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Insulin Action in Brain Regulates Systemic Metabolism and Brain Function

Insulin receptors, as well as IGF-1 receptors and their postreceptor signaling partners, are distributed throughout the brain. Insulin acts on these receptors to modulate peripheral metabolism, including regulation of appetite, reproductive function, body temperature, white fat mass, hepatic glucose output, and response to hypoglycemia. Insulin signaling also modulates neurotransmitter channel activity, brain cholesterol synthesis, and mitochondrial function. Disruption of insulin action in the brain leads to impairment of neuronal function and synaptogenesis. In addition, insulin signaling modulates phosphorylation of tau protein, an early component in the development of Alzheimer disease. Thus, alterations in insulin action in the brain can contribute to metabolic syndrome, and the development of mood disorders and neurodegenerative diseases.

Since the discovery of insulin over 90 years ago, much progress has been made in unraveling the mechanism of insulin action and its physiological functions, especially in classic target tissues, such as liver, muscle, and fat. Over the past decade, work from our laboratory and others has shown that insulin action is also important in tissues previously thought to be insulin insensitive, including the brain, β-cell, and vascular endothelium (1). In this review, we will describe recent discoveries regarding insulin action in the brain, and illustrate how this can affect both the brain and peripheral metabolism. In addition, we will explore how insulin action in the brain can affect brain function and the pathogenesis of neurodegenerative diseases.

INSULIN SIGNALING MACHINERY IN THE BRAIN

Insulin and the closely related IGF-1 and IGF-2 mediate their biological effects through two highly related tyrosine kinase receptors—the insulin receptor (IR) and the IGF-1 receptor (IGF-1R) (2). Both IRs and IGF-1Rs are expressed throughout the brain. Previous studies using in situ hybridization and immunohistochemistry have demonstrated differential distribution throughout the brain. In the mouse, the highest expression of the IR is in the olfactory bulb, followed by the cortex, hippocampus, hypothalamus, and cerebellum, with relatively low levels in the striatum, thalamus, midbrain, and brainstem (3–5). In contrast, the IGF-1R has the highest expression in the cortex, hippocampus, and thalamus, whereas moderate expression is observed in the olfactory bulb, hypothalamus, and cerebellum, with the lowest expression in the striatum, midbrain, and brainstem (3). To directly compare the expression of these receptors across the brain and to each other, we assessed transcript copy numbers found in areas of dissected brain using quantitative real-time PCR against standard curves from plasmids containing IR and IGF-1R cDNAs (Fig. 1). IR mRNA levels ranged from 250 copies/ng in the raphe nucleus to ~475 copies of IRs in the cerebellum, whereas the IGF-1R mRNA copy number ranged from ~600 copies in the hypothalamus to almost 1,000 in the cerebellum (i.e., about twice the number of IRs) (Fig. 1). How this relates to protein levels of these two receptors, and how expression varies among the individual cells and cell types in each region of the brain remains to be determined.

Most importantly, these two receptors have different physiological functions in the brain, as shown by genetic knockout in mice. Mice with genetic deletion of IRs in the brain (i.e., neuron-specific IR knockout [NIRKO]) have normal brain size and development, but exhibit metabolic phenotypes, including mild obesity and insulin resistance
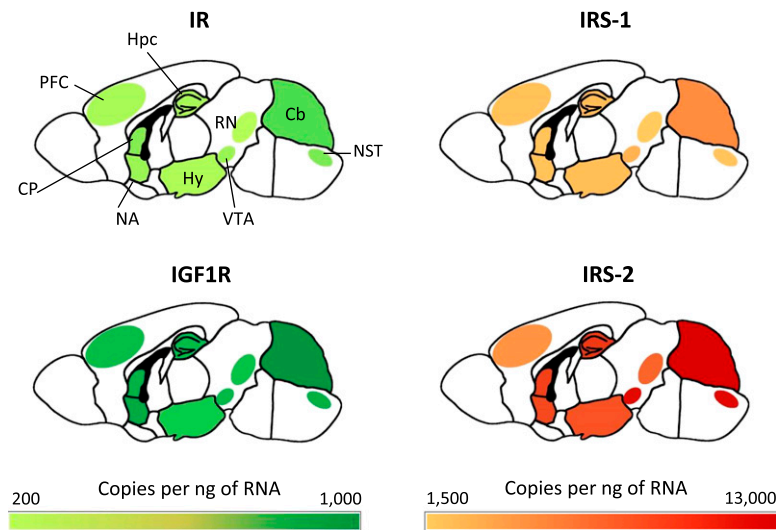
Figure 1—Expression of IR, IGF-1R, IRS-1, and IRS-2 in the brain. The expressions of IR, IGF-1R, IRS-1, and IRS-2 were determined by quantitative real-time PCR of brain regions dissected from male C57BL/6 mice. Serial dilutions of plasmids encoding the cDNA sequences of corresponding genes were used for the standard curves. Data are expressed as the copy number per nanograms of RNA transcribed. Cb, cerebellum; CP, caudate putamen; Hpc, hippocampus; Hy, hypothalamus; NA, nucleus accumbens; NST, nucleus tractus solitaries; PFC, prefrontal cortex; RN, raphe nucleus; VTA, ventral tegmental area.

(6). Knockout of IGF-1R in the brain, on the other hand, results in reduced brain size, generalized growth retardation, and behavioral changes (7). Interestingly, heterozygous deletion of IGF-1R in the brain does not affect brain growth, but results in a complex phenotype that includes reduced body weight, but increased fat mass, impaired glucose tolerance, and extended life span (7).

