Review

MOLECULAR METABOLISM

Insulin in the nervous system and the mind: Functions in metabolism, memory, and mood

Seung-Hwan Lee [1,2,5], Janice M. Zabolotny [1,5], Hu Huang [1,3], Hyon Lee [4], Young-Bum Kim [1,*]

ABSTRACT

Background: Insulin, a pleotrophic hormone, has diverse effects in the body. Recent work has highlighted the important role of insulin's action in the nervous system on glucose and energy homeostasis, memory, and mood.

Scope of review: Here we review experimental and clinical work that has broadened the understanding of insulin's diverse functions in the central and peripheral nervous systems, including glucose and body weight homeostasis, memory and mood, with particular emphasis on intranasal insulin.

Major conclusions: Implications for the treatment of obesity, type 2 diabetes, dementia, and mood disorders are discussed in the context of brain insulin action. Intranasal insulin may have potential in the treatment of central nervous system-related metabolic disorders.

Keywords Insulin; Intranasal insulin; Memory; Metabolism; Mood

1. INTRODUCTION

A fundamental metabolic action of insulin is to control blood glucose concentration by stimulating glucose transport into muscle and adipose tissue, and inhibiting hepatic glucose output [1]. It is now clear that the brain is recognized as an insulin-sensitive organ that is responsible for physiologic changes in altered metabolic disorders such as obesity and type 2 diabetes [2,3]. Insulin's actions are triggered by binding to its cell-surface receptor, which is present in virtually all mammalian cells [4]. In the brain, the insulin receptor is broadly expressed in regions including the hypothalamus, hippocampus, and cerebral cortex, all of which are involved in the metabolic control of insulin action, including feeding behavior, body weight homeostasis, neuronal development and cognitive function [3,5]. Insulin also plays important roles in neuronal circuitry formation, synaptic maintenance, neuronal survival, dendritic arborization, as well as learning and memory [6]. In this article, we review experimental and clinical studies that have demonstrated a new function of insulin in metabolism, memory, and mood. We also highlight emerging evidence that delivery of insulin to the central nervous system (CNS) via intranasal administration affects CNS-related metabolic disorders that are linked to impaired insulin action.

2. INSULIN ACTION IN THE BRAIN

2.1. Brain is an insulin-responsive organ

Crucial experimental evidence showing that the brain-specific deletion of the insulin receptor in mice leads to obesity, hyperphagia, and systemic insulin resistance clearly demonstrates the important function of brain insulin signaling in regulating metabolic homeostasis [7]. Emerging data also reveal that brain insulin signaling plays a pivotal role in regulating peripheral metabolism via the modulation of autonomic nervous system outflow to peripheral tissues [8,9]. For example, intracerebroventricular infusion of insulin in the murine brain suppresses hepatic glucose production (HGP) independent of circulating insulin and glucose levels, and these effects were abolished by inactivation of the insulin receptor in the brain [8]. Furthermore, activation of hypothalamic insulin signaling inhibited lipolysis and stimulated de novo lipogenesis by dampening sympathetic nervous system outflow to adipose tissue, whereas mice lacking the neuronal insulin receptor showed unrestrained lipolysis and decreased de novo lipogenesis in adipose tissue, highlighting the functional link of insulin signaling in the axis of the brain and periphery [9]. Thus, these peripheral metabolic responses driven by brain insulin signaling could be a decisive indicator for assessing brain insulin resistant states. Proving this issue in humans, however, is technically beyond the scope of

[1]Division of Endocrinology, Diabetes, and Metabolism, Department of Medicine, Beth Israel Deaconess Medical Center, and Harvard Medical School, 330 Brookline Ave., Boston, MA 02216, USA [2]Division of Endocrinology and Metabolism, Department of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, 222 Banpo-daero, Seocho-gu, Seoul 06591, South Korea [3]Human Performance Laboratory, Department of Kinesiology and Physiology, East Carolina Diabetes and Obesity Institute, East Carolina University, 115 Heart Dr., Greenville, NC 27858, USA [4]Department of Neurology, Neuroscience Research Institute, Gachon University Gil Medical Center, 21 Namdong-daero 774 beon-gil, Namdong-gu, Incheon 21565, South Korea

[5] Seung-Hwan Lee and Janice M. Zabolotny contributed equally to this work.

*Corresponding author. Beth Israel Deaconess Medical Center, 330 Brookline Ave., CLS-745, Boston, MA 02216, USA. Tel.: +1 617 735 3216; fax: +1 617 735 3323.

E-mails: hwanx2@catholic.ac.kr (S.-H. Lee), jzabolot@bidmc.harvard.edu (J.M. Zabolotny), huangh@ecu.edu (H. Huang), hyonlee@gilhospital.com (H. Lee), ykim2@bidmc.harvard.edu (Y.-B. Kim).

Received May 25, 2016 • Revision received June 21, 2016 • Accepted June 22, 2016 • Available online 29 June 2016

http://dx.doi.org/10.1016/j.molmet.2016.06.011

reach. On the other hand, by applying insulin intranasally to the human brain, the impacts of central insulin action on whole body metabolism can be evaluated.

In addition to these metabolic roles of the brain insulin receptor, a recent study further demonstrated that insulin resistance in brain induces dopaminergic dysfunction leading to anxiety and behavioral disorders [10], indicating a new role for insulin signaling in neuronal regulation. Along with this, a study with mice lacking brain insulin receptor substrate 2 (IRS2), one of the major downstream signaling pathways for the insulin receptor, suggests a potential role of IRS2 in the regulation of hippocampal synaptic function and plasticity in mice, which could be mediated via the N-methyl-D-aspartate (NMDA) receptor and the phosphoinositide 3-kinase (PI3K) signaling pathway [11]. It is therefore likely that defective insulin signaling in the brain is one of the key features in the pathogenesis of insulin resistance that is found in obesity, type 2 diabetes, memory impairement, cognitive dysfunction, and mood disorders (Table 1) [3,12].

2.2. Transport of endogenous insulin across the blood brain barrier

To gain access to its receptor in the CNS, insulin produced by pancreatic beta-cells is transported across the blood brain barrier (BBB) [13,14]. The BBB is composed of specialized capillary endothelial cells that are interconnected with tight junctions, which are impermeable to toxins, bacteria, viruses, and most substances in the blood (cells/proteins). The cells composing the BBB are unique in that the cell membranes are exposed to the bloodstream and the CNS, allowing integration of signals from the periphery and the brain [6]. The transport of insulin from the bloodstream across the BBB is

Table 1 — Animal models of CNS insulin receptor deficiency.

CNS function	IR deficient model	Phenotype	Observations	Refs
Metabolism	Mouse IR knockout in nestin expressing neurons	Weight	Increased food intake in female NIRKO (brain-specific insulin receptor deficient) mice. Development of diet-sensitive obesity with increases in body fat and mild insulin resistance in both male and female mice.	[7]
	Rat hypothalamic IR antisense knockdown	Weight	Rapid onset of hyperphagia, increased fat mass, and impaired hepatic insulin action. No significant change in body weight.	[38]
	Rat hypothalamic IR antisense knockdown	Weight	Increased body weight and fat mass. No changes in glucose tolerance and glucose—stimulated insulin release.	[39]
	Mouse IR knockout in tyrosine hydroxylase expressing neurons	Weight	Increased body weight, fat mass, and hyperphagia. Altered response to cocaine under food-restricted conditions.	[138]
	Rat hypothalamic IR antisense knockdown	Glucose homeostasis	Impaired ability of circulating insulin to inhibit glucose production.	[8]
	Mouse IR knockout in nestin expressing neurons	Glucose homeostasis	Defective counterregulatory response to hypoglycemia	[139]
	Mouse IR knockout in POMC expressing neurons	Glucose homeostasis	Unaltered energy and glucose homeostasis.	[41]
	Mouse IR knockout in AgRP expressing neurons	Glucose homeostasis	Unaltered energy homeostasis. Impaired insulin-induced suppression of hepatic glucose production. Reduced insulin-stimulated IL-6 expression in the liver.	[41]
	Knockin IRs in AgRP or POMC neuron on hypothalamic deficiency of insulin receptors (L1 mouse)	Glucose homeostasis	Restoration of insulin action in AgRP neurons and normalized insulin suppression of HGP. Restoration of insulin action in POMC neurons and increased HGP. Increased energy expenditure and locomotor activity by POMC-specific IR knock-in.	[50]
	Mouse IR knockout in nestin expressing neurons	Glucose homeostasis	Glycemia-dependent impairment in the sympathoadrenal response to hypoglycemia due to deletion of IR in the brain.	[140]
	Rat VMH IR antisense knockdown	Glucose homeostasis	Glucose intolerance and islet dysfunction. No effect on weight.	[40]
	Mouse IR knockout in nestin expressing neurons	Lipid homeostasis	Unrepressed lipolysis and reduced de novo lipogenesis in white adipose tissue.	[9]
	Mouse IR knockout in nestin expressing neurons	Hyperthermia	Defective IGF-1 mediated hyperthermic response.	[141]
Memory	Mouse IR knockout in nestin expressing neurons	Neuronal function	No alteration in neuronal proliferation/survival, memory, or basal brain glucose metabolism.	[114]
	Mouse IR kinase +/−	Behavioral function	Impaired recognition of familiarized objects; poor performance on both short-term (1 h) and long-term (24 h) memory tests in IR kinase +/− mice.	[117]
	Mouse IR knockout in nestin expressing neurons in Tg2676 AD mouse model	Neuronal function	Protection from premature death in the presence of decreased Aβ accumulation specifically in the hippocampus formation in nIGF-1R(−/−)Tg2576 mice with no influence on lethality of Tg2576 mice.	[115]
	Mouse IR knockout in nestin expressing neurons in Tg2676 AD mouse model	Neuronal function	Decreased Aβ burden without rescue from premature mortality of Tg2576 mice.	[142]
Mood	Rat hypothalamic IR antisense knockdown	Behavioral function	Increase in immobility time with corresponding decrease in active behaviors and increases in anxiety-like behaviors	[134]
	Mouse IR knockout in nestin expressing neurons	Behavioral function	Development of age-related anxiety and depression-like behavioral changes that were reversed with antidepressant treatment	[10]

AD, Alzheimer's disease; AgRP, agouti-related peptide; CNS, central nervous system; HGP, hepatic glucose production; IGF-1, insulin-like growth factor-1; IR, insulin receptor; POMC, proopiomelanocortin; VMH, ventromedial hypothalamus.

accomplished via a saturable transport system [13]. Insulin crosses the BBB into the hypothalamus, pons-medulla, hippocampus, striatum, parietal cortex and frontal cortex but not into the midbrain, thalamus, and occipital cortex [14]. Numerous animal studies have demonstrated that impairment in the insulin transport system of the BBB is found in obesity-associated insulin resistance as well as various physiological extremes, including starvation, hyperglycemia, activation of the immune system, and hibernation, suggesting an important function of the BBB in maintaining normal metabolic homeostasis [15]. In this regard, a recent study with human subjects further suggests a significant role for the insulin transport system in brain insulin action, as revealed by findings that cerebrospinal fluid (CSF) and circulating insulin levels are closely correlated with whole-body insulin sensitivity in insulin-sensitive humans but not in insulin-resistant humans [16]. Furthermore, another human study indicated that cerebrocortical activity in response to hyperinsulinemia, assessed by magnetoencephalography, was impaired in obese insulin-resistant humans who had an IRS1 Gly972Arg polymorphism [17], a candidate gene for developing type 2 diabetes. This effect could be due to an impaired insulin transport system in the BBB of humans with obesity. However, identification of the molecular mechanisms for this phenomenon remains to be elucidated and will be an important subject of future studies. Nevertheless, it is likely that a defective insulin transport system in the BBB is linked to peripheral insulin resistance may contribute to the pathogenesis of metabolic disorders such as obesity and type 2 diabetes.

