Diabetes, Insulin and Alzheimer's Disease

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Diabetes, Insulin and Alzheimer's Disease

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Forword

The importance of insulin in the regulation of corporal aging has been established by the dramatic increases in longevity experienced by animals in which the adipose insulin receptor or the insulin-related daf genes have been genetically modified. However, a long-held belief, described as recently as ten years ago in endocrinology textbooks, declared that the brain was an insulin-insensitive organ. This pervasive belief was challenged by leaders like Jesse Roth, Daniel Porte, and others, who established the existence of insulin receptors in the central nervous system and a clear role for insulin in CNS control of feeding. New research demonstrates that, analogous to its influence on corporal aging, insulin also makes important contributions to brain aging and the expression of late-life neurodegenerative disease. Insulin plays a key role in cognition and other aspects of normal brain function. Insulin resistance induces chronic peripheral insulin elevations and is associated with reduced insulin activity both in periphery and brain. The insulin resistance syndrome underlies conditions such as Type 2 diabetes mellitus and hypertension, which are associated with age-related cognitive impairment and Alzheimer's disease.

This volume contains the proceedings of the 24th *Colloque Médecine et Recherche dedicated to Alzheimer's disease* organized by *the Fondation IPSEN* entitled "Diabetes, Insulin and Alzheimer's Disease" which brought together experts from basic and clinical science to provide a broad survey of the role of insulin in the brain, and to discuss the mechanisms through which insulin dysregulation contributes to the development of cognitive impairment and late-life neurodegenerative disease. Each author has greatly furthered our understanding of the relationships among insulin, diabetes, and Alzheimer's disease, moving us far beyond the belief that the brain is an insulin-insensitive organ. Given the recent pandemic of conditions associated with insulin resistance, it is imperative that we achieve a comprehensive knowledge of the mechanisms through which insulin resistance affects brain function in order to develop therapeutic strategies to address these effects.

Suzanne Craft
Yves Christen

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Insulin Action in the Brain and the Pathogenesis of Alzheimer's Disease

C. Ronald Kahn and Ryo Suzuki

Abstract Over 24 million people in the U.S. have diabetes mellitus, and about 90% of these have the type 2 form of the disease. In addition, an estimated 40–60 million people have pre-type 2 diabetes, impaired glucose tolerance or the cluster of abnormalities referred to variably as the metabolic syndrome or syndrome X (Reaven 1988). In all of these disorders, a central component of the pathophysiology is insulin resistance. Insulin resistance is also closely linked to other common health problems, including obesity, polycystic ovarian disease, hyperlipidemia, hypertension and atherosclerosis (Biddinger and Kahn 2006). Recent data also indicate a link between insulin resistance, type 2 diabetes and Alzheimer's disease (Craft 2007). Cross-sectional studies have suggested an association between type 2 diabetes and cognitive decline, especially in aspects of verbal memory (Strachan et al. 1997). Longitudinal studies have revealed that patients with type 2 diabetes have a 1.5-fold greater change over time in measures of cognitive function than those without diabetes (Cukierman et al. 2005). While some of this change may certainly be due to the increased prevalence of atherosclerosis in diabetic patients, there is increasing evidence that insulin resistance itself may affect CNS function and risk of Alzheimer's disease. In this review we will explore this relationship, focusing on experiments we have performed in mice.

1 The Insulin Signaling System

The insulin/IGF-1 signaling system is evolutionarily very ancient. Homologues of these receptors have been identified in *Drosophila*, *C. elegans*, *Porifera* and many other species (Petruzzelli et al. 1986; Skorokhod et al. 1999; Dorman et al. 1995;

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Renteria et al. 2008).The insulin receptor (IR) was initially identified using ^{125}I-insulin binding (Freychet et al. 1972). Biosynthetic and affinity labeling revealed the IR to be glycoproteins, consisting of two 135 kDa α-subunits and two ~95 kDa β-subunits linked by disulfide bonds to form an $\alpha_2\beta_2$ heterotetramer (Massague et al. 1980; Kasuga et al. 1982a). On pulse-chase labeling, both subunits were derived from a single chain precursor or proreceptor (Hedo et al. 1983). In 1982, we demonstrated that the IR possessed tyrosine kinase activity, placing it biochemically in the family of receptor tyrosine kinases and opening up new avenues in the study of insulin signaling (White et al. 1987; Kasuga et al. 1982b). In 1985, two groups succeeded in cloning the cDNA of the human IR, confirming these structural features (Ullrich et al. 1985; Ebina et al. 1985).

The IR gene is present on chromosome 19p13.3 in humans and chromosome 8 in the mouse. In both, the gene is >120 kb in length and is composed of 22 exons, which to some extent encode functional domains of the receptor (Fig. 1). The IR cDNA in both humans and rodents possesses an open reading frame of 4,146 nucleotides that encodes the 1,382-amino acid precursor of the receptor (Ullrich et al. 1985; Ebina et al. 1985), including a 27-amino acid signal peptide, a 721-amino acid α-subunit, a four-amino acid processing site, and a β-subunit of 619 amino acids. During the biosynthesis of the proreceptor, both subunits undergo glycosylation, disulfide bond formation and proteolytic cleavage by a furin-related protease to give the mature receptor (Hedo et al. 1983).