Differences in gel mobility and biochemical studies of IR and IGF-1R subunits isolated from the brain indicate differences in glycosylation of these receptors from those in peripheral tissue (8,9). Also, in contrast to the peripheral tissues, which express predominantly the B isoform (+exon 11) of the IR, the brain expresses predominantly the A isoform (−exon 11), which has a higher affinity for IGF-2 (2). As in peripheral tissues, IRs and IGF-1Rs can heterodimerize, and in rabbit brain approximately half of IGF-1Rs and the majority of IRs appear to exist as heterodimers (10). This is thought to favor a response to IGF-1 over insulin. Thus, it is likely that in the brain, insulin and the IGFs exhibit considerable cross-talk in signaling and biological effects.

Both IR and IGF-1R use similar intracellular signaling machinery, and all of the major components present in peripheral tissue are present in the brain. Among the IR substrates (IRSs), IRS-2 mRNA is most abundant, ranging from 5,000 copies/ng in the prefrontal cortex to 13,000 copies/ng in the cerebellum, compared with IRS-1, which ranges from 1,800 copies/ng in the raphe nucleus to 5,300 copies/ng of reverse-transcribed RNA in the cerebellum (Fig. 1). Interestingly, both IRS-1 and IRS-2 are expressed at much higher levels than IR or IGF-R, at least at the mRNA level. Again, it will be important to understand the specific cellular distribution of these proteins. Recently, it has been shown that IRS-4, which is mainly expressed in

embryonic development, is also expressed in the brains of adult mice, especially the hypothalamus (11,12). Numerous studies have shown that IRS-1 and IRS-2 have both overlapping and distinct functions. At the whole-body level, IRS-1 appears to be critical for growth, since IRS-1-null mice are runted (13). However, brain size remains near normal, resulting in an increased brain-to-body ratio. On the other hand, IRS-2 is important for brain development, and IRS-2-null mice exhibit a reduced brain-to-body ratio (14). Despite this, IRS-2-null mice are long lived (15), consistent with a role of central insulin/IGF signaling in control of life span in mammals. IRS-4 seems to be at least partially functionally redundant to IRS-1 and IRS-2 and may synergistically cooperate with IRS-2 in the hypothalamus to control food intake, energy expenditure, and glucose metabolism (16).

One of the major downstream pathways of IRS proteins is the PI3K/Akt cascade (17). This, in turn, targets multiple downstream pathways, including mTORC1, GSK3β, and the FoxO family of transcription factors (3). Many of these pathways have been shown to play pivotal roles in normal brain function. For instance, mTORC1-mediated protein synthesis is important for synaptic plasticity (18) and the regulation of autophagy, a major mechanism to degrade misfolded proteins and damaged organelles in neurons (19). Dysregulation of mTORC1-dependent autophagy in neurons results in neuronal cell death and the onset of neurodegenerative diseases. GSK3β regulates multiple aspects of neuronal functioning, including neural progenitor cell proliferation, neuronal polarity, and neuroplasticity (20). GSK3β can also phosphorylate tau protein, a process involved in the pathogenesis of Alzheimer disease (AD). Insulin stimulates

phosphorylation of GSK3β, and this reduces its enzymatic activity. Brain-specific knockout of IR or IRS-2 results in decreased GSK3β activity and increased tau phosphorylation (14,21) (see below). FoxOs play diverse and important roles in the central nervous system (CNS), including controlling energy homeostasis and leptin sensitivity, as well as locomotor activity (22,23).

Insulin/IGF-1 signaling also activates the Grb2-SOS-Ras-MAPK cascade, which plays a direct role in cell proliferation, differentiation, gene expression, and cytoskeletal reorganization. This pathway also contributes to the normal function and survival of neuronal cells (24). Inhibition of MAPK has also been shown to block insulin and leptin stimulation of hypothalamic neuroprogenitor cells (25).

Taken together, these studies demonstrate that IR and IGF-R, as well as their common downstream pathways, are present in the brain. More importantly, these pathways function as regulators of neurogenesis, brain function, and whole-body energy balance and metabolism.

HOW DOES INSULIN GET TO THE BRAIN?

Although the presence of insulin in the cerebrospinal fluid (CSF) is well documented, the origin of "brain" insulin is controversial. Insulin levels in the CSF are ~25% of those in the blood and increase proportionally after meals or with peripheral insulin infusion, suggesting that a fraction of plasma insulin is able to cross the blood-brain barrier via a saturable transport process, possibly the IR on vascular endothelium (26,27). It also appears that there are regions of the brain, such as the hypothalamus, that lack an effective barrier, allowing insulin more ready access. Indeed, studies (28) have shown rapid activation of insulin signaling in these regions after peripheral insulin injection.

Whether insulin can also be locally synthesized in the brain, however, remains under debate. In lower organisms, like *Caenorhabditis elegans* and *Drosophila*, insulin-like peptides not only are produced in neurons, but neurons are the primary source of these peptides (3). Older experiments using a radioimmunoassay on extracts of whole rat brains show significantly higher insulin concentrations than plasma (29); however, these have not been supported by more recent studies. On the other hand, some in vitro experiments (30) have shown that insulin can be synthesized and secreted by rabbit CNS neuronal cells in culture, and studies (31–34) using the mouse or rat insulin 2 promoter to drive transgenic gene expression have also shown the expression of reporter genes in the hypothalamus. Nevertheless, no evidence has definitively demonstrated that insulin synthesis in the brain in rodents or humans is physiologically important, although there still remains a possibility that local insulin production is important for specific regional effects.

INSULIN ACTION ON NEURONAL FUNCTION AND SYNAPTIC PLASTICITY

The best-studied effects of insulin signaling in the brain are those regarding food intake and energy expenditure.