Of note, some evidence indicates that insulin is also synthesized in the brain. Detection of C-peptide immunoreactivity in the neurons of human CNS [18,19] and proinsulin or preproinsulin mRNA in animals and cell culture systems [20,21] suggests the possibility of local insulin synthesis in the brain. However, the source of CNS insulin, whether it is peripheral, central or both, is still debated and more evidence is needed to confirm this in humans.

2.3. Intranasal administration delivers insulin into the CNS

Several regions of the brain lack a blood brain barrier, including the olfactory bulb, which has been exploited therapeutically to deliver insulin into the CNS directly from the periphery via the nasal epithelium. In humans, intranasal delivery of insulin allows direct access to the brain without affecting peripheral glucose or insulin levels [22]. Two possible routes have been proposed: 1) an intraneuronal pathway that involves the internalization of the peptide into olfactory neurons, followed by axonal transport into the brain parenchyma and 2) an extraneuronal pathway in which the peptides diffuse into the subarachnoid space by passing through patent intercellular clefts in the olfactory epithelium [23–25]. Although the efficiency of intranasal delivery by insufflations seems to be restricted, it is considered a novel and promising strategy for the treatment of diseases with CNS involvement [26]. The effect of intranasal insulin on hyperglycemia, obesity, memory and cognitive impairment has been widely tested as described in Table 2.

3. CNS INSULIN ACTION AND METABOLISM

> "*We have treated fifteen patients in the hospital over periods of time varying from two weeks to two months and have been able to keep them relatively sugar free by the intranasal method. Whether this method of administration is practical in the treatment of diabetic patients further observations alone can determine. The treatment may prove too expensive to be practical and we may also discover great variations in absorption in different patients. The fact that insulin under certain conditions can be absorbed from mucous membranes is, however, of more than academic interest.*" *(Major RH, 1935)* [27]

3.1. Brain insulin resistance accompanies obesity and diabetes

Several lines of evidence suggest the existence of abnormal brain insulin action in diabetes and obesity. Abnormalities in CNS insulin action reflect defects in insulin transport across the BBB or impaired insulin signaling in insulin receptor-expressing cells of the CNS, or a combination of the two. CNS insulin levels are significantly reduced in high-fat diet-induced obese dogs [28] and genetically obese rats [29]. A down regulation of brain capillary insulin receptors could be a potential mechanism for these effects [30]. Consistent with this, insulin-resistant human subjects displayed a lower CSF/serum insulin ratio compared to insulin-sensitive subjects, suggesting insulin-resistance impairs insulin transport across the BBB in humans [16]. In the periphery, an impairment of the proximal components of the insulin signaling pathways, IR/PI3K/Akt, is thought to be a molecular mechanism responsible for insulin resistance in animals and humans [4,31]. Similar observations were found in the hypothalamus of genetically obese and diet-induced obese insulin-resistant rats [32,33], suggesting an essential role for insulin signaling in the CNS in metabolic disorders. Of note, given that insulin resistance is a pathological condition in which cells fail to respond to the metabolic actions of insulin, brain insulin-responsive physiological outcomes, including feeding behavior, hepatic glucose production, fat mass mobilization, hypothermia, responsiveness to hypoglycemia, and neuronal function, can be used as indicators of alterations in the brain insulin-resistant state.

3.2. Hypothalamic insulin action in glucose and energy homeostasis

Pioneering experiments evaluating the central effect of insulin in regulating glucose metabolism and energy homeostasis were conducted by Porte and colleagues more than 40 years ago. Intracerebroventricular injection of insulin increased pancreatic insulin secretion in dogs [34] and decreased food intake and body weight in baboons [35]. Likewise, intranasal insulin also decreased circulating glucose concentrations in dogs [36] and rhesus monkeys [37]. The knockout of the insulin receptor in the murine brain following the introduction of cre-loxP recombinant engineering, has demonstrated the role of central insulin resistance in energy homeostasis, fuel metabolism, and reproduction. Brain-specific insulin receptor inactivation did not affect brain development or neuronal survival but caused diet-sensitive obesity with increases in body fat [7].

The hypothalamus is an important mediator of energy balance, food intake, and glucose homeostasis within the brain. Rodents with selective hypothalamic insulin resistance, achieved by an intrahypothalamic injection of antisense oligodeoxynucleotides specific for the insulin receptor, failed to suppress hepatic glucose production and food intake, suggesting that the hypothalamic insulin resistance plays a critical role in insulin's effects on energy and glucose metabolism [8,38]. Selective knockdown of the insulin receptor in the rat hypothalamus also increases body weight and adiposity [39]. More recently, studies showed that chronic reduction of the insulin receptor in the ventromedial hypothalamus (VMH) of rats led to glucose intolerance due to islet dysfunction, though body adiposity was not affected, suggesting that VMH insulin signaling may regulate glucose but not energy homeostasis [40]. Metabolic observations from animal models lacking or inhibiting the insulin receptor in the brain are summarized in

Table 2 — Intranasal insulin treatment outcomes.

CNS function	Clinical subjects	Intranasal insulin	Phenotype	Treatment outcomes	Refs
Metabolism	Diabetics — fed Diabetics — fasted	1 or 2 doses (100 IU) Single dose (25 IU)	Glycemia	Lowered blood glucose in all patients. Rare occurrence of hypoglycemic shock via intranasal method.	[27]
	Normal subjects	Single dose (20—60 IU)	Glycemia	Induced hypoglycemia.	[59]
	Insulin-dependent diabetics	Single dose		Improved postprandial hyperglycemia.	
	Healthy subjects Type 1 and 2 diabetics	Single dose (0.5 IU/kg)	Glycemia	Reduced blood glucose concentrations with insulin-deoxycholate aerosol.	[60]
	Normal subjects Type 1 diabetic men and women	Single dose (1 IU/kg) 3 months	Glycemia	Lowered fasting and postprandial glucose levels. Improved long-term glycemic control.	[61]
	Non-obese type 2 diabetic men and women	Single dose (30 IU)	Glycemia	Hypoglycemic effect persisted for less than 2 h in the fasting state. Reduction in postprandial hyperglycemia persisted for 4 h.	[63]
	Type 1 diabetic men and women	3 doses (up to 120 IU each) t.i.d. at meals	Glycemia	Controlled glycemia in half of patients with intranasal insulin when combined with daily ultralente insulin.	[62]
	Fasted normal men	Single dose (1 IU/kg)	Glycemia	Blood glucose concentration decreased at 45 min.	[64]
	Type 2 diabetics	Single dose (60 or 120 IU)	Glycemia	No hypoglycemia; effective at reducing post-prandial hyperglycemia.	[65]
	Type 2 diabetics with oral drug failure	4 months (up to 240 IU/day, t.i.d.)	Glycemia	Similar diabetes control to conventional treatment with twice daily NPH insulin.	[66]
	Normal weight men or women	single dose (160 IU)	Glycemia	Increased plasma insulin and decreased plasma glucose. Increased fMRI activity in the hypothalamus correlated with increased HOMA-IR at 30 min post treatment. Decreased fMRI activity in the putamen, right insula and orbitofrontal cortex correlated with decreased HOMA-IR at 120 min post treatment.	[57]
	Type 2 diabetics	single dose (15 IU)	Glycemia	No improvement in post-prandial hyperglycemia.	[67]
	Insulin-dependent diabetics	1 month (at mealtimes, various doses)	Glycemia	Metabolic control deteriorated compared to subcutaneous insulin therapy.	[68]
	Healthy subjects	1.5 h (20 IU, every 15 min)	Glycemia	No effect on glycemia and insulin levels.	[69]
	Normal weight men	single dose (160 IU)	Glycemia	No effect on postprandial glucose concentrations.	[70]
	Normal weight men or women	single dose (160 IU)	Weight	Decreased food intake in men, not in women.	[72]
	Normal weight pre and post menopausal women	single dose (160 IU)	Weight	No effect on food intake.	[73]
	Obese men	8 weeks (40 IU, q.i.d.)	Weight	No effect on weight or body fat.	[80]
	Normal weight women	single dose (160 IU)	Weight	Decreased appetite, food intake and rated palatability after postprandial insulin.	[75]
	Normal weight men	single dose (40 IU)	Weight	Increased brain ATP and phosphocreatine. Change in cerebral energy content correlated inversely with subsequent calorie intake.	[76]
	Normal weight men or women	single dose (160 IU)	Weight	Reduced food image-cued activity in the fusiform gyrus, hippocampus, and temporal superior and frontal middle cortex. No effect on nonfood image-cued brain activity.	[77]
	Normal weight women	single dose (160 IU)	Weight	Modulation of hypothalamus and orbitofrontal cortex activity by insulin. Correlation of prefrontal cortex and anterior cingulate cortex insulin response with BMI.	[78]
	Normal weight men or women	8 weeks (40 IU, q.i.d.)	Weight	Reduced weight and body fat in men. No effect in women.	[74]
	Normal weight men or women	single dose (160 IU)	Lipolysis	Suppressed systemic FFA levels without affecting lipolytic proteins in subcutaneous adipose tissue.	[143]
	Normal weight men	single dose (160 IU)	Thermogenesis	Increased postprandial diet-induced thermogenesis.	[70]
	Lean and obese men	two days (160 IU per day)	Insulin sensitivity	Improved peripheral insulin sensitivity in lean but not obese men.	[71]
	Lean and type 2 diabetic men or women	1 week (160 IU per day)	Hepatic fat and energy metabolism	Rapid improvement of hepatic energy metabolism without affecting hepatic insulin sensitivity in healthy humans, independent of peripheral insulinemia. Blunted response in patients with type 2 diabetes.	[144]
	Lean and obese or overweight men	single dose (160 IU)	Cerebral blood flow	Selectively impaired brain insulin action in the prefrontal cortex in overweight and obese adults and in the hypothalamus in participants with high visceral adipose tissue.	[79]