Functionally, the IR behaves as a classic allosteric enzyme. The α-subunit of the IR serves as both the insulin binding subunit and the regulatory subunit. Insulin binding to the α-subunit induces conformational changes in the receptor and activates the kinase activity in the β-subunit. The β-subunit is a transmembrane protein linked by disulfide bonds to the α-subunit and contains the tyrosine kinase activity critical for insulin action (Kasuga et al. 1982b). Following stimulation, the β-subunit undergoes autophosphorylation on seven Tyr residues in an ordered cascade; three of these at Tyr 1158, 1162, 1163 result in activation of the receptor kinase toward other substrates (Feener et al. 1993; Hubbard 1997). The IR occurs as two splice variants based on inclusion (IR-B) or exclusion (IR-A) of a 12-residue segment encoded by exon 11 and inserted between residues 716 and 717 (IR-A numbering) near the C-terminus of the α-subunit. In the brain, the major isoform of the insulin receptor is the A isoform (Kenner et al. 1995). The molecular weights of the denatured α- and β-subunits from brain insulin receptors are 5-10 kDa smaller than their counterparts in other tissues, which appears to be due to differences in N-linked glycosylation (Heidenreich et al. 1983). Whether they are differences in IR isoform splicing or glycosylation in Alzheimer's brain versus normal brain has not been studied.

cDNA cloning and functional studies have revealed two other members of the IR family: the highly homologous IGF-1 receptor (Ullrich et al. 1986; Abbott et al. 1992) and the IR-related receptor (IRR; Shier and Watt 1989; Zhang and Roth 1992). Insulin, IGF-1 and IGF-2 can bind to both the IR and IGF-1R, albeit with differing affinities. No ligand has thus far been identified for the IRR, and thus its physiological function is unknown. All three receptors are normally disulfide-linked

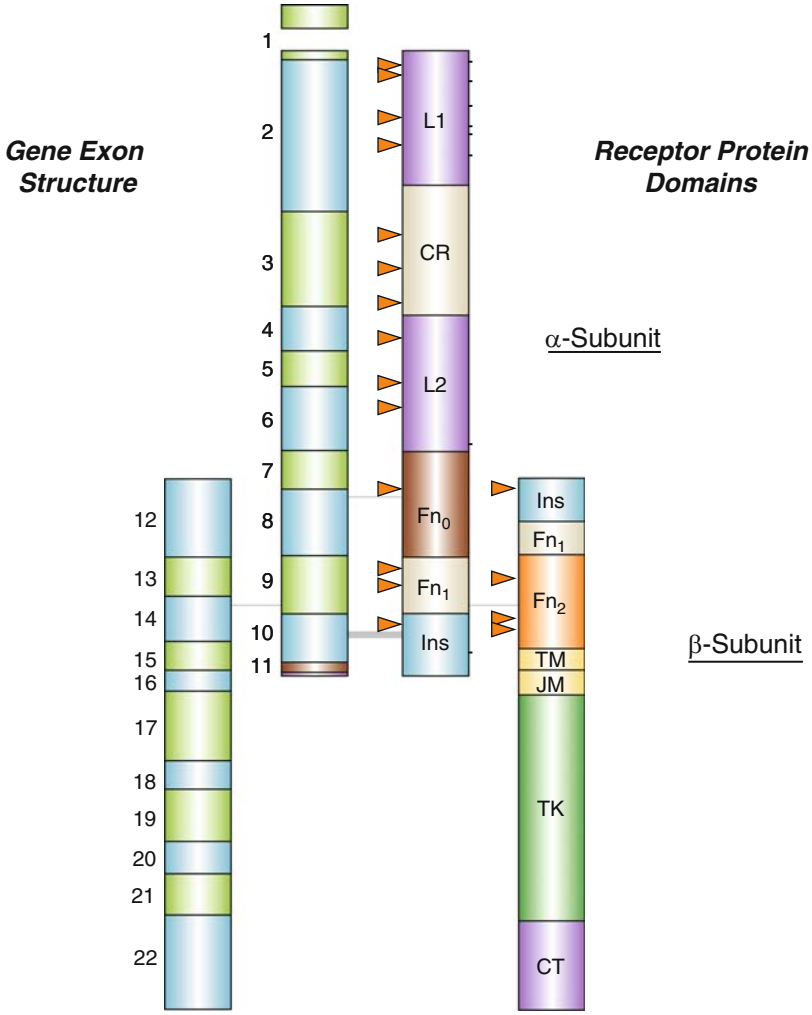

Fig. 1 Modular structure of insulin receptor (IR) gene and protein. Schematic of the $\alpha_2\beta_2$ structure of the IR. On the left, the half-receptor heterodimer is depicted by its genomic structure, which is encoded by the 22-exon sequences. On the right, the half-receptor heterodimer is depicted by predicted protein modules. L1: large domain 1; CR: cystein-rich domain; L2: large domain 2; Fn: fibronectin type III domains; Ins: Insert; TM: transmembrane domain; JM: juxtamembrane domain; TK: tyrosine kinase domain; CT: C-terminal domain. The orange arrowheads indicate the N-glycosylation sites. Adapted from De Meyts and Whittaker (2002)