As early as 1979, Woods et al. (35) showed that intracerebroventricular infusion of insulin in baboons could markedly decrease food intake and body weight gain. Insulin administration into the third ventricle in rodents has been shown to decrease food intake by decreasing expression of the orexigenic neuropeptides neuropeptide Y (NPY) and Agouti-related peptide (AgRP), and by increasing the expression of anorexigenic neuropeptides proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CaRT) in the arcuate nucleus, which together result in increased activity of α-melanocyte-stimulating hormone in neurons in the paraventricular nucleus (Fig. 2A) (36). Conversely, in the hypothalamus of insulin-deficient streptozotocin (STZ)-treated mice, there is an increase in NPY and AgRP accompanied by a decrease in POMC and CaRT (Fig. 2B). As a result, brain-specific deletion of IR in mice leads to increased food intake and mild obesity (6). This anorexigenic effect of insulin is, at least in part, a result of PI3K-dependent activation of ATP-dependent potassium channels in hypothalamic neurons, which hyperpolarize and inactivate the orexigenic AgRP neurons (37).

A number of lines of evidence have shown that central insulin action is also associated with alterations in cognitive function in the brain. Patients with both type 1 and type 2 diabetes are at higher risk for behavioral changes and accelerated cognitive decline with aging, and patients with type 2 diabetes have a higher rate and more rapid progression of AD (38). In humans with either type 1 or type 2 diabetes, imaging studies have demonstrated smaller hippocampi, as well as changes in the functional connectivity between regions of the brain (39–43). Multiple rodent models of diabetes have memory impairment (44,45), whereas central insulin infusion in rats significantly improves memory tasks, including performance in the passive avoidance task and Morris water maze (46,47). All of these point to the possible role of IR in neuronal signal transmission.

The effects of insulin differ in different neuronal populations. Insulin reduces the activity of AgRP neurons, whereas it increases the activity of dopamine neurons (37,48). Insulin also regulates the transmission of N-methyl-D-aspartate (NMDA) receptors in hippocampal neurons through the tyrosine phosphorylation of specific subunits of the NMDA receptors NR2A and NR2B (49), and appears to trigger the membrane recruitment of NMDA receptors into excitatory synapses (50). As a result, insulin-dependent enhancement of NMDA receptor activity in the postsynaptic terminal contributes to the development of long-term potentiation in the hippocampus (51), an important step in learning and memory. Insulin has also been shown to regulate α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in the hippocampus and cerebellum, triggering the clathrin-dependent endocytosis of AMPA receptors via the PI3K-PKC pathway (52,53). In the hippocampal CA1 neurons, this down-regulation of AMPA receptor activity in excitatory synaptic terminals plays a role in insulin-induced long-term
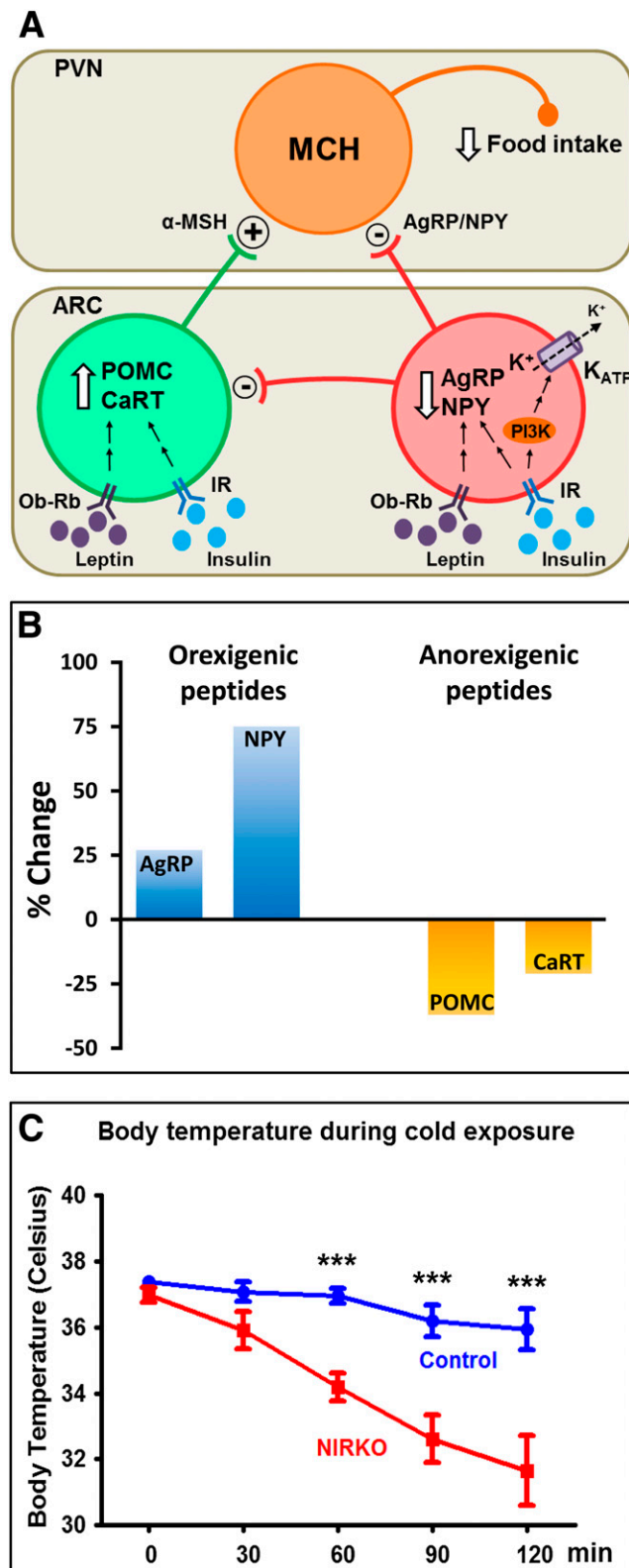
Figure 2—Insulin regulates orexigenic and anorexigenic peptides and body temperature. *A*: Insulin regulates appetite through the hypothalamus. In the arcuate nucleus (ARC) of the hypothalamus, insulin binds to IRs on POMC neurons to increase the expression of anorexigenic peptides, POMC, and CaRT, which results in increased activity of α-melanocyte–stimulating hormone (α-MSH) on melanin-concentrating hormone (MCH) neurons in the paraventricular nucleus (PVN). Conversely, insulin acts on AgRP neurons to

