The Role of Insulin, Insulin Growth Factor, and Insulin-Degrading Enzyme in Brain Aging and Alzheimer's Disease

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ABSTRACT

Most brain insulin comes from the pancreas and is taken up by the brain by what appears to be a receptor-based carrier. Type 2 diabetes animal models associated with insulin resistance show reduced insulin brain uptake and content. Recent data point to changes in the insulin receptor cascade in obesity-related insulin resistance, suggesting that brain insulin receptors also become less sensitive to insulin, which could reduce synaptic plasticity. Insulin transport to the brain is reduced in aging and in some animal models of type 2 diabetes; brain insulin resistance may be present as well. Studies examining the effect of the hyperinsulinic clamp or intranasal insulin on cognitive function have found a small but consistent improvement in memory and changes in brain neuroelectric parameters in evoked brain potentials consistent with improved attention or memory processing. These effects appear to be due to raised brain insulin levels. Peripheral levels of Insulin Growth Factor-1 (IGF-I) are associated with glucose regulation and influence glucose disposal. There is some indication that reduced sensitivity to insulin or IGF-I in the brain, as observed in aging, obesity, and diabetes, decreases the clearance of Aβ amyloid. Such a decrease involves the insulin receptor cascade and can also increase amyloid toxicity. Insulin and IGF-I may modulate brain levels of insulin degrading enzyme, which would also lead to an accumulation of Aβ amyloid.

KEYWORDS

literature review, diabetes, glucose regulation, hyperinsulinemia, impaired glucose tolerance, cognitive function. dementia, vascular disease, memory

INTRODUCTION

Over the last few years, there has been a re-evaluation of the role of insulin and insulin growth factors in brain functions. This re-evaluation has been driven by a number of observations and hypotheses that encompass the control of feeding and adiposity, the impact of diabetes on the brain and more recently, the interactions among insulin, diabetes, Alzheimer's disease, and amyloid deposition. Several recent reviews have addressed the role of brain insulin in the control of metabolism and food intake (Gerozissis 2003. Porte et al., 2005). Among several recent reports that support such a role of insulin is the demonstration that mice with conditional knockout of insulin receptors in the brain are overweight, insulin resistant, and glucose intolerant (Bruning et al., 2000). Furthermore, blockade of hypothalamic

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insulin receptors by antisense oligonucleotides produced hyperphagia, insulin resistance, and weight increase (Obici et al., 2002). These examples clearly show that the brain insulin plays an important role in feeding, peripheral metabolism, and weight regulation. Recent evidence of a down-regulation of brain insulin receptors in obesity and diabetes suggest that insulin resistance may also involve the brain (Mielke, 2005).

The aim of this review is to put together various recent data that describe the possible role of insulin, insulin receptors, and insulin growth factors in learning and memory. The role of insulin-related peptides is examined in the context of aging, diabetes, and Alzheimer's disease The general hypotheses that are entertained in this review are that

1. type 2 diabetes and obesity lead to insulin resistance in the brain,
2. insulin resistance leads to cognitive impairments in older diabetic patients and
3. insulin resistance increase amyloid deposition in Alzheimer's disease patients.

We first examine what is known about insulin's access to the brain and the impact of diabetes on the transfer of insulin from blood to the brain. We then present a brief summary of the results of studies that examined the interactions between diabetes, aging, and brain with reference to recent reviews for an in-depth analysis of the neurocognitive deficits in type 2 diabetes (Awad et al., 2004; Messier, 2003; Messier et al., 2004). Following this, we review studies that have tested the ability of insulin to modulate brain functions with a particular attention to learning and memory. For an in-depth review of the role of insulin in metabolism, feeding, and weight control, the reader can consult several excellent reviews (Gerozissis 2003. Porte et al., 2005. Woods, et al., 2003). Because insulin and insulin growth factor 1 (IGF-I) partially share receptor activity and interact under certain circumstances, we briefly discuss several aspects of IGF-I in the adult and the aging brain. Finally, we examine the interaction of insulin and IGF-I with amyloid deposition and processing as a possible mechanism in the deleterious effect of diabetes on Alzheimer's disease progression. We now turn to the examination of the source of insulin in the brain.

INSULIN TRANSPORT THROUGH THE BLOOD-BRAIN BARRIER

As discussed by Banks (2004), the nature of insulin resistance in the brain remains unclear. In the periphery, most insulin resistance is associated with receptor or post-receptor effects in specific tissues. In the brain, insulin resistance can manifest itself through changes in brain insulin uptake and by changes in the insulin receptor function as well. To understand these changes, we will briefly review what is known about the transport of insulin through the blood-brain barrier.

