolism. In addition to the role of insulin signalling in the CNS with respect to controlling feeding and energy expenditure, it makes sense that the same neuronal signalling pathways controlling nutrient intake should also control nutrient fluxes within the body.

Insulin administered directly into the hypothalamus or into the ARC attenuates the ability of peripheral insulin to suppress hepatic glucose production (HGP), an effect that is lost in NIKKO mice.\textsuperscript{144,145,161} Consistent with this, i.c.v. infusion of IR antisense oligonucleotides or neutralising insulin antibodies also attenuate the ability of peripheral insulin to suppress HGP.\textsuperscript{145} These effects appear to be mediated via AgRP neurones because the conditional deletion of the IR in AgRP neurones blunts the ability of both systemic and i.c.v. insulin to attenuate HGP, as assessed in hyperinsulinaemic euglycaemic clamped mice.\textsuperscript{161} Although the ablation of IR in POMC neurones has no overt effect on glucose metabolism, combined IR and LepRb deletion in POMC neurones results in profound deregulation of peripheral glucose metabolism.\textsuperscript{144,178}

The efferent mechanisms coupling the action of insulin in the brain to HGP are unknown but involve the vagal efferents to the liver.\textsuperscript{179} A recent study shows that this brain-liver coupling is mediated by hepatic 7-nicotinic acetylcholine receptors.\textsuperscript{180} The current working model is that CNS innervation to the liver modulates HGP by activating Kupffer cells to release interleukin-6, which in turn acts on hepatocytes via STAT-3 to repress the expression of gluconeogenic enzymes, such as glucose-6-phosphatase and phosphoenolpyruvate carboxykinase.\textsuperscript{181} Neural tracing studies attempting to delineate the efferent projections from the hypothalamus to the liver found that trans-synaptic efferents emerge from POMC, but not AgRP neurones of the hypothalamus, inferring at least one possible route of how central insulin signalling can regulate HGP.\textsuperscript{182,183} Although these studies highlight POMC efferents, future studies using new generation transgene-mediated viral vectors will be essential to provide a more comprehensive understanding of hepatic innervation.\textsuperscript{183} In rodents, liver denervation attenuates HGP, whereas, in humans (liver transplant) and canines, denervation only blunts the counter regulatory HGP response during hypoglycaemia.\textsuperscript{184-186} It is therefore important not to overstate the actions of insulin in the CNS on HGP. A new role of AgRP neurones in the coordination of whole-body insulin sensitivity was recently described by Steculorum et al.,\textsuperscript{129} who demonstrated that the acute pharmacogenetic activation of AgRP but not POMC neurones induced the expression of myostatin within BAT, robustly impairing whole-body insulin sensitivity by selectively repressing BAT glucose uptake. Using sophisticated optogenetic techniques, distinct AgRP projections to the PVH regulate feeding behaviour were further identified, whereas projections to the LH or BNST regulate peripheral BAT insulin sensitivity and glucose metabolism.\textsuperscript{129} Other studies have also shown that distinct subpopulations of AgRP neuronal projections (mainly the BNST, PVH and LH) also independently evoke feeding behaviour.\textsuperscript{187} These neurocircuitry mapping experiments have provided initial insights into the complex neuronal networks underlying the control of feeding and insulin sensitivity.

CNS insulin signalling has also been implicated in the control of WAT lipolysis because NIKKO mice exhibit unrestrained lipolysis and decreased de novo lipogenesis in WAT.\textsuperscript{144} Moreover, ARC insulin infusion increases WAT lipogenic protein expression and inactivates WAT hormone-sensitive lipase to repress WAT lipolysis.\textsuperscript{188,189} A recent study has shown that IR signalling in POMC but not AgRP neurones may be responsible for the ability of insulin to repress WAT lipolysis.\textsuperscript{190} In humans, intranasal insulin delivery decreased plasma free fatty acid levels, consistent with the attenuation of lipolysis.\textsuperscript{159,174} When taken together with the effects of insulin in the CNS on WAT browning, it is somewhat paradoxical that the action of insulin in the CNS both promotes (WAT browning) and inhibits catabolic (WAT lipolysis) processes in the same tissue (Figure 1). Although the neuronal correlates mediating the effects of insulin in the CNS on lipolysis are unclear, it will be important to determine whether common or distinct neuronal efferents mediate these opposing metabolic actions.
7 | INSULIN RESISTANCE AND METABOLIC DISEASE

A phenomenon observed ever since the earliest studies investigating insulin signalling in obesity is the considerable attenuation of the insulin response. Because this attenuation of signalling in obesity occurs despite heightened circulating levels of insulin, this phenomenon is referred to as “insulin resistance.” Insulin resistance results from defects in insulin signalling downstream of the IR (Figure 2). This renders the peripheral target tissues, as well as the brain, insensitive to the action of insulin. Despite an emerging physiological role of insulin signalling in the CNS, the relative contributions of insulin resistance in the CNS to the development of peripheral insulin resistance and T2D are unclear.