depression, which is generally considered essential for both memory consolidation and memory flexibility (54). IRs also have been shown to regulate type A γ-aminobutyric acid (GABA) receptor activity through both membrane recruitment and protein synthesis in hippocampal neurons, thereby regulating the activity of inhibitory synapses (55). Furthermore, insulin can regulate structural plasticity in the brain, including synapse number, dendritic plasticity, and visual circuit function (56), and can induce the expression of PSD95, a scaffold protein that is essential for the formation of the postsynaptic junction (57). All of these studies provide molecular mechanisms to support the positive effects of insulin on brain function and behavior.

IR expression and action in the CNS is not restricted to neurons, but may also occur in glial cells (58). Thus far, this specific aspect of central insulin action has received little attention; however, it is important to keep in mind that in the NIRKO mouse, which is the best studied model of genetically induced CNS insulin resistance, IRs are ablated in both neurons and glial cells. Insulin has been shown to stimulate proliferation, glutamate receptor expression, and cholesterol biosynthetic genes in glial cells, indicating that these cells are insulin sensitive (59–61). Glial cells, especially astrocytes, are considered the major energy suppliers for neurons (62). Moreover, astrocytes play key roles in regulating the homeostasis of the synaptic junction microenvironment, as well as inflammation (63,64). Future studies will need to determine the potentially important roles of IR and IGF-1R on glial cells, including astrocytes, oligodendrocytes, and microglia.

CNS INSULIN SIGNALING AFFECTS PERIPHERAL TISSUES

Experiments manipulating the levels of insulin or IR in the brain have demonstrated a role of central insulin signaling in the regulation of several peripheral tissues (Fig. 3). For example, insulin suppression of glucose production in the liver (gluconeogenesis) is regulated by IRs in both the liver and brain, such that genetic inactivation

inhibit the expression of orexigenic peptides AgRP and NPY. In addition, upon insulin binding, IR activates PI3K, triggering ATP-dependent potassium (K_{ATP}) channels, K^+ efflux, and hyperpolarization of AgRP neurons. This results in the attenuation of the inhibitory effect of AgRP neurons on both MCH and POMC neurons. In parallel, insulin and leptin act to decrease food intake. *B*: Transcription of the orexigenic peptides AgRP and NPY is increased, and transcription of the anorexigenic peptides POMC and CaRT is decreased in the hypothalami of STZ-treated mice compared with controls, as determined by microarray analysis on isolated hypothalami of C57BL/6 mice 10 days after treatment with vehicle or STZ to induce diabetes. Data are expressed as the percentage change over vehicle-treated mice. $P < 0.01$ for all genes. *C*: Brain IR knockout (NIRKO) mice show a significant drop in body temperature compared with controls when exposed to 4°C cold stress, indicating a defect in thermogenesis. Experiments were performed on 17-month-old female mice. ***$P < 0.001$.

of IRs in either of these tissues causes a loss of insulin suppression of hepatic glucose production (65,66). In the brain, this appears to involve IRs specifically in the AgRP neurons (37,65,67). Insulin action at the brain acts on gluconeogenesis also in part through stimulation of tyrosine phosphorylation of STAT3 in the liver (63). This results in an increase in hepatic interleukin-6 production, which inhibits hepatic glucose production, contributing further to the brain-liver control of gluconeogenesis. Conversely, CNS injection of insulin increases hepatic insulin sensitivity, and this occurs through central pathways involving PI3K and ATP-dependent potassium channels (66). Additionally, administration of insulin into the CNS promotes lipogenesis and peripheral fat accumulation (68). Insulin action in the brain also modulates the counter-regulatory response to hypoglycemia (69). Consistent with this, mice with a knockout of IR in the brain display a blunted sympathoadrenal response to hypoglycemia (67,70), showing that central insulin signaling directly alters glucose sensing in hypothalamic neurons. Central insulin does not, however, appear to affect glucose uptake in skeletal muscle or adipose tissue.

Insulin in the brain also impacts the control of body temperature. Thus, the injection of insulin or IGF-1 into the preoptic area can activate brown adipose tissue and induce hyperthermia. This effect is lost in the NIRKO mouse, indicating that it is mediated by IR (71). Consistent with this, NIRKO mice have an impaired response to cold exposure (Fig. 2C). Intranasal administration of insulin has also been shown to enhance postprandial thermogenesis in humans (72). Interestingly, sympathetic

nervous system outflow from the brain to brown adipose tissue stems from very insulin-sensitive regions of the hypothalamus (73). In view of the recent finding of active brown fat in humans and reduced activity of this fat in obese individuals, it will be important to determine the role of brain insulin resistance in this response.