The mechanisms by which insulin gains access to the CNS have recently been reviewed (Banks 2004). Although insulin and its receptors are widely spread throughout the brain (Dore et al., 1997. Schulingkamp et al., 2000. Zhao et al., 1999), apparently no significant insulin synthesis occurs in the CNS (Woodset et al., 2003). However, this conclusion has to be tempered by the presence of insulin mRNA in the brain and the possibility of restricted insulin synthesis in the brain (Devaskar et al., 1994). Most insulin is now thought to enter the brain through a receptor-based, saturable transporter that is largely saturated by serum levels of insulin that do not induce hypoglycemia (Banks et al., 1997a–c). Transport of insulin in the brain is highest in the olfactory bulb (8 times that found in the brain as a whole) followed by the pons, medulla, and hypothalamus (Banks et al., 1999). This level is consistent with the observation that the olfactory bulb has the highest concentration of insulin (Baskin et al., 1987).

Evidence also suggests that insulin is released in the hypothalamic extracellular space in anticipation of a scheduled meal in rats. Such insulin release is independent of blood insulin levels, showing that certain brain regions can release insulin in relation to a learned association (Orosco et al., 1995). Additionally, a microdialysis study showed that intra-hypothalamic infusion of glucose increases extracellular insulin and that aging and obesity significantly reduce the glucose-mediated insulin release (Gerozissis et al., 2001).

Thus, elevations in blood insulin levels will likely have different effects on different brain regions at different concentrations, owing to the disparity in insulin uptake into the brain. It is likely that in brain areas where insulin uptake is greatest, insulin could have regulatory effects whereas in areas where uptake is smaller and saturates at lower concentrations, insulin may have other effects, perhaps linked to a neurotrophic action.

Therefore, any theories involving brain insulin resistance will have to take into account not only the regional variations in transport mechanisms but also the concentrations of insulin and insulin receptors in the brain. As a result, insulin resistance in the brain could be due to changes in the receptor-based insulin carrier that would lead to reduced access of insulin to the brain or could be due to changes in insulin receptor function within the brain. One also has to take into account the large variations in blood insulin levels that follow ingestion of food and the feedback-related inhibition of insulin transport during hyperglycemia. We now turn to the effect of diabetes and aging on insulin transport and receptor function.

Hyperglycemia (in normal animals), obesity, and obesity-induced diabetes (in Zucker rats) decrease insulin transport into the brain (Banks et al., 1997a-b. Baskin et al., 1985. Kaiyala et al., 2000). Consistent with decreased insulin transport, obese Zucker rats have reduced brain levels of insulin (Baskin et al., 1985). However, in streptozotocin- or alloxan-induced diabetes, an increase in insulin transport to the brain occurs that is associated with the very low systemic insulin levels in these models. Such low levels of insulin result from the destruction of pancreatic beta cells. In animals, insulin receptors are highly expressed during development but decline during aging (Zhao et al., 1999). One human post-mortem study found that brain insulin, c-peptide, and insulin receptors are reduced in older individuals (Frolich et al., 1998).

Few studies have directly addressed the hypothesis of brain insulin resistance. In a model of metabolic syndrome (fructose-fed hamsters), which leads to obesity and impaired glucose tolerance, a downregulation of neural insulin signaling was noted, as measured by the stimulated tyrosine phosphorylation of the insulin receptor. The downregulation was accompanied by a reduced insulin-mediated long-term depression (LTD), although no change occurred in the presynaptically-mediated paired-pulse facilitation or in the expression of LTP, indicating normal hippocampal electrical activity. Thus, the establishment of neural insulin resistance leads to a reduction of insulin-induced changes in neural activity (Mielke et al., 2005). Other reports that identified the presence of insulin receptors in post-synaptic densities (Abbott et al., 1999) and showed that insulin can rapidly recruit $GABA_A$ receptors from the intracellular pool to the post-synaptic domain, suggesting a role for insulin in neural plasticity (Wan et al., 1997). Similarly, insulin has been shown to facilitate clathrin-mediated internalization of AMPA receptors, causing a form of long-term depression (Ahmadian et al., 2004). Also, insulin inhibits hippocampal pyramidal neurons in isolated brain slices at doses consistent with the presence of high affinity binding sites for insulin in the hippocampus (Palovick et al., 1984).

A recent study examined the effect of water maze training on the state of insulin receptors in

the hippocampus of rats (Zhao et al., 1999). The authors found (a) an upregulation of insulin receptor mRNA in the CA1 and dentate gyrus of the hippocampus, (b) an increased accumulation of insulin receptor protein in the hippocampal crude synaptic membrane fraction, and (c) a decrease in the cytosolic fraction. This result suggests that insulin receptors may be recruited to the synaptic membrane following neuronal activation. In the CA1 pyramidal neurons, changes in the distribution pattern of IR in particular cellular compartments, such as the nucleus and dendritic regions, were observed only in trained animals.