Table 2 — (*continued*)

Memory	Amnestic MCI or early AD or healthy	single dose (20 IU or 40 IU)	Verbal memory	Facilitated recall on two measures of verbal memory in memory-impaired APOE-ε4- adults. These effects were stronger for memory-impaired APOE-ε4- subjects than for memory-impaired APOE-ε4+ subjects and normal adults.	[15]
	Amnestic MCI or early AD	3 weeks (20 IU)	Verbal memory	Enhanced verbal memory, selective attention, and functional status. Raised fasting plasma A beta 40/42 ratio.	[123]
	Amnestic MCI or AD or healthy subjects	5 days (10, 20, 40, or 60 IU)	Verbal memory	Facilitated recall on two measures of verbal memory in memory-impaired APOE-ε4- adults. Differentially modulated plasma amyloid-β for memory-impaired subjects and normal controls, with effects that differed by APOE genotype.	[121]
	Healthy men	8 weeks (40 IU, q.i.d.)	Declarative memory	Improved declarative memory.	[122]
	Healthy men	8 weeks (40 IU, q.i.d.)	Declarative memory	Improved declarative memory and attention.	[119]
	Obese men	8 weeks (40 IU, q.i.d.)	Declarative memory	Improved declarative memory.	[80]
	Amnestic MCI or mild to moderate AD	4 months (20 or 40 IU)	Dementia	Improved delayed memory with 20 IU intranasal insulin.	[124]
			Testing	Preserved cognition and functional abilities with 20 and 40 IU insulin. Correlation between effects on memory and function with CSF Aβ42 and tau/Aβ42.	
	APOE-ε4 carriers with mild-moderate AD	Single dose (40 IU)	Memory	No impact on cognition; serum insulin levels dropped post treatment, but peripheral glucose levels were unchanged.	[145]
	Healthy men	Single dose (40 IU)	Memory	Improved odor-cued reactivation of spatial memory.	[146]
	Normal weight men or women	Single dose (160 IU)	Working memory	Improved hippocampus-dependent memory and working memory in women, not men.	[72]
	Normal weight pre and post menopausal women	Single dose (160 IU)	Working memory	Enhanced prefrontal cortex-dependent working memory.	[73]
	MCI or mild to moderate AD	3 weeks (20 or 40 IU)	Working memory	Improved memory composite (in APOE-ε4 carriers), verbal working memory and visuospatial working memory with 40 IU of insulin detemir.	[147]
	Non-diabetic and diabetic men and women	Single dose (40 IU)	Visio-spatial memory	Improved visiospatial memory acutely, correlated with regional vasoreactivity and cerebral vasodilatation.	[120]
	Older adults with type 2 diabetes	Single dose (40 IU)	Cognitive performance	Increased resting-state functional connectivity between the hippocampal regions and default mode network.	[148]
Mood	Obese men	8 weeks (40 IU, q.i.d.)	HPA axis	Improved mood and reduces HPA axis activity.	[80]
	Healthy men	Single dose (40 IU)	HPA axis	Reduced saliva and plasma cortisol after experimental social stress.	[136]
	Healthy men	8 weeks (40 IU, q.i.d.)	Mood	Enhanced mood and self-confidence, reduces anger.	[119]
	Bipolar disorder, euthymic	8 weeks (40 IU, q.i.d.)	Executive function	Improved executive function, as measured by the Trail Making Test-Part B. No effect on other neurocognitive tests.	[137]

AD, Alzheimer's disease; CNS, central nervous system; CSF, cerebrospinal fluid; FFA, free fatty acid; fMRI, functional magnetic resonance imaging; HOMA-IR, homeostasis model assessment-insulin resistance, HPA, hypothalamic-pituitary-adrenal; MCI, mild cognitive impairment; t.i.d., ter in die (three times a day); q.i.d., quarter in die (four times a day).

Table 1. These studies led scientists to map the neuronal circuitry and molecular mechanisms involved in hypothalamic insulin action on energy homeostasis and glucose tolerance.

3.3. Neuronal circuitry mediating hypothalamic insulin action on glucose and energy homeostasis

Several recent studies have focused on elucidating the neuronal circuitry that underlies insulin action on body weight and glucose homeostasis in the hypothalamus. The arcuate nucleus of the mediobasal hypothalamus, adjacent to the third ventricle and the median eminence, contains important populations of neurons that respond to afferent signals from hormones and nutrients. Insulin receptors are highly expressed in neurons expressing proopiomelanocortin (POMC) or agouti-related peptide (AgRP) and neuropeptide Y (NPY) in the arcuate nucleus [41—43]. These first order neurons project to second order neurons in other hypothalamic areas (e.g. the paraventricular hypothalamus, lateral hypothalamus, and ventromedial hypothalamus) or extrahypothalamic areas (e.g. the nucleus tractus solitarius) to ultimately alter feeding behavior or energy metabolism. Insulin decreases expression of orexigenic AgRP and NPY, leading to decreased food intake [44]. Additionally, insulin increases expression of POMC, resulting in increased levels of α-melanocyte stimulating hormone (α-MSH), which promotes anorexia and increases energy expenditure [45], presumably via melanocortin-4-receptors (MC4R) expressing neurons [46]. In AgRP neurons, once Akt translocates into the nucleus in response to insulin, it inhibits forkhead box protein O1 (FOXO1) transcriptional activity by phosphorylating FOXO1, and leads to the exclusion of FOXO1 from the

nucleus resulting in the reduction of AgRP expression [47]. In unstimulated POMC neurons, FOXO1 enhances recruitment of histone acetylases and co-suppressors on the promoter region of POMC genes to suppress its expression. Upon insulin stimulation, phosphorylated FOXO1 translocates from the nucleus resulting in de-inhibition of the POMC promoter, thereby increasing POMC expression [47–49]. Interestingly, regulation of HGP is affected by hypothalamic insulin signaling [8]. A branched-pathway model suggests that insulin receptor knock-in in POMC neurons promotes HGP and activates melanocortinergic energy expenditure whereas insulin receptor knock-in in AgRP neurons decreases HGP [50].

However, in contrast to brain-wide insulin receptor knockout [7] or disruption of POMC- or AgRP-expressing neurons [51,52], selective inactivation of the insulin receptor in either POMC- or AgRP-expressing neurons failed to exhibit altered food intake or body weight, raising the question of whether insulin action in these neurons is necessary for energy homeostasis [41]. Because other neuronal populations are known to regulate energy balance in response to leptin (e.g. steroidogenic factor-1 (SF-1) neurons of the VMH [53,54]), insulin action in these neuron populations may also contribute to its role in the central nervous system. A recent study indicated that insulin-dependent PI3K activation in SF-1 expressing VMH neurons contributed to diet-induced obesity by reducing firing frequency [55]. Another potential explanation is that both insulin and leptin signals are required for normal glucose homeostasis [43] because they stimulate different populations of POMC-expressing neurons [56].

3.4. Intranasal insulin regulates human glucose homeostasis

The introduction of the intranasal application of insulin provides us with a tool to evaluate insulin's role in the human CNS on whole-body metabolism. From the perspective of insulin's glucose-lowering action, one of the most intriguing questions is whether the administration of insulin via the intranasal route alters circulating glucose levels in humans directly or indirectly. Experimental data in humans demonstrated that intranasal insulin administration led to increased insulin levels in the CSF but not in the bloodstream and did not induce change in blood glucose levels [22]. These observations suggested that insulin directly entered the CSF, bypassing the bloodstream without affecting circulating insulin and glucose levels. However, some studies revealed that administration of intranasal insulin increased circulating insulin levels and decreased glucose levels, although it remained with the range of euglycemia [57,58]. The difference between these studies could be due to either the intranasal insulin dose used or the duration of intranasal insulin therapy.

Several studies examined the effect of intranasal insulin treatment on glucose homeostasis in normal and type 1 or type 2 diabetic patients (Table 2). Reduction in glucose levels was observed by a single or multiple administrations with various doses [27,57,59–66], while some studies failed to demonstrate this effect [67–70]. Given that one of insulin's actions in the hypothalamus is to regulate HGP [38], it is conceivable that brain insulin in humans plays a role in regulating systemic blood glucose levels through the brain-liver axis. It has also been suggested that CNS insulin exerts a positive feedback on the pancreas to increase insulin secretion [34]. Of note, a recent study demonstrated that intranasal-induced insulin delivery in the brain improves peripheral insulin sensitivity by increasing hypothalamic activity and parasympathetic output in lean men [71]. Further studies will be needed to elucidate the mechanism of intranasal insulin's action and to find an efficient way to deliver insulin leading to favorable outcomes. In addition, because previous studies used different amounts of or delivery schemes for insulin application which might have affected the conflicting results, investigating the optimal dose, frequency and duration of intranasal insulin treatment will be crucial.

3.5. Intranasal insulin regulates brain activity to modulate human energy homeostasis

Extensive studies have been conducted to identify the impact of intranasal insulin administration on energy homeostasis. In normal weight adults, intranasal insulin (160 IU) acutely decreased food intake in men, however body weight was not altered under these conditions in pre or post menopausal women [72,73]. Similar results were observed in subjects receiving long-term intranasal insulin (40 IU, four times a day [q.i.d.], for 8 weeks) [74]. Nevertheless, postprandial administration of intranasal insulin (160 IU) was found to attenuate appetite, food intake and food palatability in women [75]. In healthy men, intranasal insulin (160 IU) acutely increased postprandial energy expenditure and decreased circulating insulin [75]. Functional magnetic resonance imaging (fMRI) studies have begun to reveal the insulin-mediated changes in activity in the human brain related to food intake. In normal weight men, intranasal insulin (40 IU) increased brain ATP and phosphocreatine, and changes in brain energy content were inversely proportional to subsequent caloric intake, suggesting that intranasal insulin may play a role in meal termination [76]. In normal weight adults, intranasal insulin acutely reduced food image-cued activity in the left and right fusiform gyrus, right hippocampus, and right temporal superior and frontal middle cortices, areas involved in object processing and memory. Of note, insulin did not affect nonfood image-cued brain activity [77]. Lastly, in normal weight women, intranasal insulin (160 IU) acutely modulated hypothalamic and orbitofrontal cortex activity, and the prefrontal cortex and anterior cingulate cortex's insulin response correlated with body mass index [78].