Insulin resistance is associated with impaired female reproduction and is a central feature in the pathogenesis of polycystic ovary syndrome. In addition, central obesity is associated with both insulin resistance and menstrual disorders, indicating that metabolic disorders may affect fertility. The best evidence that insulin in the brain has a direct effect on reproductive function comes from NIRKO mice, in which both sexes display hypothalamic hypogonadism, with reduced fertility due to hypothalamic dysregulation of luteinizing hormone, which can be corrected by the injection of gonadotropin-releasing hormone and follicle-stimulating hormone (6). Mice lacking IRS-2 also have reduced serum levels of luteinizing hormone and show reduced fertility (74). Female mice with knockout of IR in gonadotropin-secreting pituitary cells, on the other hand, display a paradoxical increase in fertility in the context of obesity (75), highlighting the complexity of insulin action at different levels of the CNS on the reproductive axis.

INSULIN ACTION ON BRAIN AND NEURODEGENERATIVE DISEASES

Over the past decade, a number of studies (76,77) have shown an association between AD and decreased insulin signaling in the CNS, suggesting that reduced insulin
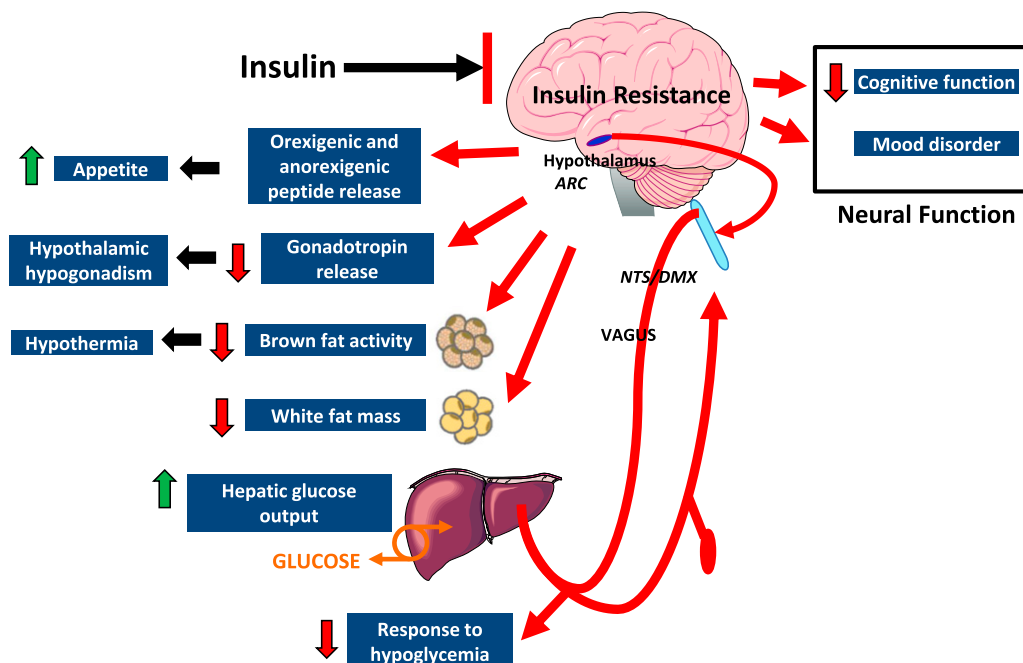
Figure 3—Effects of insulin signaling in the brain on central and peripheral function. Brain insulin resistance results in increased food intake, hypothalamic hypogonadism, hypothermia, decreased white fat mass, increased hepatic glucose output, impaired response to hypoglycemia, and impaired neural function. ARC, arcuate nucleus; DMX, dorsal motor vagal nucleus; NTS, nucleus of the solitary tract.

action might play a role in the pathogenesis of this disease. Patients with AD exhibit reduced expression and activation of IR, IGF-1R, and IRS-1 proteins in the brain, in particular in the hippocampus and hypothalamus, as well as increased serine phosphorylation in IRS-1, which is generally regarded as inhibitory (78,79). They also have lower insulin concentrations in the CSF, but higher insulin concentrations in plasma, suggesting reduced insulin action in the CNS. In line with this possibility, a small clinical trial of intranasal insulin administration to individuals with AD decreased the rates of cognitive decline (80).

Whether insulin resistance is a cause or consequence of AD is still not clear; however, insulin action has been shown to play a role in several important parts of the progressive pathogenesis of AD. Aggregated amyloid-β (Aβ) fibrils and hyperphosphorylation of tau protein causing amyloid plaques and helical neurofibrillary tangles are hallmarks of AD, and these can be ameliorated by insulin signaling (81). Mechanistically, insulin and IGF-1 treatment have been shown to decrease the intracellular Aβ burden by increasing the trafficking and clearance of Aβ via the MAPK pathway, which alleviate gliosis and cognitive deficits in a mouse model of AD (82–84).

We have shown that deletion of brain IRs (NIRKO) results in increased phosphorylation of tau protein, one of the major components of pathologic neurofibrillary tangles (21). As noted above, tau hyperphosphorylation occurs secondary to a decrease in GSK3β phosphorylation, which results in increased GSK3β activity. Despite the increased phosphorylation of tau, NIRKO mice do not develop memory disorders. This may be due to opposing effects at other levels in the multistage pathogenesis of this disease (Fig. 4). Moreover, knockout of IR has been

shown to reduce amyloid burden in a mouse model of AD, and knockout of IRS-2 reduces mortality in a mouse model of AD (85,86). On the other hand, the treatment of mice and rats with drugs that stimulate insulin secretion, such as exendin-4 or intranasal insulin, has been shown to reduce neurological pathologies in AD mice (87–89). Synaptic loss, a phenotype of AD patients, can also be rescued with insulin treatment of cultured neurons (90). Insulin and Aβ are both substrates of insulin-degrading enzyme, and it has been suggested that hyperinsulinemia inhibits the degradation of Aβ by competitively blocking insulin-degrading enzyme (91). Finally, the administration of intranasal insulin in humans, which allows for high doses of insulin to be delivered to the brain, can result in improved cognitive function in certain groups of AD patients, while suppressing lipolysis in healthy adults (80,92). The cognitive response in AD patients seems to be dependent on sex, insulin dose, and apolipoprotein (Apo)Eϵ4 status, underscoring the complexity of insulin action in the brain.