Mice with conditional inactivation of the insulin receptor gene (brain/neuron specific knockout) have a complete loss of insulin-mediated inhibition of neuronal apoptosis (and also a partial inhibition of the IGF-I induced inhibition of apoptosis, suggesting that the anti-apoptosis effect of IGF-I may be partly mediated by insulin receptor activation). However, these knockout mice showed no deficiencies in learning and memory and no difference in cerebral metabolism as measured by 18F-FDG micro-PET. Although Tau phosphorylation was increased, the increase was not accompanied by increased neurofibrillary tangles up to 18 months of age (Schubert et al., 2004).

In summary, brain insulin, most of which comes from the pancreas, is taken up by the brain by what appears to be a receptor-based carrier. Moreover, insulin is transported at various rates through the blood-brain barrier to different brain regions. Type 2 diabetes animal models, which are associated with insulin resistance, show a reduced uptake of insulin to the brain. Recent data point to changes in the insulin receptor cascade in obesity-related insulin resistance, suggesting that brain insulin receptors also undergo changes similar to those observed in the periphery. Finally, insulin appears to modulate the membrane inclusion of GABA and AMPA receptors, suggesting a role in synaptic plasticity. The absence of insulin receptors in the brain of conditional knockout mice does not lead to any major deficit in learning and memory This observation suggests that insulin and insulin receptors have modulatory roles and their absence can be compensated by other mechanisms. We now turn to the various studies that have tried to link insulin to memory and cognitive functions in animals and humans.

Diabetes, aging, and the brain

A number of recent reviews have examined the impact of diabetes on brain functions and the possible link between diabetes and dementia (Awad et al., 2002. Messier, 2003. Messier et al., 2004. Strachan et al., 1997). The conclusions were that (a) type 2 diabetes is associated with an increased incidence of cognitive decline, particularly in patients older than 70 years, and (b) that diabetes appears to be a significant risk factor for vascular dementia and Alzheimer's disease. Several studies have also linked diabetes with brain atrophy. A Japanese MRI study found that brain atrophy was 40% more frequent in diabetic patients than in control participants, even though no difference in vascular pathology occurred, as revealed by the MRI (Araki et al., 1994). Similar findings were obtained in a study in the Netherlands showing that the volumes of the amygdala and hippocampus, two important structures for memory processing, were reduced in diabetic patients, regardless of vascular pathology (den Heijer et al., 2003). A smaller study also found a relation between an indirect measure of temporal lobe volume and fasting blood glucose in diabetic patients with a smaller temporal volume associated with drug-treated diabetic patients compared with diet-treated ones (Soininen et al., 1992). Finally, in normal aged humans, memory and hippocampal volume were found to be correlated to measures of glucose tolerance (Convit et al., 2003). Taken together, these studies suggest that the brain may be affected by changes in insulin levels in the

periphery, and a number of recent studies have addressed the role of insulin on cognitive functions.

Another way to determine whether type 2 diabetes is associated with cognitive deficits is to evaluate the long-term effects of diabetes on cognitive function and whether changes over time are observable in repeated-measures designs. Longitudinal studies allow for such an assessment and limit the between-subjects variability observed in cross-sectional studies.

Two recent longitudinal studies have been conducted to evaluate the relation between cognitive decline and type 2 diabetes. Gregg et al. (2000) compared the performance of 682 diabetic women (mean duration of type 2 diabetes = 10.2 years. SD = 9.5 years) to the performance of 8997 age-matched women (mean age = 72 years) who were assessed on two occasions: at baseline and 3 to 6 years following baseline assessment. Variables such as age, education, depression, hypertension, and cardiovascular disease were methodologically controlled. The neuropsychological assessment included a modified version of the Mini-Mental State Examination as a brief cognitive screening measure, a psychomotor speed test, and a measure of frontal lobe/executive function. The results of this study revealed an increased odds of major cognitive decline in type 2 diabetic patients (defined as the greatest 10^{th} percentile reduction in performance from initial to follow-up score) on the psychomotor speed test and the measure of frontal lobe/executive function—Digit Symbol test (Wechsler 1981) and Trails B test (Spreen & Strauss 1998).

A second longitudinal study compared the performance of 55 type 2 diabetes participants with the performance of 103 participants with impaired fasting glucose and 768 age-matched controls (mean age = 65 years) on several neuro-psychological tests. These tests included the Mini-Mental State Examination, as well as measures of verbal and visual memory, facial recognition, executive function, motor function, arithmetic ability, processing speed, and logical reasoning (Fontbonne et al., 2001). The authors controlled for age, gender, education, depression, hypertension, and body mass index. They reported, however, that diabetic participants had higher levels of triglycerides, as well as lower levels of total cholesterol and HDL cholesterol compared with controls. The authors concluded that the odds ratios for 'serious worsening' were greater than 2 for measures of verbal memory, facial recognition, processing speed, and motor function. Serious worsening is defined as a change in memory scores down to the lowest 15% of the distribution of the observed changes for the whole sample. Taken together, these longitudinal studies suggest that participants with type 2 diabetes are at higher risk for cognitive decline compared with age-matched controls.