homodimers but may also function as heterodimer hybrids, like IR/IGF-1R hybrids, in tissues that express both receptors (Slaaby et al. 2006; Benyoucef et al. 2007). IGF-1 receptors are abundant in brain and widely distributed therein and they have a somewhat different distribution from IRs (Dore et al. 1997; Baron Van Evercooren et al. 1991). IRR mRNA is also found in brain, but its distristribution is

highly restricted to the forebrain, primarily cholinergic neurons and neurons that coexpress trkA, a high-affinity receptor for nerve growth factor (Tsuji et al. 1996).

2 Linking the Receptor to the Insulin and IGF-1 Signaling Pathways

Following ligand binding, the activated IRs and IGF-1 receptors initiate signaling networks that share many similarities and critical nodes of signal divergence and regulation (Taniguchi et al. 2006; Fig. 2). The primary action of these receptors is to phosphorylate a family of at least 12 intracellular substrate proteins. The first four of these identified were designated IRS-1 to IRS-4 (IR substrates 1-4; Taniguchi et al. 2006; White 1998). These IRS proteins are 60 to 180 kDa and are characterized by a pleckstrin homology (PH) domain, a phosphotyrosine binding (PTB) domain, which account for their high affinity for the IR, and up to 20 potential tyrosine phosphorylation sites spread throughout the molecule. All four IRS proteins have been identified in brain, although the major forms appear to be IRS-1 and

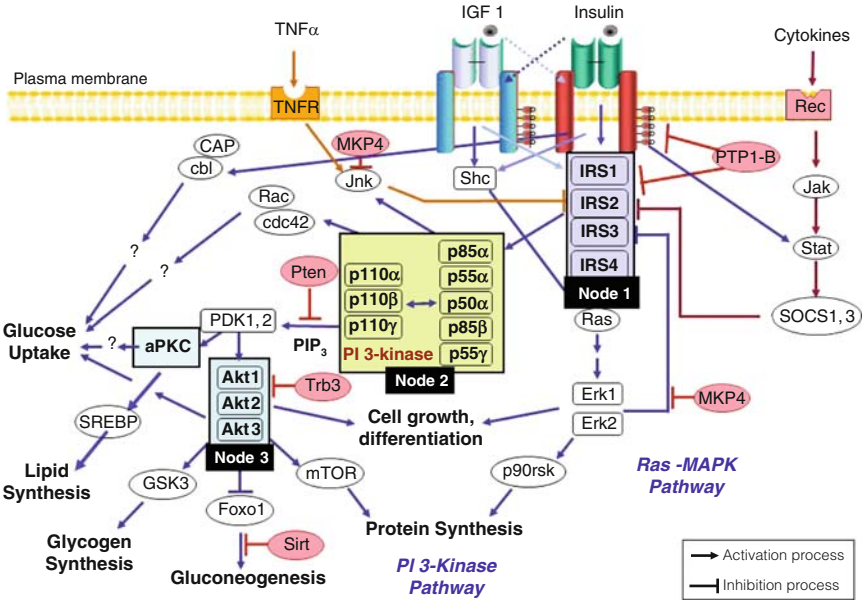

Fig. 2 Signaling networks of insulin and IGF-I receptors. The insulin and IGF-1 signaling networks are complex and contain at least three critical nodes. The three major nodes in this pathway are the IR coupled to IRS proteins (purple box), PI 3-Kinase (green box), and Akt (blue box). Plain arrows represent an activation process, blocked arrows represent an inhibition process, and dashed arrows represent an activation process with less intensity. Cytokine (TNFα, IL6, leptin) signaling pathways have been shown to interfere with insulin signaling and are also represented on this figure (orange and red arrows). Adapted from Taniguchi et al. (2006)