Brain insulin resistance is also associated with Parkinson disease (PD). In some studies, patients with type 2 diabetes exhibit an almost twofold increased risk of PD compared with control subjects (93). PD patients also show reduced expression of IRs, IGF-1Rs, and their endogenous ligands in various brain regions, along with increased numbers of markers of insulin resistance, inflammation, and mitochondrial dysfunction (94). Type 2 diabetes mouse models express more α-synuclein, the protein involved in Lewy body formation in PD patients, and exhibit increased susceptibility to toxin-induced dopaminergic loss (95). Mutant α-synuclein, which is present in one form of genetic PD, can inhibit AKT activation, indicating that this pathology
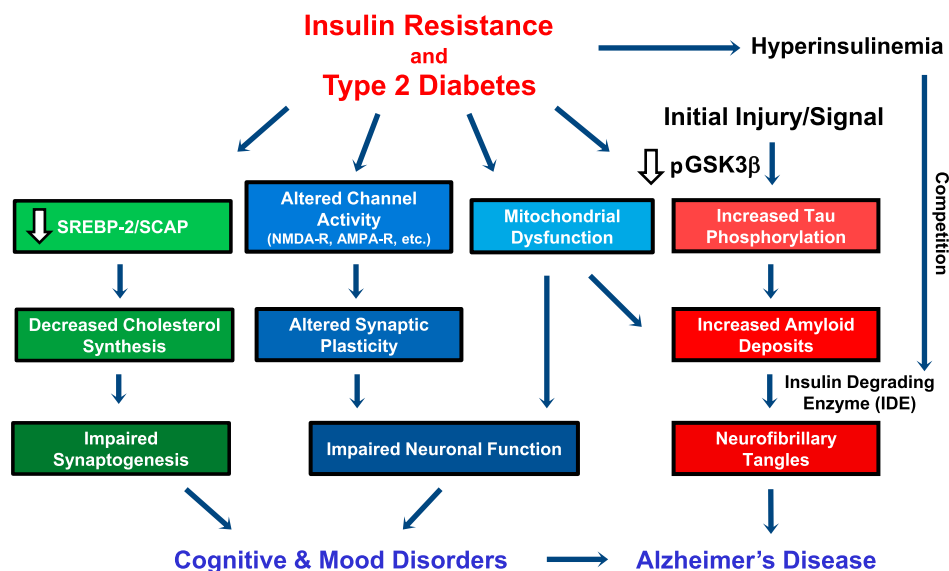
Figure 4—Insulin resistance in the brain influences neurological function through multiple pathways. Impaired insulin signaling in the brain leads to decreased SREBP-2/SCAP-dependent cholesterol synthesis, altered synaptic plasticity, mitochondrial dysfunction, and increased tau phosphorylation, all of which may contribute downstream to impaired neurological functioning, including AD and mood disorders. AMPA-R, AMPA receptor; NMDA-R, NMDA receptor.

can also influence insulin sensitivity (96). Thus, it is intriguing to hypothesize that the increase in AD and PD pathogenesis seen later in life is driven, in part, by the age-related decline observed in IGF-1 and insulin concentrations and receptor expression.

ALTERATIONS IN CHOLESTEROL METABOLISM AND MITOCHONDRIA IN DIABETES AND AD

We and others have shown that another possible link between diabetes and AD is altered cholesterol metabolism. ApoE is the major apolipoprotein and the principal carrier of cholesterol in the brain. The genetic variant ApoEε4 is the most common risk factor for late-onset AD, but the mechanism behind this observation remains largely unknown (97). In vitro experiments coculturing astrocytes and neurons suggest that cholesterol is synthesized in astrocytes, packaged into ApoE particles, and secreted to be taken up by neurons and incorporated into membranes to augment synaptogenesis and vesicle formation (98). We have shown that diabetic mice, including models of both type 1 and type 2 diabetes, have decreased cholesterol biosynthesis in the brain due to decreased expression and/or function of SREBP-2, the major regulator of cholesterologenic gene transcription (61). These mice also have decreased levels of SREBP cleavage-activating protein (SCAP), a sterol-sensing molecule, which contributes to altered processing of immature SREBP-2 to its mature form, leading to a further defect in cholesterol synthesis (61,99). The decrease in cholesterol synthesis that occurs in STZ diabetic mice can be reversed by the administration of insulin into the third ventricle at levels that do not affect blood glucose levels or peripheral metabolism, indicating that these changes in cholesterol metabolism are due to a direct action of insulin on the brain. Interestingly, we found that primary cultures of both neurons and glia express all of the enzymes of cholesterol synthesis and that both can be stimulated by insulin in vitro, implicating both neurons and glia in the maintenance of normal cholesterol levels in the brain.

The brain is a very cholesterol-rich organ with cholesterol densely packed into the cell membranes, contributing to membrane fluidity, vesicle formation, and synaptogenesis (100). To understand the consequences of modest decreases in cholesterol synthesis similar to those seen in diabetic mouse brains without the other accompanying metabolic changes associated with diabetes, we studied mice with a heterozygous deletion in the brain of SCAP, and performed a knockdown of SREBP-2 in isolated neurons in vitro. We found that mice with a 50% reduction in SCAP had reductions in SREBP-2 and in vivo cholesterol synthesis similar to those seen in diabetic mice (99). This resulted in defects in synaptic transmission, including alterations in long-term potentiation, implying a memory defect. On behavioral testing, SCAP knockout mice also demonstrated impaired learning and abnormal stress responses (Fig. 5), further implicating insulin regulation of brain cholesterol metabolism as another link between

diabetes and AD. Importantly, the knockdown of SREBP-2 in isolated neurons in culture also resulted in a decrease in the formation of synapses and synaptic vesicles (Fig. 5) (61). Thus, insulin regulation of cholesterol synthesis may be an important component of the effects of diabetes on altered brain and nerve function.