The findings of these longitudinal studies are further substantiated by studies evaluating risk of co-existence of diabetes and dementia (reviewed in Messier, 2003). Some studies evaluating the co-existence of these two diseases demonstrated a moderate association between diabetes and Alzheimer's disease, with odds ratios ranging from 1.3 to 1.9 (Brayne et al., 1998. Ott et al., 1996. Ott et al., 1999. Peila et al., 2002). This finding has not been consistently observed, however, as other studies have not reported such associations (Odds ratios ranging from 1.0 to 1.3. (Curb et al., 1999. Leibson et al., 1997. Luchsinger et al., 2001). Recently, Peila et al. (2002) demonstrated that the odds ratio for the co-existence of diabetes and Alzheimer's disease significantly increased only for subjects with the ApoE4 allele. Post-mortem examination of these subjects revealed that Alzheimer's patients who were diabetic had an increased number of infarcts and that the ApoE4 allele was associated with increased neuro-fibrillary tangles in the hippocampus and cortex, as well as increased cerebral amyloid angiopathy. Although several reports found that Alzheimer's

disease was associated with hyperinsulinemia (Bucht et al., 1983. Craft et al., 1993. Craft et al., 1996. 1998), others did not (Fujisawa et al., 1991. Kilander et al., 1993). Two studies found that compared with ApoE2 bearers, bearers of the ApoE4 allele tend to have lower insulin levels (Craft et al., 1998. Gonzalez et al., 1999).

The relation between diabetes and Alzheimer's disease remains unclear, but could be mediated by the insulin-degrading enzyme (IDE), which also degrades beta-amyloid protein. Given that insulin competes with beta-amyloid for the insulin-degrading enzyme, it has been proposed that in certain cases, Alzheimer's disease amyloid deposition can be increased by excessive levels of insulin (Cook et al., 2003. Craft et al., 2000. Ling et al., 2002). However, there is no indication that high levels of insulin or diabetes, as such, independently produce amyloid deposition of neurofibrillary tangles (Heitner & Dickson 1997. Peila et al., 2002), suggesting that diabetes is not a cause of Alzheimer's pathology. Rather, insulin may further aggravate the pathologies already present in Alzheimer's patients. This possibility will be discussed further below.

In the preceding sections, we have examined the possibility that the insulin resistance found in muscle and fat tissues in people with peripheral insulin resistance and type 2 diabetes may also be found for insulin receptors in the brain. Together, the observations that suggest that insulin transport to the brain is reduced in aging, and in some animal models of type 2 diabetes, has led to the proposal that stimulation of the brain's insulin receptors could facilitate brain function and, more precisely, learning and memory. This hypothesis has been tested using several methodologies, as described in the next section.

Effect of insulin on cognitive functions

Very few studies have shown a beneficial effect of peripheral insulin injections on brain functions owing to the hypoglycemic action of insulin and the deleterious effect of hypoglycemia on brain function (Kopf & Baratti 1995, 1999). One experiment, however, showed that insulin injected in conjunction with glucose improved memory in rats (Messier & White 1987). Similarly, the peripheral injection of small doses of insulin reversed the amnesia produced by scopolamine in a food-motivated operant task (Blanchard & Duncan 1997. Messier & Destrade 1994). Intracerebroventricular injection of insulin was shown to improve the performance on an avoidance task (Park et al., 1995), although no effect on memory was found following intrahippocampal insulin injection in rats (Paulus et al., 2005).

The observations that insulin, insulin receptors, and C-peptide levels in cerebral spinal fluid (CSF) appear to be reduced in aging, (Frolich et al., 1998) along with the finding that Alzheimer's patients have lower levels of insulin in the CSF (Craft et al., 1998) suggest impaired transport of insulin into the CSF. The results of these studies have prompted several studies examining the effect of insulin in young and old persons.

The effect of insulin on brain function in humans has been studied using two experimental protocols. In the first, the hyperinsulinemic clamp procedure, increased blood insulin levels are maintained while glucose is infused intravenously to keep blood glucose levels close to normal fasting levels. One study showed that verbal memory is improved in Alzheimer's patients but not in normal age-matched controls during the hyperinsulinemic clamp procedure (Craft et al., 1996).