IRS-2, similar to most insulin-sensitive tissues. Other direct substrates of the insulin/IGF-1 receptor kinases include the various isoforms of Shc, DOK-4 and DOK-5 (also referred to as IRS-5 and IRS-6), Gab-1, p62dok, Cbl, FAK, Sam68, DAPP1, and CEACAM1 (Ribon et al. 1998; Poy et al. 2002; Najib and Sanchez-Margalet 2002; Okamura-Oho et al. 2001; Wick et al. 2001; Cai et al. 2003). Following phosphorylation, these substrates function as key intermediates in signal transduction by interacting with other intracellular molecules. The best-characterized of these are SH2 domain proteins that bind to phosphotyrosines in specific sequence motifs on the IRS proteins. These SH2 proteins fall into two major categories: adaptor molecules, such as the regulatory subunit of PI 3-kinase, Grb2, which associates with SOS to activate the Ras-MAP kinase pathway (Baltensperger et al. 1993; Valverde et al. 2001), CrkII (Karas et al. 2001) and Nck2 (Tu et al. 2001), and enzymes, such as the phosphotyrosine phosphatase SHP2 (Rocchi et al. 1996) and the tyrosine kinase Fyn (Sun et al. 1996). The IRS proteins also interact with proteins that do not contain SH2 domains, including the calcium ATPases SERCA 1 and 2 (Algenstaedt et al. 1997), SV40 large T antigen (Prisco et al. 2002), Rho-kinases (Begum et al. 2002), PH domain-interacting protein (PHIP; Farhang-Fallah et al. 2000), IRAS (Sano et al. 2002) and others (Kruger et al. 2008; Hanke and Mann 2009). Through extensive studies, each of these has been shown to play important roles in the downstream actions of insulin and IGF-1, with the enzyme PI 3-kinase forming the most important link in insulin signaling to its metabolic effects (reviewed in Taniguchi et al. 2006).

In addition to these primary pathways of insulin/signal transduction, there are a number of other pathways activated, including pathways involving Cbl, CAP and the GTPase TC10 (Ribon et al. 1998; Chang et al. 2007), activation of GTPase of the Rac and Rho family (Usui et al. 2003), and interactions with the adaptor protein APS (Barres et al. 2006). Indeed, in collaboration with Mathias Mann using phosphoproteomics, we have identified as many as 40 proteins involved in insulin/IGF-1 action via tyrosine phosphorylation (Kruger et al. 2008).

3 Regulation of the IRS and IGF-1 Receptors in Physiology and Pathophysiology

The insulin/IGF-1 signaling pathway is subject to regulation at multiple levels in normal physiology and disease states. Over 100 patients with syndromes of severe insulin resistance have been reported with mutations in the IR gene (Taylor et al. 1994; Rouard et al. 1999). In addition to mutation, there are a number of mechanisms that play a role in acquired alterations of IR signaling in disease. The most common mechanism is down-regulation of the IR, which occurs to variable degrees in all hyperinsulinemic states (Gavin et al. 1974; Haft et al. 1994). This down-regulation occurs through internalization and subsequent degradation of the receptor. It is not clear if the brain shows similar down-regulation of the IR in obesity and

type 2 diabetes in humans; in rodents, studies on this point have provided conflicting results (Figlewicz et al. 1986; Havrankova et al. 1979)

In addition to changes in receptor concentration, inhibition of receptor kinase activity can occur in diabetes and obesity secondary to phosphorylation of the IR or its substrates by serine kinases activated by increased levels of cytokines, such as TNFα and IL-6 (Hotamisligil et al. 1993; Fernandez-Real et al. 2000; Takayama et al. 1988). IR and IGF-1 receptor function may also be modified by protein-protein interaction. Interacting proteins include the suppressors of cytokine signaling (SOCS) proteins (Emanuelli et al. 2000; Ueki et al. 2004), the growth factor receptor-bound proteins Grb10 and Grb14 (He et al. 1998; Kasus-Jacobi et al. 1998) and PC-1, also termed ectonucleotide pyrophosphatase phosphodiesterase 1 (ENPP1; Goldfine et al. 2008).

4 Creation and Characterization of the Brain IR Knockout Mouse

IRs are present on virtually all tissues in mammals, including the classic insulin-responsive tissues (muscle, fat and liver) and non-classical tissues, such as brain, β-cells, endothelial cells, etc. A major initiative of the past 10 years was based on the use of the Cre-*lox* system to create tissue-specific IR knockout (KO) mice and to use these to define more precisely the role of insulin action in each tissue of the body. Since IRs are widely distributed throughout the central nervous system (CNS; Havrankova et al. 1978) and have been suggested to play a role in feeding behavior (Schwartz et al. 2000), we decided to study the physiological role of insulin in the brain. We created mice with a neuron-specific disruption of the IR gene (NIRKO mice) using the nestin promoter (Bruning et al. 2000). Inactivation of the brain IR had no impact on brain development but, as expected, resulted in a loss of insulin-stimulated PIP_3 in the hypothalamus, while response to leptin remained normal (Fig. 3, top; Schubert et al. 2004). As a result of CNS insulin resistance, NIRKO