Mitochondrial stress has also been proposed as both a contributor to and consequence of the pathologies seen in AD (101). Recent data demonstrate that in the brains of obese diabetic mice and diabetic humans there is decreased expression of the mitochondrial chaperone heat shock protein 60 (Hsp60) (28). In mice, this appears to be due, at least in part, to a lack of proper leptin action in the hypothalamus. Mice with a heterozygous deletion of Hsp60 have increased mitochondrial dysfunction, which is accompanied by insulin resistance in the hypothalamus. Further, mitochondrial dysfunction induced by the knockout of mitofusin 1 or 2 in AgRP neurons results in impaired neuronal firing in mice exposed to a high-fat diet (102).

Taken together, these data indicate that insulin action in the brain can alter neuronal and glial function in multiple ways and also can modify the pathogenesis of neurodegenerative diseases like AD at multiple steps (Fig. 4). Coupled with a preliminary study (80) showing that intranasal insulin application may reduce cognitive decline in AD patients, the insulin signaling pathway is an important target for future AD research.

INSULIN IN THE BRAIN AND MOOD DISORDERS

Multiple studies have shown an association between depression and both type 1 and type 2 diabetes beyond what is associated with living with a chronic disease (103). The mechanisms by which diabetes can influence depression remain elusive but may relate to effects of insulin on neurotransmission, as well as indirect effects linked to insulin resistance, such as inflammation and increased cytokine production (103). Several brain areas have been implicated in the pathogenesis of mood disorders, including the prefrontal cortex, nucleus accumbens, striatum, amygdala, and raphe nucleus, all of which are areas of the brain that express IR (Fig. 1). Insulin signaling has been shown to modulate the dopaminergic and serotonergic systems, which are pathways that play a pivotal role in depression (104,105). For example, central insulin administration can increase dopamine transporter protein expression in rats via a PI3K-dependent pathway (106–108), thereby fine-tuning dopamine signaling. Additionally, the ablation of IR in catecholaminergic neurons attenuates insulin-induced excitability in dopaminergic neurons, indicating that insulin might modulate their neuronal activity, affecting depression (106,107,109). On the other hand, insulin has also been shown to increase serotonin levels in the brain, suggesting a beneficial effect of insulin signaling on the dopamine and serotonin signaling cascade, and thus possibly on mood (110). Both type 1 and type 2 diabetes animal models exhibit depressive-like behavior (111,112), which can be ameliorated by treatment with insulin or insulin sensitizers
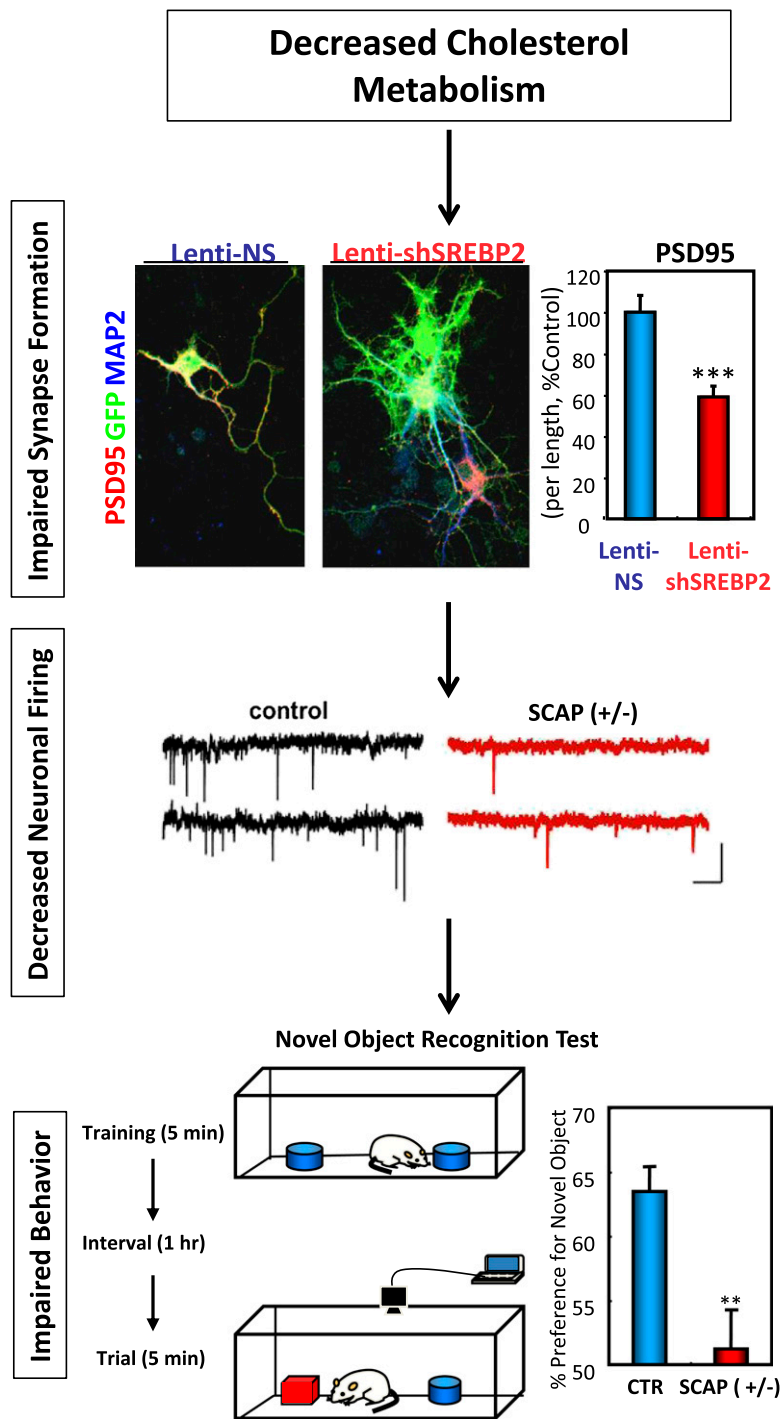
Figure 5—SCAP and SREBP-2 are important for synapse formation, nerve firing, and memory tasks. *Top*: SREBP-2 was silenced with a green fluorescent protein (GFP)-lentiviral construct (Lenti-shSREBP-2 [short hairpin SREBP-2]) in primary cultured mouse hippocampal cells. The PSD95 marker is represented in red, and the neuronal marker MAP2 is shown in blue. Adapted from Suzuki et al. (61). *Middle*: Mice with heterozygous deletion of SCAP show a decrease in spontaneous neuronal activity, as determined by basal recordings of miniature excitatory postsynaptic current in area CA1 neurons (adapted from Suzuki et al. [99]). *Bottom*: Impaired memory task performance. For the novel object recognition test, mice were trained for 5 min with two identical objects. One hour later, they were placed back in the same cage with one familiar and one novel object, and were monitored by video for 5 min. Mice with a heterozygous deletion of SCAP in the brain showed no exploratory preference for the novel object. Adapted from Suzuki et al. (99). CTR, control.