A second study that also used the hyperinsulinemic clamp showed that insulin improves the memory of younger patients for emotional words, and also produces changes in the evoked potentials P300 latencies, suggesting an influence of insulin on attentional-cognitive functions (Kern et al., 2001). Another study examined the impact of different blood insulin levels using the hyperinsulinemic clamp in Alzheimer patients and

aged-matched controls. Patients homozygotic for the APOE4 genotype and healthy controls had better recall at the low insulin levels (150 pmol/L), whereas patients with no APOE4 alleles had better recall at higher insulin levels (219 pmol/L) (Craft et al., 2003), suggesting a dose-response curve specific for each population.

In an FDG-PET study using the euglycemic clamp, the effect of insulin together with a glucose infusion produced an increase in cerebral glucose uptake (Bingham et al., 2002). In this study, somatostatin was given to suppress endogenous insulin secretion, and intravenous glucose was used to keep blood glucose levels at 5 mmol/L. Little glucose was infused in the control condition, but an average of 292.45 mg/kg of glucose was infused to keep glucose levels stable during the insulin infusion. As a result, the study actually demonstrated the combined effect of exogenous insulin and glucose on cerebral glucose uptake. This effect may be exerted either through a metabolic effect of insulin on brain glucose uptake or through a neuronal activation produced by the combined glucose-insulin infusion.

Another approach that has been taken to introduce insulin into the brain is intranasal administration. Experimental studies using mice (Gizurarson et al., 1995), rats (Thorne, et al., 2004) or monkeys (Anand et al., 1974. Gopinath et al., 1978) showed that peptides delivered into the nasal cavity via, or close to, the olfactory epithelium are transported into the brain within 10 to 15 minutes. Various mechanisms are thought to allow peptides to enter the brain through the intranasal route, although the precise mechanisms have not been elucidated. The mechanism most likely to provide rapid entry into the brain involves an extraneuronal pathway through perineural channels that deliver peptides to the parenchyma and/or the CSF (Frey, 2002).

Another possible route is along the trigeminal neural pathway (Thorne et al., 2004). A detailed analysis of the transfer of IGF-I from the nose to the brain showed significant IGF-I concentrations in most brain regions after 30 min, with olfactory structures having the highest concentration (Thorne et al., 2004). Detectable IGF-I concentrations were found in the hippocampus, midbrain, cerebellum, medulla, and cervical spinal cord, showing a wide distribution in the brain 30 min after intranasal administration. A double-blind crossover study looking at the safety of intranasal insulin revealed no adverse effects after three weeks of daily 60IU intranasal insulin administration (Kupila et al., 2003). More-over, no effect on blood glucose levels was found, indicating that intranasal insulin does not induce hypoglycemia.

A study examined the effect of 20IU of insulin administered in the nasal cavities every 15 min for 2.5 h in adult male volunteers of normal weight (Kern et al., 1999). At 30 min and 2 h, an EEG was recorded to assess auditory-evoked brain potentials. Baseline-to-peak amplitudes of the N1 (but not P2) component of auditory-evoked brain potentials were reduced during insulin infusions for the central (Cz) and parietal (Pz) locations. The amplitude of the P3 component was also reduced during insulin infusion at the frontal, parietal, and central location with the frontal location showing the greatest amplitude changes, whereas the central and parietal locations were reduced only within 500 ms of the stimulus presentation. The results of these studies are similar to those obtained previously by the same groups using the hyperinsulinemic/euglycemic clamp, for which insulin levels are kept elevated while glucose levels are kept at fasting levels through intravenous glucose infusion (Kern et al., 1998) or during insulin-induced hypoglycemia (Kern et al., 1990. Tamburrano et al., 1988).

The results of these studies suggest that insulin itself is the cause of the observed neuroelectric changes. Another study used the hyperinsulinemic/euglycemic clamp to study the effect of low and high levels of blood insulin on evoked brain potentials, as well as on neuropsychological performance (Kern et al., 2001). The results

revealed no difference between the N1 and P2 components elicited during the presentation of frequent tones under low and high insulin levels. An increase in P3 latency at the frontal and parietal placement was observed but, contrary to the intranasal study, no clear baseline-to-peak P3 amplitude increase. There was an increase in slow wave in the frontal regions during the high insulin condition and a small increase in the number of emotional and food-related words recalled toward the end of the 6 h of testing, which was concurrent with an increase of correct responses during the interference condition of the Stroop test and increases in the feeling of hunger.

Lastly, an experiment examined the effect of 8 weeks of intranasal insulin on cognitive performance and mood (Benedict et al., 2004). A 40IU insulin dose was infused into the nose 4 times a day. The participants were first tested during the placebo phase of the trial, after the infusion of the first insulin solution, and 1 week before the end of the trial. No difference was found between the per-formance of the insulin and saline groups for memory, except for delayed recall of words tested 1 week after the last testing session. Furthermore, no effect of insulin on the Stroop test or on word stem completion was found. There were, however, increases in the self-rating mood scores for self-confidence and well-being , as well as a reduced self-rating of depression after receiving insulin for 8 weeks (Benedict et al., 2004). Finally, one recent trial showed that intranasal insulin (20 IU) im-proved declarative memory in Alzheimer's patients and, to a lesser degree, in older adults (Baker et al., 2003).