Few studies have examined the effects of intranasal insulin on body weight in patients with obesity, diabetes, or metabolic syndrome. One day 160 IU of intranasal insulin reduced cerebral blood flow in the prefrontal cortex in lean participants, but not in overweight/obese participants, suggesting impaired brain insulin action in the latter group. Furthermore, behavioral changes for craving sweet foods were not observed in the overweight/obese group [79]. In obese men, long-term intranasal insulin (40 IU q.i.d., for 8 weeks) did not reduce body weight or body fat, unlike in lean men, suggesting that in obesity, pathways or brain areas that mediate the effects of insulin on body weight are resistant to the effects of insulin [80]. Taken together, these studies support an important role for insulin in the CNS in regulating energy balance. Further studies are needed to determine whether the effect of intranasal insulin to reduce body adiposity in obesity is limited.

4. CNS INSULIN ACTION, MEMORY AND COGNITIVE FUNCTION

Interest in the effects of insulin on cognition date to the 1920–30's with the advent of *Insulinshockbehandlung* in Europe [81]. Originally tested as a methodology to change the mental status of patients with delirium tremens or morphine addiction, insulin shock therapy or insulin coma therapy was advanced by Manfred Sakel as a treatment for psychosis in dementia praecox (or premature dementia, a diagnosis that today would most closely refer to schizophrenia), and later occasionally in depressive disorders [81–83]. While insulin shock therapy was largely abandoned in the 1950's consequent to the emergence of antipsychotic medications and a lack of evidence that insulin was the therapeutic component of coma therapy [81,84], it

ushered in an era of studies of the effects of insulin on learning and memory, originally stemming from the mistaken belief that insulin shock therapy promoted the unlearning of newly acquired psychotic behaviors [85]. Despite its inauspicious beginning, interest in the role of the hormone insulin in the CNS on cognitive and affective disorders has persisted, with renewed interest in the initial years of this century.

4.1. Is dementia a metabolic disorder?

Besides regulating neural circuits involved in maintaining energy homeostasis, insulin also influences cognitive functions through its actions on synaptic plasticity and long-term potentiation in the hippocampus and other brain regions involved in learning and memory [86,87]. Recent studies also have indicated a strong association between Alzheimer's disease and CNS insulin resistance [88,89]. Alzheimer's disease is a neurodegenerative disease causing progressive deterioration of memory and cognitive function and is the most common form of dementia, accounting for more than 50% of cases [90]. Although aging is the most prominent risk factor, now there is ample evidence that people with glucose intolerance, insulin resistance and metabolic syndrome are at higher risk for cognitive impairment and dementia compared to age- and gender-matched controls [91–94]. A meta-analysis and a large-scaled pooled analysis demonstrate that diabetes is associated with an approximately 60–70% increased risk of all types of dementia [95,96]. Therefore, Alzheimer's disease is sometimes referred as type 3 diabetes, a brain specific impairment of insulin signaling [89]. Multifactorial pathogeneses that can be linked to brain insulin signaling defects, such as oxidative stress due to hyperglycemic toxicity, chronic inflammatory processes, mitochondrial dysfunction, abnormal cholesterol metabolism, adverse vascular changes and severe hypoglycemia are thought to trigger the development of dementia in people with metabolic disturbances (reviewed in [97,98]).

4.2. Brain insulin resistance is implicated in memory impairment and cognitive dysfunction

Insulin resistance was associated with progressive atrophy in cortical regions affected by Alzheimer's disease, and this corresponded to worse cognitive performance in asymptomatic, late middle-aged adults [99]. Dysregulation of brain insulin signaling has been proposed as a causal mechanism. A recent study highlighted that serine phosphorylation of IRS1 is a common pathophysiologic mechanism of Alzheimer's disease and diabetes. The levels of IRS1 serine phosphorylation and their activated kinases showed a positive correlation with levels of oligomeric β-amyloid (Aβ) plaques and an inverse association with memory and cognition [100]. A study using autopsied frontal cortices showed that the expression levels of several components of the insulin-PI3K-Akt signaling pathway were decreased in subjects with type 2 diabetes and/or Alzheimer's disease [101]. Similar findings were also observed in a rat model of sporadic Alzheimer's disease [102]. This was associated with an over-activation of glycogen synthase kinase-3β (GSK-3β), which in turn hyperphosphorylates microtubule-associated protein tau, a major component of neurofibrillary tangles that disrupt neuronal function [101–103]. GSK-3β is regarded as the key signaling molecule regulating tau phosphorylation, and its activity is negatively regulated by its phosphorylation with Akt at Ser9. In addition to hyperphosphorylation, tau can also undergo *O*-GlcNAcylation, a post-translational protein modification by *O*-linked N-acetyl-D-glucosamine (*O*-GlcNAc). Because this modification is regulated by glucose availability via the hexosamine biosynthesis pathway, downregulation of *O*-GlcNAcylation by impaired brain glucose metabolism facilitates tau hyperphosphorylation [104]. Conversely, an *O*-GlcNAcase inhibitor increased tau *O*-GlcNAc which correlates with decreased tau aggregates and neuronal cell death [105]. Therefore, brain insulin resistance is now considered as a causal factor of neurofibrillary degeneration.

Of note, Alzheimer's disease itself can induce or augment insulin resistance, thus participating in type 2 diabetes pathogenesis or other metabolic dysfunctions. Aβ oligomers stimulate tumor necrosis factor (TNF)-α signaling, which activates the c-Jun N-terminal kinase (JNK) pathway resulting in serine phosphorylation of IRS1 and defective insulin signaling [106]. Aβ oligomers also induced substantial loss of neuronal surface insulin receptors and inhibited neuronal response to insulin, which was associated with Akt phosphorylation at Ser473, in hippocampal neurons [88]. These findings suggest that the link between insulin resistance and Alzheimer's disease could be bidirectional.

4.3. Diet and diabetes alter CNS insulin action and cognitive function in animals

Diet may play an important part in the development of insulin resistance in the brain. In hamsters, a diet high in fructose induces peripheral as well as neural insulin resistance, as evidenced by decreased insulin-mediated IR, IRS1, and Akt phosphorylation and elevated protein-tyrosine phosphatase 1B (PTP1B) expression in the cerebral cortex and hippocampus [107]. Insulin-induced long-term depression, a functional measure of synaptic plasticity, was also attenuated in the brains of these animals, suggesting that brain insulin resistance may contribute to cognitive impairment [107]. High fat feeding of rats also impaired neuronal insulin signaling and long-term depression in the CA1 region of the hippocampus [108]. Rosiglitazone, an insulin sensitizer, reversed high-fat diet-induced neuronal insulin resistance and associated impairments in insulin-induced long-term depression and increased neuronal Akt serine phosphorylation in response to insulin [109]. Treatment of high-fat fed mice with vildagliptin, a dipeptidyl peptidase-4 inhibitor (anti-diabetic drug), also restored insulin-induced neuronal IR, IRS1, and Akt activation and long-term depression, improved brain mitochondrial dysfunction, and enhanced cognitive function measured by the Morris water maze test [110]. Furthermore, insulin treatment rescued impaired hippocampal neuron proliferation in mice with experimental diabetes [111]. Taken together, insulin's action in the CNS is crucial in maintaining cognitive function in animals.

4.4. Insulin's role in learning, memory, and cognition: animal studies

Diet-induced impaired neural insulin signaling in animal models of Alzheimer's disease-like neuropathology promoted amyloidogenic $A\beta_{1-40}$ and $A\beta_{1-42}$ peptide generation, increased Alzheimer's disease-type amyloid plaque burden in the brain and impaired performance in a spatial water maze task [112]. Aβ is implicated in initiating a deterioration of synapse function, composition, structure, and plasticity in Alzheimer's disease. In hippocampal neuron cultures, Aβ causes loss of IR on dendrites and interferes with insulin receptor signaling and long term potentiation [88,113]. In brain/neuron-specific insulin receptor knockout (NIRKO) mice, neuronal proliferation, neuronal survival, basal brain glucose metabolism or memory was not altered [114]. Neuronal-specific insulin receptor deletion also did not affect lethality of mice expressing the Swedish mutation of the amyloid precursor protein (APP^{SW}), a model for Alzheimer's disease [115]. However, in NIRKO mice, Akt and GSK3β phosphorylation was markedly reduced, tau phosphorylation substantially increased, and

insulin-stimulated PI3-kinase activation and neuronal apoptosis were blocked or inhibited, respectively, raising the possibility that insulin receptor deficiency in the brain may influence the risk of developing Alzheimer's disease independent of neuronal apoptosis or survival [114]. Moreover, intranasal insulin administration in mice improved short- and long-term object memory recognition [116]. Of note, the effects of intranasal insulin on memory were blunted by diet-induced obesity [116]. Similarly, mice heterozygous for the insulin receptor gene demonstrated poor performance on both short-term (1 h) and long-term (24 h) memory tests in comparison to that of wild-type mice [117]. Impaired brain insulin signaling and tau hyperphosphorylation in a rat model of type 2 diabetes were normalized by intranasal insulin [118]. Experimental research suggesting that insulin plays an important role in memory are further supported by clinical studies examining the impact of intranasal insulin on memory in healthy subjects as well as patients with diabetes, amnesic mild cognitive impairment, or Alzheimer's disease.

4.5. Intranasal insulin regulates brain activities involved in learning and memory in human

Besides its effect on peripheral energy metabolism, intranasal insulin has been proposed as a novel therapy to improve memory and cognition in humans. Several studies involving both healthy and cognitively impaired subjects have evaluated the effect of intranasal insulin on cognitive function (Table 2). Although variable results were observed according to the dose of insulin (160 IU vs. lower dose), duration of treatment (acute vs. long-term), timing of assessment (immediate recall vs. delayed recall), measures of cognitive function (word list recall, digit span or object recognition) and gender, potential beneficial effects were suggested. Numerous studies demonstrated improvement in memory or cognitive functions after the administration of intranasal insulin, with the effect being more prominent in women than in men [15,72,73,80,119–122]. These data suggest that intranasal insulin has the potential to enhance working memory performance even in healthy non-diabetic subjects.

In subjects with amnestic mild cognitive impairment or probable Alzheimer's disease, acute administration of 20 IU insulin improved immediate and delayed verbal memory [123]. Another clinical trial evaluated the effect of a 4-month treatment of 20 IU or 40 IU intranasal insulin in adults with amnestic mild cognitive impairment or Alzheimer's disease. Memory and recall tasks were markedly improved with 20 IU of insulin, and general cognition and functional abilities were preserved with both doses of insulin [124]. Studies by Reger et al. showed that the effect on verbal memory was greater in participants without the APOE-epsilon4 allele, a risk factor for Alzheimer's disease, suggesting a differential dose-response reaction of intranasal insulin in groups with different genetic backgrounds [15,121]. These results, along with an excellent safety profile and compliance, provide the basis for future clinical trials to establish a new role of insulin for the treatment of dementia.