(113,114). At a mechanistic level, insulin decreases the activity of monoamine oxidase, the enzyme responsible for degrading serotonin and dopamine, thereby increasing the activity of those systems (113). Ongoing studies in our laboratory reveal that NIRKO mice exhibit altered behavior, such as an increase in anxiety and depression-like behaviors with aging, indicating that central insulin signaling can affect complex behaviors and mood (A. Kleinridders, unpublished observations).

INSULIN ACTION IN THE BRAIN AND LONGEVITY

The effect of insulin signaling on life span may also involve the brain. This was first shown in *C. elegans* where hypofunctional mutations in daf-2 (the homolog of mammalian IRs and IGF-1Rs) were shown to increase longevity by up to 250%. Several other alterations in the insulin/IGF-1 pathway, such as overexpression of FoxO1 and inactivation of Akt, support the initial observation that reduced insulin/IGF-1 signaling in eukaryotes prolongs life span (115,116). A similar beneficial effect of reduced insulin/IGF-1 signaling on longevity is found in flies (117). In both worms and flies, these effects can be cell nonautonomous, and have been shown to involve loss of insulin signaling in neurons, as well as in adipose tissue in the fly and the gut in worms (116).

Studies in mammals suggest that both IR and IGF-1R may have effects on longevity, but the relationship is complex, since deletion or hypofunction of either receptor profoundly affects growth and physiology, making it difficult to elucidate any direct effect on aging. The most direct evidence that insulin and/or IGF-1 signaling in the brain can extend life span comes from studies of mice with knockout of IRS-2 in the brain and whole-body IRS-2 heterozygous mice on both a wild-type and Huntington disease background (15,118), both of which show an increased life span, confirming that in mammals decreased insulin/IGF-1 signaling can be beneficial for survival. However, brain-specific knockouts of the IR do not appear to have increased longevity, but this may be the result of other defects that negatively affect life span. However, fat-specific IR knockout mice (119) and whole-body IGF-1R heterozygous mice (120) both have an extended median and maximum life span (~15–20%). Increased levels of Hsp60, a known regulator of central insulin action (121), in the brain are seen in long-lived animal species, further suggesting a role for central insulin signaling and longevity (122). Also, genetic variation within the *FoxO3a* gene, a transcription factor downstream of the IR, is associated with human longevity (123), but how this relates to central insulin signaling is unknown. Further studies will be required to understand the full role of insulin versus IGF-1 signaling at the level of the brain in longevity.

CONCLUSIONS AND FUTURE PERSPECTIVES

Insulin is a key homeostatic factor in the brain, acting through both the IR and IGF-1R to maintain the health of the brain and to influence systemic metabolism. Current data demonstrate that insulin signaling in the brain is vital in the fine-tuning of brain activity. Through direct neuronal effects, and regulation of cholesterol synthesis, mitochondrial function, tau phosphorylation, and Aβ processing, defects in insulin signaling provide a link between diabetes and CNS disorders. Recent work (124,125) also suggests that insulin may play a role in the severity of strokes, further expanding the therapeutic potential for insulin. This also raises important questions as to how to treat these abnormalities and the role of intranasal insulin or alternative routes of administration for increasing central insulin action as a component in the management of AD and other neurocognitive disorders. The effects of insulin action on brain lipid metabolism, not only cholesterol as described here, but free fatty acids and triglycerides as evidenced by the effects of brain lipoprotein lipase knockout (126), need to be better understood, as does their relationship to the ApoEε4 phenotype. Also, it will be important to determine how the role of insulin in glial cells can modify the effects of insulin on appetite, energy balance, systemic metabolism, and brain function. Ultimately, we want to know whether insulin action in the brain may also be a potential therapeutic site for mood disorders in the diabetic and/or nondiabetic populations. It is hoped that continued investigation of the mechanisms of insulin action in the brain in conjunction with ongoing clinical trials will answer these questions.

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