In summary, studies that examined the effect of the hyperinsulinic clamp on cognitive function found a small but consistent improvement in memory and changes in brain neuroelectric parameters in evoked brain potentials, consistent with improved attention or memory processing. One PET study using the hyperinsulinemic/euglycemic clamp showed that brain glucose uptake increased during that procedure, suggesting that this increase may underlie the effect of the clamp procedure on brain functions. Studies using intra-nasal insulin reported cognitive improvements similar to those described with the hyperinsulin-emic clamp, suggesting that the effects of this procedure are likely due to increased brain insulin levels.

The final sections of this review deals with the possible interaction between the brain's insulin and IGF-I signaling pathways, as well as their possible role in brain aging and Alzheimer's disease. The literature on insulin growth factors and their roles in development and brain maturing is extensive and will not be reviewed here except for a brief presentation of key facts. The reader is referred to the comprehensive review by Juul (2003) for an exhaustive overview of IGF-I roles, physiology, and biochemistry.

INSULIN AND INSULIN GROWTH FACTOR-1

Insulin, insulin growth factor 1 (IGF-I) and insulin growth factor 2 (IGF-II) are ubiquitous molecules that have important roles in maintaining homeostasis, in postnatal growth, and in development (Juul, 2003). Insulin, IGF-I, and IGF-II each has its own receptor but cross-interact with low affinity with each others' receptors. In blood, insulin is found in picomolar concentrations whereas IGF-I is found in nanomolar concentrations. In theory, this would lead to a much higher impact of IGF-I on all three receptors if not for the finding that much of the IGF is bound to IGF binding proteins (IGFBP-3) in the blood, which blocks its biological activity (Baxter et al., 1998). Both IGF-I and IGFBP-3 decrease monotonically after puberty (Juul et al., 1994).

Insulin and IGF-I are genetically related polypeptides that share substantial amino acid sequences. Although insulin is synthesized in the pancreas, IGF-I is produced by the liver, and its expression is enhanced by pituitary growth

hormone. IGF-I is also synthesized in the brain, where growth hormone does not influence its expression (Bondy & Cheng 2004). In rats, the expression of mRNA for IGF-I is maximal during development of the nervous system but remains expressed in the adult brain in many regions including the hippocampus, olfactory bulb, hypothalamic area, and cerebellum, as well as in epithelial and ependymal cells of the choroids plexus (Rotwein et al., 1990).

Overexpression of IGF-I in transgenic animals results in increased brain size and myelination (Carson et al., 1993), whereas IGF-I knockout animals have smaller brains, a smaller granule cell layer in the dentate gyrus, and lower numbers of oligodendrocytes and myelinated axons (Beck et al., 2004). The role of IGF-I is also illustrated by a recent report showing that when weaver mutant mice, who exhibit apoptosis of cerebellar granule neurons, are cross-bred with transgenic mice overexpressing IGF-I, an increase in surviving granule cells and a decrease in apoptotic cells reduced the impact of the weaver mutation (Zhong et al., 2005). IGF-I transgenic animals were also shown to have greater number of synapses during and after post-natal development (O'Kusky et al., 2000. 2003).

Another series of experiments ex-amined the effect of IGF-I on cell differentiation in multipotent adult neural progenitor cells and found that IGF-I played an instructive role by shifting the differentiation process from the production of astrocytes to oligodendrocytes and, to a lesser extent, neurons (Hsieh et al., 2004). This effect was thought to be dependent on the indirect inhibition of the bone morphogenetic protein (BMP) signaling by the upregulation of BMP antagonists Noggin, Smad6, and Smad7.

Although much remains to be done in elucidating the role of IGF-I using transgenic animals, the general view is that IGF-I controls and/or enables the growth of new brain cells including neurons and glia, reduces developmental apoptosis, and increases synaptogenesis during early post-natal development (D'Ercole et al., 2002).

A number of observations have led researchers to study the role of IGF-I in aging and dementia. In a small study, it was found that IGF-I blood levels are correlated with MMSE performance in older persons (65 years and over), with lower levels of IGF-I being associated with lower performance in the MMSE (Rollero et al., 1998). Plasma insulin-like growth factor I (IGF-I) level was reduced in family members carrying the Swedish amyloid precursor protein (APP) 670/671 mutation with Alzheimer's disease (AD) compared with age-matched controls from the same family carrier without AD (Mustafa et al., 1999).