5. CNS INSULIN ACTION AND MOOD

5.1. Depression is a common co-morbidity of diabetes and involves abnormalities in insulin action

> *"In our work with diabetic children, we have always been impressed with the marked symptoms of depression shown by these patients. We have observed that the most noticeable reaction from the use of insulin was the clearing of the depression. This observation led us to make some investigations into the possible effect of insulin in states of true mental depression." (Cowie DM,* et al.*, 1924)* [125]

Recognition of an association between diabetes and depression predates the modern diagnostic conceptualization for either disorder. Indeed, the British physician Thomas Willis, who was one of the first to recognize glycosuria as a symptom of diabetes, postulated that diabetes was caused by 'sadness or long sorrow and other depressions and disorders' [126]. Major depressive disorder (MDD) is a common co-morbidity of diabetes [126,127], although the biological basis of MDD is not clearly understood. Evidence suggests that the relationship between MDD and diabetes is bidirectional [127,128]. Insulin resistance, hypothalamic-pituitary-adrenal (HPA) axis dysregulation, and inflammation have been implicated as mechanistic links between the two disorders [128]. As in diabetes [129] and dementia [130], cerebral glucose metabolism is markedly altered in MDD [131]. Moreover, systemic insulin resistance has been described as a pathogenic feature of MDD [128]. Indeed, in a recent plasma proteomics study aimed at identifying peripheral markers of MDD, the analyte with the greatest differential expression and statistical significance between healthy and MDD subjects was insulin [132]. Moreover, emerging experimental and clinical data suggest that insulin action in brain may play a direct role in controlling mood regulation and cognition in MDD.

5.2. Brain insulin signaling is involved in depression-like states in animals

Recent studies in animals lend support to the concept that insulin action in the CNS has important effects on mood. High fat feeding of rats elevates neuronal corticosterone and impairs neuronal insulin signaling and long-term depression in the CA1 region of the hippocampus, indicating pathophysiological links between metabolic overload, neuronal stress and neuronal insulin resistance [108]. In rats exposed to chronic unpredictable mild stress (CUMS) to induce a depression-like state, insulin-stimulated IRS2 tyrosine phosphorylation and PI3K activation are suppressed, and suppressor of cytokine signaling 3 (SOCS3) is overexpressed in the arcuate nucleus of the hypothalamus. These effects were reversible by treatment with the antidepressant fluoxetine [133]. Moreover, in rats, lentivirus-mediated hypothalamic insulin receptor-knockdown promoted anhedonia and behavioral despair, as measured by the sucrose preference test and forced swim test, respectively [134]. Furthermore, pretreatment of mice with the insulin sensitizer dicholine succinate prior to social defeat stress diminished anhedonia and behavioral despair. Dicholine succinate pretreatment also reduced the anxiety scores of stressed mice in the dark/light box paradigm and blocked stress-induced impairments of long-term contextual memory in the step-down avoidance test, while preserving hippocampal gene expression of insulin-like growth factor 2 [135]. NIRKO mice also develop age-related anxiety and depression-like behaviors accompanied by central mitochondrial dysfunction, increased oxidative stress, and increased lipid and protein oxidation in the striatum and nucleus accumbens [10]. Insulin receptor deficiency increases brain monoamine oxidase (MAO) A and B in these brain regions, leading to an increase in dopamine turnover. The depression-like behaviors NIRKO mice exhibit are reversible via treatment with MAO inhibitors and other antidepressants [10]. Collectively, these data support the idea that impairments in CNS insulin receptor signaling may contribute to depression-like behaviors in animals. Further clinical studies are

needed to determine whether impaired CNS insulin action contributes to mood dysregulation in MDD.

5.3. Intranasal insulin treatment improves mood and attenuates HPA axis hyperactivity in clinical studies

In healthy subjects, administration of intranasal insulin (40 IU) prior to a psychosocial stressor was found to diminish saliva and plasma cortisol without affecting heart rate or blood pressure stress reactivity, suggesting that intranasal insulin blunts the responsiveness of the stress-induced HPA axis [136]. Longer-term treatment with intranasal insulin (eight weeks, 40 IU, q.i.d.) not only improved declarative memory and attention, but also enhanced mood and self-confidence, and reduced anger in healthy subjects [119]. Importantly, although obese subjects were resistant to the effects of longer-term intranasal insulin (eight weeks, 40 IU, q.i.d.) to reduce weight or adiposity, they remained sensitive to insulin's effects to improve declarative memory and mood [80]. Of note, intranasal insulin therapy also acutely and chronically lowered plasma adrenocorticotropic hormone (ACTH) and serum cortisol in obese subjects, suggesting that HPA axis activity was reduced [80]. The potential impact of intranasal insulin on mood in patients with depression has not yet been investigated. However, in an initial study of euthymic bipolar disorder patients, adjunctive intranasal insulin treatment (eight weeks, 40 IU q.i.d.) improved one measure of executive function [137]. Collectively, these studies suggest that insulin action in the brain may be an important component of the neuroendocrinology of mood regulation, in part via blunting hyperactivity of the HPA axis. Further clinical studies are needed to determine whether intranasal insulin or insulin sensitizers may have the potential to normalize mood and HPA axis activity in affective disorders and treat MDD.

6. CONCLUSIONS

The field of metabolic physiology has been greatly advanced by the elucidation of insulin's function in the brain regulating systemic energy metabolism, memory, and mood. Over the past decade, extensive research has shed light on a new role of insulin in CNS-related metabolic disorders, including dementia, memory impairment, and cognitive dysfunction. In addition, accumulating evidence demonstrates that intranasal insulin administration represents a potential treatment option for these conditions. However, knowledge on this area needs to be further advanced in order to understand the precise role of insulin in the brain, and its effect on metabolic and neurodegenerative diseases. Because assessing brain insulin resistance is still technically limited, development of better measures or technology to identify the action of brain insulin would be important. Furthermore, large-scale clinical trials for pharmacological treatments, including intranasal insulin, that promote brain insulin sensitivity may provide novel therapeutic possibilities for the treatment of cognitive and mood disorders in the future.

FUNDING

This work was supported by grants from the National Institutes of Health (R01DK083567 to Y.B.K.) and the Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (HI14C3417 to S.H.L).

CONFLICT OF INTEREST

The authors have no conflicts to declare.

REFERENCES

[1] Samuel, V.T., Shulman, G.I., 2012. Mechanisms for insulin resistance: common threads and missing links. Cell 148:852–871.
[2] Schwartz, M.W., Porte Jr., D., 2005. Diabetes, obesity, and the brain. Science 307:375–379.
[3] Plum, L., Schubert, M., Bruning, J.C., 2005. The role of insulin receptor signaling in the brain. Trends in Endocrinology and Metabolism 16:59–65.
[4] Choi, K., Kim, Y.B., 2010. Molecular mechanism of insulin resistance in obesity and type 2 diabetes. Korean Journal of Internal Medicine 25:119–129.
[5] Havrankova, J., Roth, J., Brownstein, M., 1978. Insulin receptors are widely distributed in the central nervous system of the rat. Nature 272:827–829.
[6] Banks, W.A., Owen, J.B., Erickson, M.A., 2012. Insulin in the brain: there and back again. Pharmacology & Therapeutics 136:82–93.
[7] Bruning, J.C., Gautam, D., Burks, D.J., Gillette, J., Schubert, M., Orban, P.C., et al., 2000. Role of brain insulin receptor in control of body weight and reproduction. Science 289:2122–2125.
[8] Obici, S., Zhang, B.B., Karkanias, G., Rossetti, L., 2002. Hypothalamic insulin signaling is required for inhibition of glucose production. Nature Medicine 8:1376–1382.
[9] Scherer, T., O'Hare, J., Diggs-Andrews, K., Schweiger, M., Cheng, B., Lindtner, C., et al., 2011. Brain insulin controls adipose tissue lipolysis and lipogenesis. Cell Metabolism 13:183–194.
[10] Kleinridders, A., Cai, W., Cappellucci, L., Ghazarian, A., Collins, W.R., Vienberg, S.G., et al., 2015. Insulin resistance in brain alters dopamine turnover and causes behavioral disorders. Proceedings of the National Academy of Sciences of the United States of America 112:3463–3468.
[11] Costello, D.A., Claret, M., Al-Qassab, H., Plattner, F., Irvine, E.E., Choudhury, A.I., et al., 2012. Brain deletion of insulin receptor substrate 2 disrupts hippocampal synaptic plasticity and metaplasticity. PLoS One 7:e31124.
[12] Defronzo, R.A., 2009. Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. Diabetes 58:773–795.
[13] Banks, W.A., Jaspan, J.B., Kastin, A.J., 1997. Selective, physiological transport of insulin across the blood-brain barrier: novel demonstration by species-specific radioimmunoassays. Peptides 18:1257–1262.
[14] Banks, W.A., Kastin, A.J., 1998. Differential permeability of the blood-brain barrier to two pancreatic peptides: insulin and amylin. Peptides 19:883–889.
[15] Reger, M.A., Watson, G.S., Frey 2nd, W.H., Baker, L.D., Cholerton, B., Keeling, M.L., et al., 2006. Effects of intranasal insulin on cognition in memory-impaired older adults: modulation by APOE genotype. Neurobiology of Aging 27:451–458.
[16] Heni, M., Schopfer, P., Peter, A., Sartorius, T., Fritsche, A., Synofzik, M., et al., 2014. Evidence for altered transport of insulin across the blood-brain barrier in insulin-resistant humans. Acta Diabetologica 51:679–681.
[17] Tschritter, O., Preissl, H., Hennige, A.M., Stumvoll, M., Porubska, K., Frost, R., et al., 2006. The cerebrocortical response to hyperinsulinemia is reduced in overweight humans: a magnetoencephalographic study. Proceedings of the National Academy of Sciences of the United States of America 103:12103–12108.
[18] Dorn, A., Bernstein, H.G., Rinne, A., Ziegler, M., Hahn, H.J., Ansorge, S., 1983. Insulin- and glucagonlike peptides in the brain. Anatomical Record 207:69–77.
[19] Dorn, A., Bernstein, H.G., Rinne, A., Hahn, H.J., Ziegler, M., 1982. Insulin-like immunoreactivity in the human brain- a preliminary report. Histochemistry 74:293–300.
[20] Ghasemi, R., Haeri, A., Dargahi, L., Mohamed, Z., Ahmadiani, A., 2013. Insulin in the brain: sources, localization and functions. Molecular Neurobiology 47:145–171.