The constant availability of highly palatable nutrient-dense foods, together with the trend towards a more sedentary lifestyle in western societies, underpins the obesity and T2D epidemics. Strikingly, the consumption of a high-fat diet for as short as 72 hours is sufficient to reduce hypothalamic insulin sensitivity, independent of changes in body weight and fat mass in rodents. Moreover, consumption of saturated fatty acids in lean mice attenuates the ability of CNS administered insulin to regulate food intake and body weight. At a molecular level, saturated fatty acids such as palmitate or stearate cross the BBB where they activate local inflammatory signalling within the hypothalamus resulting in CNS cellular insulin and leptin resistance. Inflammation and the hyperleptinaemia in obesity are known to drive reactive gliosis, endoplasmic reticulum (ER) stress and the expression of negative regulators of insulin signalling, such as SOCS3, PTP1B and TCPTP within the hypothalamus (Figure 2b3). The hyperleptinaemia seen in obesity is considered to increase the hypothalamic expression of TCPTP, PTP1B and SOCS3, whereas ER stress and the obesity-associated inflammation drives the expression of PTP1B and SOCS3. These negative regulators may be instrumental in the initiation/exacerbation and/or maintenance of cellular leptin and insulin resistance (Figure 2b3). In the case of SOCS3, increased expression is seen as early as 2 days in AgRP neurons and 2 weeks in POMC neurons. Deletion of TCPTP and PTP1B or SOCS3 in POMC neurons, or SOCS3 in AgRP neurons, enhances hypothalamic insulin signalling and improves whole-body glucose metabolism in diet-induced obese mice. The inducible constitutive expression of X-box-binding protein 1 (Xbp1, a key regulator of the unfolded protein response to ER stress) within the POMC neurons alone protects against ER stress and diet-induced obesity and improves glucose homeostasis, effects that may be dependent on the expression of SOCS3 and PTP1B.

In addition to defective IR signalling, obesity is also associated with a decreased BBB permeability, thus potentially restricting access to hormones such as insulin and leptin to the brain (Figure 2b1). Insulin CSF concentrations are lower in insulin-resistant individuals despite higher circulating levels of insulin, suggesting an impaired delivery into the brain. The relative importance of impaired insulin delivery to the brain in metabolic disease remains to be determined. Furthermore, diet-induced obesity results in reactive gliosis in the ARC in both rodents and humans. This gliosis effectively makes POMC and AgRP neurones less accessible and responsive to insulin in the surrounding ISF (Figure 2b). Understanding the relative contribution of elevated PTP1B, TCPTP and SOCS3 expression, defective BBB permeability or reactive gliosis in the development of insulin resistance in the CNS and its influence in obesity and T2D remains to be determined.

8 | CONCLUSIONS AND FUTURE PERSPECTIVES

An important function of the brain is to ensure a steady supply of energy substrate to maintain the body’s internal milieu. To accomplish this task, divergent signals must be integrated, processed and transduced into homeostatic adjustments of food intake, energy expenditure and glucose and lipid metabolism. Insulin signalling in the brain plays several distinct roles in regulating feeding behaviour, energy expenditure, and peripheral processes central to the control of lipid and glucose metabolism. Many of the actions of insulin in the CNS are coordinated by the AgRP and POMC neurones in the ARC. Deconstruction of the neuronal subsets responding to peripheral insulin and the delineation of how these neurones transduce the varied actions of insulin throughout the brain represents a future challenge. Beyond the hypothalamus, recent work has highlighted tantalising new roles for CNS insulin signalling in aspects of mood, food preference, olfaction and memory formation. Understanding insulin signalling in this context is still in its infancy; however, exploring the molecular mechanisms of how insulin orchestrates such responses will shed light on how energy expenditure and glucose metabolism may be integrated with changes in mood and behaviour. Moreover, defining the relative contribution of insulin resistance in the CNS to the development of obesity and T2D remains a key unanswered question. What is apparent, however, is that when the brain becomes insulin resistant, the crucial modulatory effects of the brain on energy and glucose homeostasis are impaired. Future therapeutic advances targeting insulin signalling in the CNS may therefore have far reaching implications for the prevention and treatment of metabolic disease.

REFERENCES


118. Takahashi KA. Cone RD. Fasting induces a large, leptin-dependent increase in the intrinsic action potential frequency of orexigenic arcuate nucleus neuropeptide Y/Agouti-related protein neurons. Endocrinology. 2005;146:1043-1047.