Also, IDDM and severe insulin resistance may cause acquired IGF-I resistance through a decrease in IGF-I availability or altered second messenger systems (Jain et al., 1998). Studies in patients with diabetes have shown that in insulin-deficient states, serum IGF-I concentrations are low and increase with insulin therapy (Bereket et al., 1996). Although total IGF-I has been found to be within normal values in type 2 diabetes, free IGF-I remains elevated in type 2 diabetic patients to a degree similar to that observed in obese individuals without diabetes (Frystyk et al., 1999). In normal subjects, low IGF-I is associated with high 2-h glucose concentrations during a glucose tolerance test (Sandhu et al., 2002), indicating that IGF-I has a role in peripheral uptake of glucose.

Studies conducted in experimental animals have shown that if IGF-I synthesis by the liver is stopped, the animals become insulin-resistant, and this situation is improved when IGF-I is administered. Likewise, deletion of the IGF-I receptor in muscle tissue in mice induces severe insulin resistance. The administration of IGF-I to patients with type 2 diabetes mellitus has been shown to result in an improvement in insulin sensitivity and a reduction in the requirement for exogenously administered insulin to maintain glucose homeostasis (Clemmons, 2004).

In summary, IGF-I is involved in brain development and maturation. Peripheral levels are associated with peripheral glucose regulation and interact with insulin mechanisms that control glucose disposal. In the next section, we briefly review the results of studies that have examined the impact of insulin and IGF-I on amyloid production and disposal in animal models and in patients with Alzheimer's disease.

INSULIN AND IGF-I AND AMYLOID ACCUMULATION

Beta-amyloid (Aβ) is a potentially deleterious compound when it accumulates in the aging brain and is associated with Alzheimer's disease (Selkoe, 2001). During aging, IGF-I levels decline, with a decreased sensitivity to insulin and IGF-I as well (Jain et al., 1998). Both hormones are presumed to help reduce beta-amyloid toxicity by increasing its cellular release (Gasparini et al., 2001), stimulating tissue clearance (Carro et al., 2002), and protecting from its toxic effects (Niikura et al., 2001).

Previous experiments had shown an increased uptake of insulin at the blood brain barrier of the Tg2576 (double transgenic APP and PS1) mouse (Poduslo et al., 2001) and when fed a high-cholesterol diet for 7 weeks, an increased amyloid load and deposit size in the Tg2576 mouse (Refolo et al., 2000). In a more recent experiment, Tg2576 mice were given access to a 60% fat diet and it was found that the diet induced insulin resistance, higher weight, and higher evoked blood glucose levels, but no change in total cholesterol occurred compared with standard-chow-fed animals (Ho et al., 2004).

However, a 5-month exposure to the diet doubled the amount of Aβ-40 and Aβ42 and also increased the number and volume of 6E10-immunopositive amyloid plaques. The high-fat diet also reduced markers of insulin receptor activity (PI3K, AKT/PKB, and Glycogen synthase kinase-3α [GSK-3α]), consistent with insulin resistance and reduced performance in the water maze. Finally, the high-fat diet also reduced IDE expression and activity.

On the other hand, the inhibition of GSK-3α by lithium chloride has been shown to decrease the production of amyloid peptides in animal models of Alzheimer's disease (Phiel et al., 2003), which would suggest that higher insulin receptor activity leads to higher amyloid production. In cell cultures, it was shown that insulin decreases intracellular levels of Aβ40 and Aβ42, which appeared to be mediated by an accelerated transfer from the Golgi/transGolgi net-work to the plasma membrane (Gasparini et al., 2001).

There is now good evidence that insulin-degrading enzyme (IDE) is one of the proteins that degrades beta-amyloid in vivo and in vitro and is selective for the Aβ monomer (Farris et al., 2003. Vekrellis et al., 2000). Additionally, a number of linkage studies have identified chromosome 10q as a site of a number of markers for increased plasma levels of beta amyloid that are close to the IDE site (Bertram et al., 2000. Ertekin-Taner et al., 2000, 2004. Prince et al., 2003), although a number of genetic studies concluded that IDE was not associated with late-onset Alzheimer's disease (Abraham et al., 2001. Boussaha et al., 2002).

Two recent studies suggested that IDE variant expres-sion may affect Alzheimer's severity rather than risk (Blomqvist et al., 2005), and there may be inefficient forms of IDE that foster poor amyloid processing (Farris et al., 2005). Some post-mortem studies have also indicated that hippocampal IDE levels and mRNA were decreased in AD brain tissue from patients with the APOE4 gene (Cook et al., 2003).