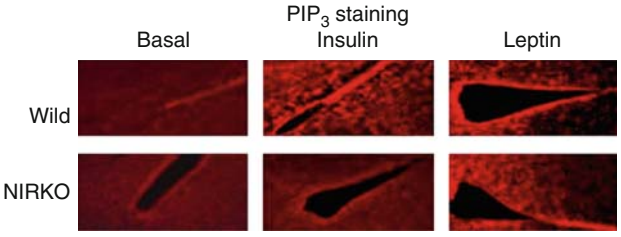

Fig. 3 Loss of insulin signaling in brain-specific IR knockout (NIRKO) mice. The ability of the brain to respond to peripherally administered insulin and leptin is demonstrated by an increase in PIP_3 staining in the paraventricular region. Stimulation by insulin is lost in the brain of the NIRKO mouse, whereas stimulation by leptin remains active. Adapted from Shubert et al. (2004)

mice showed increased food intake, and both male and female mice developed diet-sensitive obesity, with increases in body fat and plasma leptin levels, mild insulin resistance, elevated insulin levels, and hypertriglyceridemia (Fig. 3, middle). In addition, loss of insulin action in the CNS had an effect on liver metabolism. Thus, while peripheral insulin suppressed hepatic glucose production by 74% in control mice, insulin action on hepatic glucose production (HGP) was markedly blunted in NIRKO mice (Fig. 4, bottom; Fisher et al. 2005). This finding is complementary to those of Rossetti and Accili that insulin action on the brain can regulate hepatic glucose output (Obici et al. 2002; Okamoto et al. 2004). In NIRKO mice, insulin-stimulated brain glucose uptake was reduced ~46%,

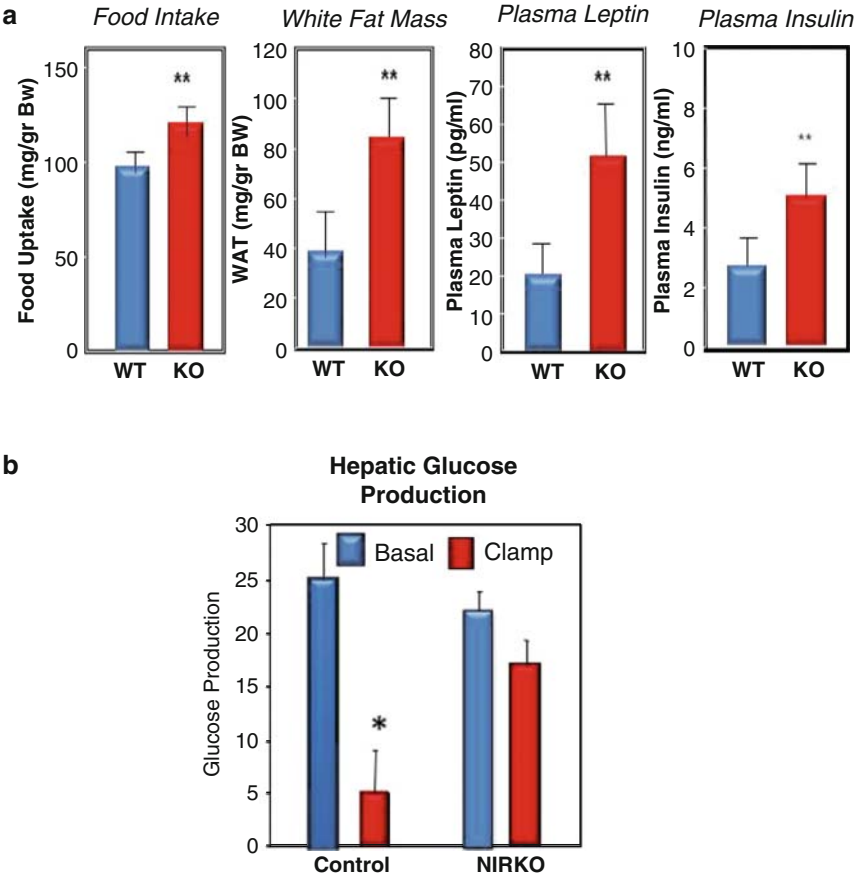

Fig. 4 Metabolic phenotypes of brain-specific IR knockout (NIRKO) mice. Knockout of the IR in the brain results in mild hyperphagia and obesity with increased leptin levels and increased plasma insulin levels (Panel a). WT: wild type; KO: knockout. The hyperglycemia is due to a defect in insulin's ability to suppress hepatic glucose output (Panel b). Adapted from Bruning et al. (2000)

whereas glucose transport in muscle or fat was not altered. Finally, NIRKO mice exhibited defects in counter-regulatory response to hypoglycemia, especially increases in epinephrine and nor-epinephrine (Fisher et al. 2005), and impaired testicular and ovarian function due to hypothalamic hypogonadism (Bruning et al. 2000). Thus, IR signaling in the CNS plays an important role in regulation of appetite, energy disposal, hepatic metabolism, hypoglycemic counter-regulation and reproduction.