[21] Birch, N.P., Christie, D.L., Renwick, A.G., 1984. Proinsulin-like material in mouse foetal brain cell cultures. FEBS Letters 168:299—302.

[22] Born, J., Lange, T., Kern, W., McGregor, G.P., Bickel, U., Fehm, H.L., 2002. Sniffing neuropeptides: a transnasal approach to the human brain. Nature Neuroscience 5:514—516.

[23] Thorne, R.G., Emory, C.R., Ala, T.A., Frey 2nd, W.H., 1995. Quantitative analysis of the olfactory pathway for drug delivery to the brain. Brain Research 692:278—282.

[24] Illum, L., 2000. Transport of drugs from the nasal cavity to the central nervous system. European Journal of Pharmaceutical Sciences 11:1—18.

[25] Renner, D.B., Svitak, A.L., Gallus, N.J., Ericson, M.E., Frey 2nd, W.H., Hanson, L.R., 2012. Intranasal delivery of insulin via the olfactory nerve pathway. Journal of Pharmacy and Pharmacology 64:1709—1714.

[26] Chapman, C.D., Frey 2nd, W.H., Craft, S., Danielyan, L., Hallschmid, M., Schioth, H.B., et al., 2013. Intranasal treatment of central nervous system dysfunction in humans. Pharmaceutical Research 30:2475—2484.

[27] Major, R.H., 1935. The intranasal application of insulin, experimental and clinical experiences. Transactions of the American Clinical and Climatological Association 51:21—27.

[28] Kaiyala, K.J., Prigeon, R.L., Kahn, S.E., Woods, S.C., Schwartz, M.W., 2000. Obesity induced by a high-fat diet is associated with reduced brain insulin transport in dogs. Diabetes 49:1525—1533.

[29] Stein, L.J., Dorsa, D.M., Baskin, D.G., Figlewicz, D.P., Porte Jr., D., Woods, S.C., 1987. Reduced effect of experimental peripheral hyperinsulinemia to elevate cerebrospinal fluid insulin concentrations of obese Zucker rats. Endocrinology 121:1611—1615.

[30] Schwartz, M.W., Figlewicz, D.P., Baskin, D.G., Woods, S.C., Porte Jr., D., 1992. Insulin in the brain: a hormonal regulator of energy balance. Endocrine Reviews 13:387—414.

[31] Kim, Y.B., Nikoulina, S.E., Ciaraldi, T.P., Henry, R.R., Kahn, B.B., 1999. Normal insulin-dependent activation of Akt/protein kinase B, with diminished activation of phosphoinositide 3-kinase, in muscle in type 2 diabetes. The Journal of Clinical Investigation 104:733—741.

[32] Clegg, D.J., Gotoh, K., Kemp, C., Wortman, M.D., Benoit, S.C., Brown, L.M., et al., 2011. Consumption of a high-fat diet induces central insulin resistance independent of adiposity. Physiology & Behavior 103:10—16.

[33] Niswender, K.D., Morrison, C.D., Clegg, D.J., Olson, R., Baskin, D.G., Myers Jr., M.G., et al., 2003. Insulin activation of phosphatidylinositol 3-kinase in the hypothalamic arcuate nucleus: a key mediator of insulin-induced anorexia. Diabetes 52:227—231.

[34] Chen, M., Woods, S.C., Porte Jr., D., 1975. Effect of cerebral intraventricular insulin on pancreatic insulin secretion in the dog. Diabetes 24:910—914.

[35] Woods, S.C., Lotter, E.C., McKay, L.D., Porte Jr., D., 1979. Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. Nature 282:503—505.

[36] Harai, S., Ikenaga, T., Matsuzawa, T., 1978. Nasal absorption of insulin in dogs. Diabetes 27:296—299.

[37] Anand Kumar, T.C., Sehgal, A., Bajaj, J.S., 1980. Intranasal administration of insulin reduces circulating levels of glucose in the Rhesus monkey. Current Science 49:548—550.

[38] Obici, S., Feng, Z., Karkanias, G., Baskin, D.G., Rossetti, L., 2002. Decreasing hypothalamic insulin receptors causes hyperphagia and insulin resistance in rats. Nature Neuroscience 5:566—572.

[39] Grillo, C.A., Tamashiro, K.L., Piroli, G.G., Melhorn, S., Gass, J.T., Newsom, R.J., et al., 2007. Lentivirus-mediated downregulation of hypothalamic insulin receptor expression. Physiology & Behavior 92:691—701.

[40] Paranjape, S.A., Chan, O., Zhu, W., Horblitt, A.M., Grillo, C.A., Wilson, S., et al., 2011. Chronic reduction of insulin receptors in the ventromedial hypothalamus produces glucose intolerance and islet dysfunction in the absence of weight gain. American Journal of Physiology. Endocrinology and Metabolism 301:E978—E983.

[41] Konner, A.C., Janoschek, R., Plum, L., Jordan, S.D., Rother, E., Ma, X., et al., 2007. Insulin action in AgRP-expressing neurons is required for suppression of hepatic glucose production. Cell Metabolism 5:438—449.

[42] Varela, L., Horvath, T.L., 2012. Leptin and insulin pathways in POMC and AgRP neurons that modulate energy balance and glucose homeostasis. EMBO Reports 13:1079—1086.

[43] Hill, J.W., Elias, C.F., Fukuda, M., Williams, K.W., Berglund, E.D., Holland, W.L., et al., 2010. Direct insulin and leptin action on pro-opiomelanocortin neurons is required for normal glucose homeostasis and fertility. Cell Metabolism 11:286—297.

[44] Sipols, A.J., Baskin, D.G., Schwartz, M.W., 1995. Effect of intracerebroventricular insulin infusion on diabetic hyperphagia and hypothalamic neuropeptide gene expression. Diabetes 44:147—151.

[45] Benoit, S.C., Air, E.L., Coolen, L.M., Strauss, R., Jackman, A., Clegg, D.J., et al., 2002. The catabolic action of insulin in the brain is mediated by melanocortins. The Journal of Neuroscience 22:9048—9052.

[46] Balthasar, N., Dalgaard, L.T., Lee, C.E., Yu, J., Funahashi, H., Williams, T., et al., 2005. Divergence of melanocortin pathways in the control of food intake and energy expenditure. Cell 123:493—505.

[47] Kim, M.S., Pak, Y.K., Jang, P.G., Namkoong, C., Choi, Y.S., Won, J.C., et al., 2006. Role of hypothalamic Foxo1 in the regulation of food intake and energy homeostasis. Nature Neuroscience 9:901—906.

[48] Kitamura, T., Feng, Y., Kitamura, Y.I., Chua Jr., S.C., Xu, A.W., Barsh, G.S., et al., 2006. Forkhead protein FoxO1 mediates Agrp-dependent effects of leptin on food intake. Nature Medicine 12:534—540.

[49] Belgardt, B.F., Okamura, T., Bruning, J.C., 2009. Hormone and glucose signalling in POMC and AgRP neurons. The Journal of Physiology 587:5305—5314.

[50] Lin, H.V., Plum, L., Ono, H., Gutierrez-Juarez, R., Shanabrough, M., Borok, E., et al., 2010. Divergent regulation of energy expenditure and hepatic glucose production by insulin receptor in agouti-related protein and POMC neurons. Diabetes 59:337—346.

[51] Yaswen, L., Diehl, N., Brennan, M.B., Hochgeschwender, U., 1999. Obesity in the mouse model of pro-opiomelanocortin deficiency responds to peripheral melanocortin. Nature Medicine 5:1066—1070.

[52] Gropp, E., Shanabrough, M., Borok, E., Xu, A.W., Janoschek, R., Buch, T., et al., 2005. Agouti-related peptide-expressing neurons are mandatory for feeding. Nature Neuroscience 8:1289—1291.

[53] Dhillon, H., Zigman, J.M., Ye, C., Lee, C.E., McGovern, R.A., Tang, V., et al., 2006. Leptin directly activates SF1 neurons in the VMH, and this action by leptin is required for normal body-weight homeostasis. Neuron 49:191—203.

[54] Kim, K.W., Sohn, J.W., Kohno, D., Xu, Y., Williams, K., Elmquist, J.K., 2011. SF-1 in the ventral medial hypothalamic nucleus: a key regulator of homeostasis. Molecular and Cellular Endocrinology 336:219—223.

[55] Klockener, T., Hess, S., Belgardt, B.F., Paeger, L., Verhagen, L.A., Husch, A., et al., 2011. High-fat feeding promotes obesity via insulin receptor/PI3K-dependent inhibition of SF-1 VMH neurons. Nature Neuroscience 14:911—918.

[56] Williams, K.W., Margatho, L.O., Lee, C.E., Choi, M., Lee, S., Scott, M.M., et al., 2010. Segregation of acute leptin and insulin effects in distinct populations of arcuate proopiomelanocortin neurons. The Journal of Neuroscience 30:2472—2479.

[57] Heni, M., Kullmann, S., Ketterer, C., Guthoff, M., Linder, K., Wagner, R., et al., 2012. Nasal insulin changes peripheral insulin sensitivity simultaneously with altered activity in homeostatic and reward-related human brain regions. Diabetologia 55:1773—1782.

[58] Stockhorst, U., de Fries, D., Steingrueber, H.J., Scherbaum, W.A., 2011. Unconditioned and conditioned effects of intranasally administered insulin vs placebo in healthy men: a randomised controlled trial. Diabetologia 54:1502—1506.