Using the Brain Efflux Index, a study showed that Aβ (1-40) elimination from the brain was reduced by 30% in old rats, whereas inhibitors of neprilysin and IDE (the two main amyloid-degrading enzymes) reduced Aβ efflux by 25% to 30% (Shiiki et al., 2004). Neprilysin knockout

mice have a two-fold increase in Aβ (1-40) and Aβ (1-42) in the brain (Iwata et al., 2000), but a 34% inhibition of Aβ-elimination rate by thiorphan, a neprilysin inhibitor, suggests that neprilysin action is non-saturable or that Aβ fragments inhibit their efflux transport (Shiiki et al., 2004). In the same study, the co-administration of insulin with Aβ (1-40) reduced the elimination rate of Aβ (1-40) and increased the level of intact Aβ (1-40) in the brain (Shiiki et al., 2004). The results of that study parallel those obtained in humans, in which systemic insulin infusions led to an increase in CSF Aβ (1-42) (Watson et al., 2003). One possibility is that insulin competes with Aβ (1-40) (Xie et al., 2002) for transcytosis transport using the insulin receptor at the BBB (Pardridge et al., 1995). However, an inhibitor of the insulin receptor kinase failed to affect Aβ elimination rate (Shiiki et al., 2004).

Another experiment using hippocampal cell cultures showed that treatment with insulin produced an increase in IDE-protein levels, which was accompanied by an increase in phosphadylinositol-3 (PI-3) (Zhao et al., 2004). The inhibition of PI-3 abolished the upregulation of IDE by insulin, suggesting that the effect of insulin on IDE may be mediated through an activation of the insulin receptor. The authors also showed that in Tg2576 mice raised on a safflower oil-based diet, a reduction in IDE mRNA and protein expression correlated with the level of beta-amyloid in the hippocampus.

Finally, a post-mortem study showed reduced IDE levels in the hippocampus and temporal cortex of Alzheimer's patients, and the highest reductions were found in patients with two copies of the ApoE4 allele (Zhao et al., 2004). Additionally, levels of IDE and neprilysin were found to be lower in the human hippocampus and cortex compared with levels in the cerebellum, a structure having fewer amyloid deposits in AD (Caccamo et al., 2005). The same study also found that 75% of IDE was oxidized in the AD hippocampus compared with 25% in the cerebellum, suggesting the possibility that the enzymatic activity of oxidized IDE may be reduced, leading to increased amyloid deposition (Caccamo et al., 2005). This result is consistent with the observation that in transgenic mice overexpressing APP, Aβ-amyloid deposition increases in a progressive, age-related fashion, despite the presence of high APP levels throughout their lifetime. Taken together, the results of these studies support the hypothesis that amyloid accumulation in the aging brain is due to deficits in degradation rather than to an increase in amyloid production, and that brain insulin, insulin receptor activity, or the insulin degrading enzyme play a role in the clearance of Aβ amyloid.

GENERAL CONCLUSIONS

Brain insulin, most of which comes from the pancreas, is taken up by the brain by what appears to be a receptor-based carrier. Insulin is transported at various rates through the blood-brain barrier to different brain regions.

Type 2 diabetes animal models associated with insulin resistance show reduced insulin brain uptake and content. Some recent data point to changes in the insulin receptor cascade in obesity-related insulin resistance, which is consistent with a reduced sensitivity of insulin receptors. Such changes are accompanied by reductions in synaptic plasticity. The absence of insulin receptors in the brain of conditional knockout mice does not reveal any major deficit in learning and memory, suggesting that insulin and insulin receptors have modulatory roles, or that compensatory mechanisms exist.

Insulin transport to the brain is reduced in aging and in some animal models of type 2 diabetes. Because brain- insulin uptake is receptor based, insulin resistance would thus lead to a reduction in both insulin uptake and the ability of

insulin to stimulate its receptors in the brain.

Studies examining the effect of the hyperinsulinic clamp or intranasal insulin on cognitive function have found a small but consistent improvement in memory and changes in brain neuroelectric parameters in evoked brain potentials. These changes are consistent with improved attention or memory processing. These effects appear to be due to raised brain insulin levels. The results also suggest that a reduction in brain insulin or brain insulin sensitivity could be the cause of some of the cognitive deficits observed in persons with impaired glucose tolerance or type 2 diabetes.

Insulin growth factor-I is involved in brain development and maturation. Peripheral levels are associated with peripheral glucose regulation and interact with insulin mechanisms that control glucose disposal. There is some indication that decreased sensitivity to insulin or to IGF-I as observed in aging, obesity, and diabetes decreases the clearance of Aβ amyloid through processes that appear to involve the insulin receptor. However, the exact sequence of cellular events for these processes remains to be elucidated. Insulin and IGF-I also appear to modulate the brain levels of insulin degrading enzyme. This enzyme has been shown to be important in the degradation of insulin and a reduction of its activity would lead to accumulation of Aβ amyloid. This hypothesis is consistent with the observation that amyloid deposition is increased both in transgenic animal models of Alzheimer's disease that are diabetic and in diabetic patients suffering from Alzheimer's disease.

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