To define the specific cells in the brain involved in control of metabolism, in collaboration with Jens Bruning, we generated mice with selective inactivation of the IR in pro-opiomelanocortin (POMC) or agouti-related peptide (AgRP)-expressing neurons (Konner et al. 2007). While neither POMC- nor AgRP- IR KO mice exhibited obesity or altered energy homeostasis, IR KO in AgRP neurons resulted in a loss of insulin's ability to normally suppress HGP. AgRP-IRKO mice also exhibited reduced hepatic IL-6 expression and increased hepatic expression of glucose-6-phosphatase. In addition, we created two mouse models with inducible IR inactivation, one in the whole body including brain (IRΔwb) and a second restricted to peripheral tissues (IRΔper) (Koch et al. 2008). While both strains developed severe hyperinsulinemia, hyperglycemia was more pronounced in IRΔwb mice, consistent with the additional role of insulin action in brain control of glucose metabolism also observed by Accili (Okamoto et al. 2004). Interestingly, the IRΔwb mice also had a more pronounced reduction in the white adipose tissue (WAT) mass than IRΔper, suggesting an additional role of central insulin action in control of fat mass (Koch et al. 2008).

5 Impairment of insulin Signaling in Brain is Linked to Neurodegenerative Disease

There is a growing body of evidence linking insulin resistance and insulin action in the brain to neurodegenerative disease, especially Alzheimer's disease (Craft 2007). Low concentrations of insulin and reduced receptor numbers and signaling events in the CNS with Altzheimer's disease have been reported (Frolich et al. 1998; Hoyer 2002). Insulin administration while maintaining euglycemia improves memory in both healthy adults and Alzhermer's disease patients (Craft et al. 1999). In addition to Alzheimer's disease, Parkinson's disease is reported to accompany insulin resistance with a high prevalence (Pressley et al. 2003). Likewise, some studies have found that patients with Huntington's disease have a higher prevalence of diabetes and insulin resistance (Farrer 1985). Since insulin has neuroprotective effects in vivo (Hui et al. 2005; Rizk et al. 2006; Collino et al. 2009), impaired insulin action in the brain may have a critical role for pathogenesis of those neurodegenerative diseases.

One specific potential molecular link between insulin and neurodegeneration is the enzyme glycogen synthase kinase 3 (GSK3; Hooper et al. 2008). GSK3 activity

is negatively modulated by insulin via an activation of Akt. GSK3 induces the hyperphosphorylation of Tau in vitro, and its overexpression in the adult brain of conditional transgenic mice causes Tau-hyperphosphorylation and neurodegeneration (Lucas et al. 2001).

To directly determine whether the brain IR is an important regulator of GSK3 in vivo, we performed additional studies in NIRKO mice. These studies revealed a markedly reduced phosphorylation of Akt and GSK3ß in the brains of NIRKO mice leading to a parallel and substantial increase in Tau-phosphorylation (Fig 5, bottom right); Schubert et al. 2004). In vitro neurons of NIRKO mice exhibit a complete loss of insulin-mediated activation of PI 3-kinase and inhibition of neuronal apoptosis. Thus, lack of insulin signaling in neurons can induce some markers of neurodegeneration and increased susceptibility to cell death. Nevertheless, NIRKO mice exhibit no alteration in neuronal survival or memory function measured by water maze test (Schubert et al. 2004), suggesting that, for development of Allzheimer's disease, some other mechanisms might be crucial besides insulin signal deficiency in the brain. Surprisingly, one model of Alzheimer's disease, the Tg2576 Swedish amyloid precursor protein mutant-overexpressing transgenic, shows improvement in premature mortality or Aß deposition when the mice lack IGF-1 receptor or IRS-2 in the hippocampus (Freude et al. 2009; Killick et al. 2009); in this model, IR deficiency did not affect mortality (Freude et al. 2009). These data suggest distinct roles for IRs and IGF-1 receptors in the hippocampus and in the pathogenesis of Alzheimer's disease.

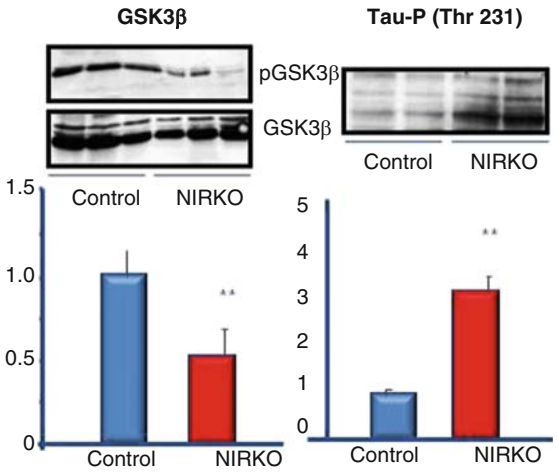

Fig. 5 Altered GSK3 and Tau phosphorlation in brain-specific IR knockout (NIRKO) mice. The left panel shows reduced GSK3β phosphorylation in the NIRKO mouse brain as determined by immunoblotting. Since phosphorylation decreases GSK3β activity, this decrease would correspond to increased kinase activity. The right panel shows increased Tau threonine-231 phosphorylation, which is presumably the result of the increased GSK3β activity. Increased Tau phosphorylation is a marker of abnormalities in Alzheimer's disease. Adapted from Shubert et al. (2004)