[59] Pontiroli, A.E., Alberetto, M., Secchi, A., Dossi, G., Bosi, I., Pozza, G., 1982. Insulin given intranasally induces hypoglycaemia in normal and diabetic subjects. British Medical Journal (Clinical Research Ed.) 284:303–306.
[60] Moses, A.C., Gordon, G.S., Carey, M.C., Flier, J.S., 1983. Insulin administered intranasally as an insulin-bile salt aerosol. Effectiveness and reproducibility in normal and diabetic subjects. Diabetes 32:1040–1047.
[61] Salzman, R., Manson, J.E., Griffing, G.T., Kimmerle, R., Ruderman, N., McCall, A., et al., 1985. Intranasal aerosolized insulin. Mixed-meal studies and long-term use in type I diabetes. The New England Journal of Medicine 312:1078–1084.
[62] Frauman, A.G., Cooper, M.E., Parsons, B.J., Jerums, G., Louis, W.J., 1987. Long-term use of intranasal insulin in insulin-dependent diabetic patients. Diabetes Care 10:573–578.
[63] Frauman, A.G., Jerums, G., Louis, W.J., 1987. Effects of intranasal insulin in non-obese type II diabetics. Diabetes Research and Clinical Practice 3:197–202.
[64] Paquot, N., Scheen, A.J., Franchimont, P., Lefebvre, P.J., 1988. The intranasal administration of insulin induces significant hypoglycaemia and classical counterregulatory hormonal responses in normal man. Diabetes & Metabolism 14:31–36.
[65] Coates, P.A., Ismail, I.S., Luzio, S.D., Griffiths, I., Ollerton, R.L., Volund, A., et al., 1995. Intranasal insulin: the effects of three dose regimens on postprandial glycaemic profiles in type II diabetic subjects. Diabetes Medicine 12:235–239.
[66] Lalej-Bennis, D., Boillot, J., Bardin, C., Zirinis, P., Coste, A., Escudier, E., et al., 2001. Efficacy and tolerance of intranasal insulin administered during 4 months in severely hyperglycaemic Type 2 diabetic patients with oral drug failure: a cross-over study. Diabetes Medicine 18:614–618.
[67] Bruce, D.G., Chisholm, D.J., Storlien, L.H., Borkman, M., Kraegen, E.W., 1991. Meal-time intranasal insulin delivery in type 2 diabetes. Diabetes Medicine 8:366–370.
[68] Hilsted, J., Madsbad, S., Hvidberg, A., Rasmussen, M.H., Krarup, T., Ipsen, H., et al., 1995. Intranasal insulin therapy: the clinical realities. Diabetologia 38:680–684.
[69] Kern, W., Born, J., Schreiber, H., Fehm, H.L., 1999. Central nervous system effects of intranasally administered insulin during euglycemia in men. Diabetes 48:557–563.
[70] Benedict, C., Brede, S., Schioth, H.B., Lehnert, H., Schultes, B., Born, J., et al., 2011. Intranasal insulin enhances postprandial thermogenesis and lowers postprandial serum insulin levels in healthy men. Diabetes 60:114–118.
[71] Heni, M., Wagner, R., Kullmann, S., Veit, R., Mat Husin, H., Linder, K., et al., 2014. Central insulin administration improves whole-body insulin sensitivity via hypothalamus and parasympathetic outputs in men. Diabetes 63:4083–4088.
[72] Benedict, C., Kern, W., Schultes, B., Born, J., Hallschmid, M., 2008. Differential sensitivity of men and women to anorexigenic and memory-improving effects of intranasal insulin. The Journal of Clinical Endocrinology & Metabolism 93:1339–1344.
[73] Krug, R., Benedict, C., Born, J., Hallschmid, M., 2010. Comparable sensitivity of postmenopausal and young women to the effects of intranasal insulin on food intake and working memory. The Journal of Clinical Endocrinology & Metabolism 95:E468–E472.
[74] Hallschmid, M., Benedict, C., Schultes, B., Fehm, H.L., Born, J., Kern, W., 2004. Intranasal insulin reduces body fat in men but not in women. Diabetes 53:3024–3029.
[75] Hallschmid, M., Higgs, S., Thienel, M., Ott, V., Lehnert, H., 2012. Postprandial administration of intranasal insulin intensifies satiety and reduces intake of palatable snacks in women. Diabetes 61:782–789.
[76] Jauch-Chara, K., Friedrich, A., Rezmer, M., Melchert, U.H., G.S.-E., H., Hallschmid, M., et al., 2012. Intranasal insulin suppresses food intake via enhancement of brain energy levels in humans. Diabetes 61:2261–2268.
[77] Guthoff, M., Grichisch, Y., Canova, C., Tschritter, O., Veit, R., Hallschmid, M., et al., 2010. Insulin modulates food-related activity in the central nervous system. The Journal of Clinical Endocrinology & Metabolism 95:748–755.
[78] Kullmann, S., Frank, S., Heni, M., Ketterer, C., Veit, R., Haring, H.U., et al., 2013. Intranasal insulin modulates intrinsic reward and prefrontal circuitry of the human brain in lean women. Neuroendocrinology 97:176–182.
[79] Kullmann, S., Heni, M., Veit, R., Scheffler, K., Machann, J., Haring, H.U., et al., 2015. Selective insulin resistance in homeostatic and cognitive control brain areas in overweight and obese adults. Diabetes Care 38:1044–1050.
[80] Hallschmid, M., Benedict, C., Schultes, B., Born, J., Kern, W., 2008. Obese men respond to cognitive but not to catabolic brain insulin signaling. International Journal of Obesity (London) 32:275–282.
[81] James, F.E., 1992. Insulin treatment in psychiatry. History of Psychiatry 3:221–235.
[82] Sakel, M., 1937. The origin and nature of the hypoglycemic therapy of the psychoses. Bulletin of the New York Academy of Medicine 13:97–109.
[83] Mack, C.W., Burch, B.O., 1939. Insulin shock therapy in dementia praecox: a report of a series of cases. California and Western Medicine 50:339–344.
[84] Crammer, J.L., 2000. Insulin coma therapy for schizophrenia. Journal of the Royal Society of Medicine 93:332–333.
[85] Berman, L., Riess, B., 1942. The effect of insulin shock on learning in the white rat. Science 95:511–512.
[86] Stranahan, A.M., Norman, E.D., Lee, K., Cutler, R.G., Telljohann, R.S., Egan, J.M., et al., 2008. Diet-induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle-aged rats. Hippocampus 18:1085–1088.
[87] Biessels, G.J., Reagan, L.P., 2015. Hippocampal insulin resistance and cognitive dysfunction. Nature Reviews Neuroscience 16:660–671.
[88] Zhao, W.Q., De Felice, F.G., Fernandez, S., Chen, H., Lambert, M.P., Quon, M.J., et al., 2008. Amyloid beta oligomers induce impairment of neuronal insulin receptors. FASEB Journal 22:246–260.
[89] de la Monte, S.M., Wands, J.R., 2008. Alzheimer's disease is type 3 diabetes-evidence reviewed. The Journal of Diabetes Science and Technology 2:1101–1113.
[90] Querfurth, H.W., LaFerla, F.M., 2010. Alzheimer's disease. The New England Journal of Medicine 362:329–344.
[91] Arvanitakis, Z., Wilson, R.S., Bienias, J.L., Evans, D.A., Bennett, D.A., 2004. Diabetes mellitus and risk of Alzheimer disease and decline in cognitive function. Archives of Neurology 61:661–666.
[92] Whitmer, R.A., Gustafson, D.R., Barrett-Connor, E., Haan, M.N., Gunderson, E.P., Yaffe, K., 2008. Central obesity and increased risk of dementia more than three decades later. Neurology 71:1057–1064.
[93] Biessels, G.J., Staekenborg, S., Brunner, E., Brayne, C., Scheltens, P., 2006. Risk of dementia in diabetes mellitus: a systematic review. The Lancet Neurology 5:64–74.
[94] Lee, J.H., Choi, Y., Jun, C., Hong, Y.S., Cho, H.B., Kim, J.E., et al., 2014. Neurocognitive changes and their neural correlates in patients with type 2 diabetes mellitus. Endocrinology & Metabolism (Seoul) 29:112–121.
[95] Chatterjee, S., Peters, S.A., Woodward, M., Arango, S.M., Batty, G.D., Beckett, N., et al., 2016. Type 2 diabetes as a risk factor for dementia in women compared with men: a pooled analysis of 2.3 million people comprising more than 100,000 cases of dementia. Diabetes Care 39:300–307.
[96] Gudala, K., Bansal, D., Schifano, F., Bhansali, A., 2013. Diabetes mellitus and risk of dementia: a meta-analysis of prospective observational studies. Journal of Diabetes Investigation 4:640–650.
[97] Blazquez, E., Velazquez, E., Hurtado-Carneiro, V., Ruiz-Albusac, J.M., 2014. Insulin in the brain: its pathophysiological implications for States related with

central insulin resistance, type 2 diabetes and Alzheimer's disease. Frontiers in Endocrinology (Lausanne) 5:161.

[98] de la Monte, S.M., 2012. Brain insulin resistance and deficiency as therapeutic targets in Alzheimer's disease. Current Alzheimer Research 9:35–66.

[99] Willette, A.A., Xu, G., Johnson, S.C., Birdsill, A.C., Jonaitis, E.M., Sager, M.A., et al., 2013. Insulin resistance, brain atrophy, and cognitive performance in late middle-aged adults. Diabetes Care 36:443–449.

[100] Talbot, K., Wang, H.Y., Kazi, H., Han, L.Y., Bakshi, K.P., Stucky, A., et al., 2012. Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. The Journal of Clinical Investigation 122:1316–1338.

[101] Liu, Y., Liu, F., Grundke-Iqbal, I., Iqbal, K., Gong, C.X., 2011. Deficient brain insulin signalling pathway in Alzheimer's disease and diabetes. The Journal of Pathology 225:54–62.

[102] Deng, Y., Li, B., Liu, Y., Iqbal, K., Grundke-Iqbal, I., Gong, C.X., 2009. Dysregulation of insulin signaling, glucose transporters, O-GlcNAcylation, and phosphorylation of tau and neurofilaments in the brain: implication for Alzheimer's disease. American Journal of Pathology 175:2089–2098.

[103] Takashima, A., 2006. GSK-3 is essential in the pathogenesis of Alzheimer's disease. Journal of Alzheimer's Disease 9:309–317.

[104] Liu, F., Iqbal, K., Grundke-Iqbal, I., Hart, G.W., Gong, C.X., 2004. O-GlcNAcylation regulates phosphorylation of tau: a mechanism involved in Alzheimer's disease. Proceedings of the National Academy of Sciences of the United States of America 101:10804–10809.

[105] Yuzwa, S.A., Shan, X., Macauley, M.S., Clark, T., Skorobogatko, Y., Vosseller, K., et al., 2012. Increasing O-GlcNAc slows neurodegeneration and stabilizes tau against aggregation. Nature Chemical Biology 8:393–399.

[106] Bomfim, T.R., Forny-Germano, L., Sathler, L.B., Brito-Moreira, J., Houzel, J.C., Decker, H., et al., 2012. An anti-diabetes agent protects the mouse brain from defective insulin signaling caused by Alzheimer's disease- associated Abeta oligomers. The Journal of Clinical Investigation 122:1339–1353.

[107] Mielke, J.G., Taghibiglou, C., Liu, L., Zhang, Y., Jia, Z., Adeli, K., et al., 2005. A biochemical and functional characterization of diet-induced brain insulin resistance. Journal of Neurochemistry 93:1568–1578.

[108] Pratchayasakul, W., Kerdphoo, S., Petsophonsakul, P., Pongchaidecha, A., Chattipakorn, N., Chattipakorn, S.C., 2011. Effects of high-fat diet on insulin receptor function in rat hippocampus and the level of neuronal corticosterone. Life Sciences 88:619–627.

[109] Pipatpiboon, N., Pratchayasakul, W., Chattipakorn, N., Chattipakorn, S.C., 2012. PPARgamma agonist improves neuronal insulin receptor function in hippocampus and brain mitochondria function in rats with insulin resistance induced by long term high-fat diets. Endocrinology 153:329–338.

[110] Pipatpiboon, N., Pintana, H., Pratchayasakul, W., Chattipakorn, N., Chattipakorn, S.C., 2013. DPP4-inhibitor improves neuronal insulin receptor function, brain mitochondrial function and cognitive function in rats with insulin resistance induced by high-fat diet consumption. European Journal of Neuroscience 37:839–849.

[111] Ho, N., Balu, D.T., Hilario, M.R., Blendy, J.A., Lucki, I., 2012. Depressive phenotypes evoked by experimental diabetes are reversed by insulin. Physiology & Behavior 105:702–708.

[112] Ho, L., Qin, W., Pompl, P.N., Xiang, Z., Wang, J., Zhao, Z., et al., 2004. Diet-induced insulin resistance promotes amyloidosis in a transgenic mouse model of Alzheimer's disease. FASEB Journal 18:902–904.

[113] Townsend, M., Mehta, T., Selkoe, D.J., 2007. Soluble Abeta inhibits specific signal transduction cascades common to the insulin receptor pathway. The Journal of Biological Chemistry 282:33305–33312.

[114] Schubert, M., Gautam, D., Surjo, D., Ueki, K., Baudler, S., Schubert, D., et al., 2004. Role for neuronal insulin resistance in neurodegenerative diseases. Proceedings of the National Academy of Sciences of the United States of America 101:3100–3105.

[115] Freude, S., Hettich, M.M., Schumann, C., Stohr, O., Koch, L., Kohler, C., et al., 2009. Neuronal IGF-1 resistance reduces Abeta accumulation and protects against premature death in a model of Alzheimer's disease. FASEB Journal 23:3315–3324.

[116] Marks, D.R., Tucker, K., Cavallin, M.A., Mast, T.G., Fadool, D.A., 2009. Awake intranasal insulin delivery modifies protein complexes and alters memory, anxiety, and olfactory behaviors. The Journal of Neuroscience 29:6734–6751.

[117] Das, P., Parsons, A.D., Scarborough, J., Hoffman, J., Wilson, J., Thompson, R.N., et al., 2005. Electrophysiological and behavioral phenotype of insulin receptor defective mice. Physiology & Behavior 86:287–296.

[118] Yang, Y., Ma, D., Wang, Y., Jiang, T., Hu, S., Zhang, M., et al., 2013. Intranasal insulin ameliorates tau hyperphosphorylation in a rat model of type 2 diabetes. Journal of Alzheimer's Disease 33:329–338.

[119] Benedict, C., Hallschmid, M., Hatke, A., Schultes, B., Fehm, H.L., Born, J., et al., 2004. Intranasal insulin improves memory in humans. Psychoneuroendocrinology 29:1326–1334.

[120] Novak, V., Milberg, W., Hao, Y., Munshi, M., Novak, P., Galica, A., et al., 2014. Enhancement of vasoreactivity and cognition by intranasal insulin in type 2 diabetes. Diabetes Care 37:751–759.

[121] Reger, M.A., Watson, G.S., Green, P.S., Baker, L.D., Cholerton, B., Fishel, M.A., et al., 2008. Intranasal insulin administration dose-dependently modulates verbal memory and plasma amyloid-beta in memory-impaired older adults. Journal of Alzheimer's Disease 13:323–331.

[122] Benedict, C., Hallschmid, M., Schmitz, K., Schultes, B., Ratter, F., Fehm, H.L., et al., 2007. Intranasal insulin improves memory in humans: superiority of insulin aspart. Neuropsychopharmacology 32:239–243.

[123] Reger, M.A., Watson, G.S., Green, P.S., Wilkinson, C.W., Baker, L.D., Cholerton, B., et al., 2008. Intranasal insulin improves cognition and modulates beta-amyloid in early AD. Neurology 70:440–448.

[124] Craft, S., Baker, L.D., Montine, T.J., Minoshima, S., Watson, G.S., Claxton, A., et al., 2012. Intranasal insulin therapy for Alzheimer disease and amnestic mild cognitive impairment: a pilot clinical trial. Archives of Neurology 69:29–38.

[125] Cowie, D.M., Parsons, J.P., Raphael, T., 1924. Insulin and mental depression. Archives of Neurology and Psychiatry 12:522–533.

[126] Rubin, R.R., Peyrot, M., 2002. Was Willis right? Thoughts on the interaction of depression and diabetes. Diabetes/Metabolism Research and Reviews 18:173–175.

[127] Musselman, D.L., Betan, E., Larsen, H., Phillips, L.S., 2003. Relationship of depression to diabetes types 1 and 2: epidemiology, biology, and treatment. Biological Psychiatry 54:317–329.

[128] Silva, N., Atlantis, E., Ismail, K., 2012. A review of the association between depression and insulin resistance: pitfalls of secondary analyses or a promising new approach to prevention of type 2 diabetes? Current Psychiatry Reports 14:8–14.

[129] Baker, L.D., Cross, D.J., Minoshima, S., Belongia, D., Watson, G.S., Craft, S., 2011. Insulin resistance and Alzheimer-like reductions in regional cerebral glucose metabolism for cognitively normal adults with prediabetes or early type 2 diabetes. Archives of Neurology 68:51–57.

[130] Chen, Z., Zhong, C., 2013. Decoding Alzheimer's disease from perturbed cerebral glucose metabolism: implications for diagnostic and therapeutic strategies. Progress in Neurobiology 108:21–43.

[131] Su, L., Cai, Y., Xu, Y., Dutt, A., Shi, S., Bramon, E., 2014. Cerebral metabolism in major depressive disorder: a voxel-based meta-analysis of positron emission tomography studies. BMC Psychiatry 14:321.

[132] Domenici, E., Wille, D.R., Tozzi, F., Prokopenko, I., Miller, S., McKeown, A., et al., 2010. Plasma protein biomarkers for depression and schizophrenia by multi analyte profiling of case-control collections. PLoS One 5:e9166.

[133] Pan, Y., Hong, Y., Zhang, Q.Y., Kong, L.D., 2013. Impaired hypothalamic insulin signaling in CUMS rats: restored by icariin and fluoxetine through inhibiting CRF system. Psychoneuroendocrinology 38:122–134.

[134] Grillo, C.A., Piroli, G.G., Kaigler, K.F., Wilson, S.P., Wilson, M.A., Reagan, L.P., 2011. Downregulation of hypothalamic insulin receptor expression elicits depressive-like behaviors in rats. Behavioural Brain Research 222:230—235.

[135] Cline, B.H., Steinbusch, H.W., Malin, D., Revishchin, A.V., Pavlova, G.V., Cespuglio, R., et al., 2012. The neuronal insulin sensitizer dicholine succinate reduces stress-induced depressive traits and memory deficit: possible role of insulin-like growth factor 2. BMC Neuroscience 13:110.

[136] Bohringer, A., Schwabe, L., Richter, S., Schachinger, H., 2008. Intranasal insulin attenuates the hypothalamic-pituitary-adrenal axis response to psychosocial stress. Psychoneuroendocrinology 33:1394—1400.

[137] McIntyre, R.S., Soczynska, J.K., Woldeyohannes, H.O., Miranda, A., Vaccarino, A., Macqueen, G., et al., 2012. A randomized, double-blind, controlled trial evaluating the effect of intranasal insulin on neurocognitive function in euthymic patients with bipolar disorder. Bipolar Disorder 14:697—706.

[138] Konner, A.C., Hess, S., Tovar, S., Mesaros, A., Sanchez-Lasheras, C., Evers, N., et al., 2011. Role for insulin signaling in catecholaminergic neurons in control of energy homeostasis. Cell Metabolism 13:720—728.

[139] Fisher, S.J., Bruning, J.C., Lannon, S., Kahn, C.R., 2005. Insulin signaling in the central nervous system is critical for the normal sympathoadrenal response to hypoglycemia. Diabetes 54:1447—1451.

[140] Diggs-Andrews, K.A., Zhang, X., Song, Z., Daphna-Iken, D., Routh, V.H., Fisher, S.J., 2010. Brain insulin action regulates hypothalamic glucose sensing and the counterregulatory response to hypoglycemia. Diabetes 59: 2271—2280.

[141] Sanchez-Alavez, M., Osborn, O., Tabarean, I.V., Holmberg, K.H., Eberwine, J., Kahn, C.R., et al., 2011. Insulin-like growth factor 1-mediated hyperthermia involves anterior hypothalamic insulin receptors. The Journal of Biological Chemistry 286:14983—14990.

[142] Stohr, O., Schilbach, K., Moll, L., Hettich, M.M., Freude, S., Wunderlich, F.T., et al., 2013. Insulin receptor signaling mediates APP processing and beta-amyloid accumulation without altering survival in a transgenic mouse model of Alzheimer's disease. Age (Dordrecht) 35:83—101.

[143] Iwen, K.A., Scherer, T., Heni, M., Sayk, F., Wellnitz, T., Machleidt, F., et al., 2014. Intranasal insulin suppresses systemic but not subcutaneous lipolysis in healthy humans. The Journal of Clinical Endocrinology & Metabolism 99: E246—E251.

[144] Gancheva, S., Koliaki, C., Bierwagen, A., Nowotny, P., Heni, M., Fritsche, A., et al., 2015. Effects of intranasal insulin on hepatic fat accumulation and energy metabolism in humans. Diabetes 64:1966—1975.

[145] Rosenbloom, M.H., Barclay, T.R., Pyle, M., Owens, B.L., Cagan, A.B., Anderson, C.P., et al., 2014. A single-dose pilot trial of intranasal rapid-acting insulin in apolipoprotein E4 carriers with mild-moderate Alzheimer's disease. CNS Drugs 28:1185—1189.

[146] Brunner, Y.F., Kofoet, A., Benedict, C., Freiherr, J., 2015. Central insulin administration improves odor-cued reactivation of spatial memory in young men. The Journal of Clinical Endocrinology & Metabolism 100:212—219.

[147] Claxton, A., Baker, L.D., Hanson, A., Trittschuh, E.H., Cholerton, B., Morgan, A., et al., 2015. Long-acting intranasal insulin detemir improves cognition for adults with mild cognitive impairment or early-stage Alzheimer's disease dementia. Journal of Alzheimer's Disease 44:897—906.

[148] Zhang, H., Hao, Y., Manor, B., Novak, P., Milberg, W., Zhang, J., et al., 2015. Intranasal insulin enhanced resting-state functional connectivity of hippocampal regions in type 2 diabetes. Diabetes 64:1025—1034.