6 Insulin-degrading Enzyme in Pathogenesis of Alzheimer's Disease and Metabolic Diseases

Another potential candidate that links insulin resistance/diabetes and Alzheimer's disease is insulin-degrading enzyme (IDE). More than 50 years ago, Mirsky and Broh-Kahn described "insulinase," a 110 kDa zinc metalloendopeptidase present in liver exract (Mirsky and Broh-Kahn 1948). This enzyme, currently named IDE or insulysin, is highly expressed in the brain, testis and muscle, as well as in the liver (Kuo et al. 1993). IDE is predominantly cytosolic, with smaller amounts in peroxisomes, endoplasmic reticulum and plasma membranes (Miners et al. 2008). Interestingly, up to 10% fraction of the total IDE is trafficked to the extracellular space, despite its lack of a classical signal peptide, presumably via an unconventional protein secretion pathway (Zhao et al. 2009). Several peptides with molecular weights of 3-10 kDa have been shown to serve as the substrates of IDE, including insulin, IGF-I, IGF-II, amylin, and Aß. The peptide substrates share little to no homology of primary amino acid sequence but have a similar secondary structure with "amyloidogenic" character (Qiu and Folstein 2006), as demonstrated by recent crystallographic data (Shen et al. 2006).

Levels of IDE protein and transcripts are reduced in the hippocampi from Alzheimer's disease patients with an apolipoprotein E (apoE)-ε4 allele compared to either patients without this allele or normal subjects (Cook et al. 2003). A recent report exhibited that Aß degradation extracellularly by IDE is facilitated by apoE (Jiang et al. 2008). The IDE region of chromosome 10q has been shown to have genetic linkage to late-onset Alzheimer's disease (Bertram et al. 2000). A lot of evidence indicates that the same region of chromosome 10q is also genetically linked to type 2 diabetes (Saxena et al. 2007; Zeggini et al. 2007). In addition, a well-characterized rat model of type 2 diabetes, Goto-Kakizaki (GK), has been found to harbor two missense mutations in IDE gene that decrease its ability to degrade both insulin and Aß (Fakhrai-Rad et al. 2000; Farris et al. 2004). Furthermore, genetic discuption of IDE gene in mice causes increased levels of cerebral Aß and glucose intolerance with hyperinsulinemia (Farris et al. 2003).

Because of the strong Aß-degrading ability of IDE, defects in IDE activity in the brain can be a direct trigger of Aß deposition to develop Alzheimer's disease (Fig. 6). Several reports suggest that insulin signaling regulates IDE expression. Incubation with insulin increases IDE protein in primary hippocampal neurons, whereas reduction of PI 3-kinase p85 subunit is correlated with a decrease of IDE in human Alzheimer's disease brains and in Tg2576 transgenic mice fed a high-fat diet (Zhao et al. 2004). Insulin-deficient diabetes induced by streptozotocin (STZ) administration also reduces IDE protein in the brain (Jolivalt et al. 2008). Thus, secondary reduction of IDE caused by insufficient insulin action in the brain might accelerate onset of Alzheimer's disease.

In contrast, insulin-resistant/glucose-intolerant phenotypes of IDE-deficient rodents and a genetic linkage of human IDE chromosomal region with type 2 diabetes susceptibility strongly suggest that IDE has a role in maintaining

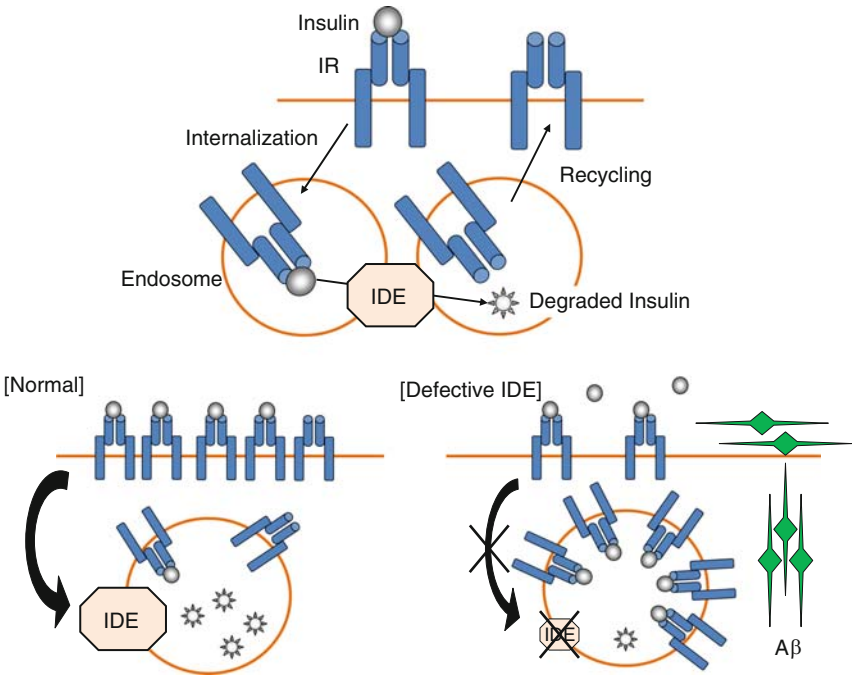

Fig. 6 Hypothetical mechanism of insulin resistance caused by IDE insufficiency. IR bound with insulin receives internalization. IDE degrades the ligand insulin at endosome. Free receptor is transferred to membrane and recycled (top). When IDE is functional, sufficient numbers of receptors are recycled to the cell surface, and the downstream signal maintains expression of IDE (bottom left). In case IDE has insufficient function, ligand-bound IRs are trapped and unable to be transferred/recycled. Reduction of available IR causes insulin resistance, and consequent impairment of insulin action causes IDE downregulation, which aggravates IDE insufficiency as a "vicious cycle"

insulin sensitivity in the body. However, the mechanism remains unclear. IDE knockout mice have about a 3-fold increase in fasting insulin levels in plasma (Farris et al. 2003), possibly as a consequence of reduced insulin degradation, but hyperinsulinemia itself does not always cause systemic insulin resistance or impaired glucose tolerance (Hennige et al. 2003). Fakhrai-Rad proposed a hypothesis that a decreased intracellular degradation of insulin bound to its receptor would inhibit receptor-mediated signal transduction by lowering the number of available receptors on the cell membrane and/or compromising the downstream signaling from the receptor (Fakhrai-Rad et al. 2000). When either IDE or IR has a defective activity a priori or posteriori, mutual regulation between IDE and insulin action in the CNS may behave as a "vicious cycle" that may trigger development of cognitive dysfunction and onset of Alzheimer's disease in diabetes patients (Fig. 6).

7 Insulin, Diabetes, and Brain Cholesterol Metabolism

Insulin plays a crucial role for glucose homeostasis, cell survival, and lipid metabolism. Both type 1 and type 2 diabetes are frequently accompanied by dyslipidemia, which can occur as a consequence of alterations in lipogenesis, lipoprotein secretion, and lipolysis in the body. A number of studies have been reported concerning the effects of insulin on circulating lipid or lipid contents in the peripheral tissues. However, lipid metabolism, especially cholesterol in the CNS with insulin resistance/diabetes, is not yet well characterized in spite of its potential importance.

In preliminary studies, we have observed a possible connection between insulin action in the brain and cholesterol metabolism. In studies using Affymetrix microarrays to identify genes differentially expressed in the hypothalami from STZ-diabetic mice (a model of type 1 diabetes), ob/ob mice (a model of type 2 diabetes) and NIRKO mice, a model of CNS insulin resistance we found that the cholesterol biosynthesis pathway was one of the most highly regulated gene sets in the hypothalamus of the STZ-diabetic mouse, with a decrease in expression of cholesterol synthesis-related genes (Fig. 7).

The brain is the most cholesterol-rich organ, containing approximately 25% of the cholesterol present in the body. Disturbances of intracellular cholesterol

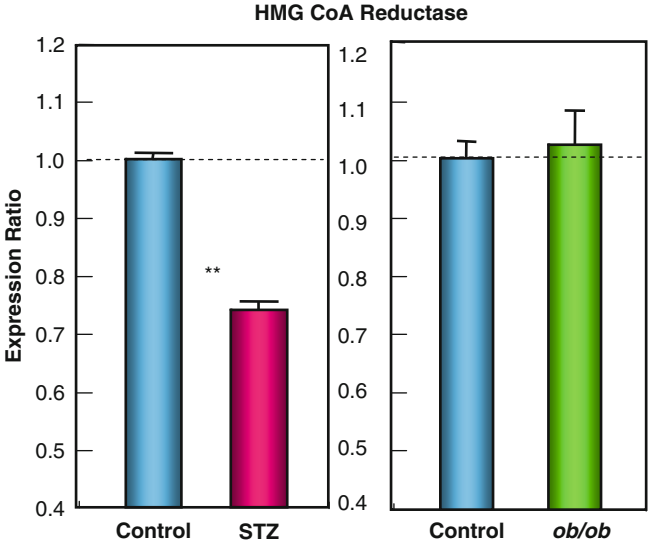

Fig. 7 Suppression of a cholesterol synthetic gene in streptozotocin-induced diabetes mouse brain. Expression of HMG-CoA reductase (HMGCR), a rate-limiting enzyme for cholesterol biosynthesis, was measured using RNA from hypothalami of brains from control mice, mice with streptozotocin (STZ)-induced diabetes (a model of type 1 diabetes) and *ob*/*ob* mice (a model of obesity and type 2 diabetes) using quantitative real-time PCR. Data are expressed relative to control levels of 1.0