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Broadening the definition of brain insulin resistance in aging and Alzheimer's disease

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ABSTRACT

It has been > 20 years since studies first revealed that the brain is insulin sensitive, highlighted by the expression of insulin receptors in neurons and glia, the presence of circulating brain insulin, and even localized insulin production. Following these discoveries, evidence of decreased brain insulin receptor number and function was reported in both clinical samples and animal models of aging and Alzheimer's disease, setting the stage for the hypothesis that neuronal insulin resistance may underlie memory loss in these conditions. The development of therapeutic insulin delivery to the brain using intranasal insulin administration has been shown to improve aspects of memory or learning in both humans and animal models. However, whether this approach functions by compensating for poorly signaling insulin receptors, for reduced insulin levels in the brain, or for reduced trafficking of insulin into the brain remains unclear. Direct measures of insulin's impact on cellular physiology and metabolism in the brain have been sparse in models of Alzheimer's disease, and even fewer studies have analyzed these processes in the aged brain. Nevertheless, recent evidence supports the role of brain insulin as a mediator of glucose metabolism through several means, including altering glucose transporters. Here, we provide a review of contemporary literature on brain insulin resistance, highlight the rationale for improving memory function using intranasal insulin, and describe initial results from experiments using a molecular approach to more directly measure the impact of insulin receptor activation and signaling on glucose uptake in neurons.

1. Introduction

Despite significant knowledge of neuronal energy expenditure in the cortex, including estimates of the metabolic cost for eliciting single action potentials and excitatory post-synaptic potentials, maintaining resting membrane potential, and buffering calcium transients in spines (Attwell and Laughlin, 2001; Howarth et al., 2012), the sensitivity of these dynamic signaling processes to insulin remains unclear. To date, it is still common to read that the brain is insulin independent despite evidence to the contrary. Indeed, many studies support the notion that insulin-dependent processes are present and, in some cases, able to influence memory or recall (Banks et al., 2012; Benedict et al., 2004; Bruning et al., 2000; de la Monte et al., 2006; Dou et al., 2005; Grillo et al., 2009; Kamal et al., 2013; Lannert and Hoyer, 1998; McNay et al., 2010; Pancani et al., 2013; Salameh et al., 2015; Stanley et al., 2016; Wickelgren, 1998; Woods et al., 1979; Zhao et al., 1999). Recently, therapeutic intranasal insulin (INI) administration has been successfully employed to ameliorate several cognitive functions (declarative memory [short-term or delayed], verbal fluency, attention, functional status, spatial memory, and several others). It is not clear, however, whether this approach functions by compensating for poorly signaling insulin receptors (IRs), for reduced insulin levels in the brain, or for reduced trafficking of insulin into the brain. Potential pathways mediating the benefits of INI include increases in cerebral blood flow (CBF),

Abbreviations: AD, Alzheimer's disease; IR, Insulin receptor; GLUT, glucose transporter; INI, intranasal insulin; MCI, mild cognitive impairment; T2DM, Type-2 diabetes mellitus; AUC, area-under-the-curve; GTT, glucose tolerance test; IRS-1, insulin receptor substrate-1; CNS, central nervous system; APP, amyloid precursor protein; PS1, presenilin 1; AHP, Afterhyperpolarization; CBF, cerebral blood flow; ICV, intracerebroventricular; BBB, blood-brain barrier; ApoE, apolipoprotein E; Aβ, amyloid beta; 2-NBDG, 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxyglucose; TBI, traumatic brain injury; FDG-PET, 2-[18F]fluoro-2-deoxy-D-glucose positron emission tomography

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reductions in inflammation, and elevations in glucose metabolism. Together, the current body of literature supports the theory of an insulin-sensitive brain and highlights the physiological importance of maintained insulin signaling, yet the exact mechanisms involved in this process appear to be highly complex and variable.

In aging and Alzheimer's disease (AD), conditions where both glucose utilization and memory recall are known to decline, it is tempting to infer causality between these two processes. However, it is only recently that region-specific brain glucose concentrations were measured to test for associations between glucose metabolism and severity of AD pathology (An et al., 2018). Interestingly, this study found that reductions in glucose metabolism were associated with elevated glucose levels and reduced numbers of glucose transporter (GLUT) type 3 in the brains of AD patients compared to aged-match controls. On initial assessment, elevated brain glucose levels together with cognitive decline may appear contradictory; however, it is possible that the increased glucose in the brains of AD patients may reflect reduced glucose utilization through either dysfunctional IR (Frolich et al., 1998), reduced GLUTs at the cell membrane (An et al., 2018), or diminished insulin levels in the central nervous system (CNS) (Craft et al., 1998; Frolich et al., 1998; Molina et al., 2002), all of which may lead to reduced insulin sensitivity.

Here, we review the current evidence supporting the theory that the brain is insulin-sensitive and address potential mechanisms underlying the age- and AD-related decrease in insulin and IR signaling, a phenomenon often referred to as brain insulin resistance. Additionally, we discuss cellular components that may participate in this process. Finally, we introduce a molecular technique designed to directly investigate the relationship between IR activity and glucose metabolism in neurons and present preliminary data supporting this as a viable technique.

2. What elements and processes inform insulin resistance in the brain?

While it is becoming clear that insulin and IR signaling play an important role in overall brain health and contribute to numerous physiological functions, the question as to how insulin and IR dysfunction lead to reductions in memory remains unanswered. A PubMed query with the terms “insulin resistance,” “brain,” and either “aged” or “Alzheimer's disease” shows a steady increase in articles published between the years 1998 and 2018, highlighting the growing scientific interest in this topic (Fig. 1). One relevant concept based on a broad phenotype characterized by reduced brain insulin levels, IR signaling, and activity of insulin-related processes, is the brain insulin resistance hypothesis (Hoyer, 1998). This hypothesis was formulated following observations of insulin resistance in AD (Frolich et al., 1998). This work showed that compared to age-matched control brains, AD brains displayed region-specific reductions in insulin concentrations together with elevations in IR densities (Frolich et al., 1998). While the elevation in IR density was surprising, this result has since been interpreted as a failed compensation, given the evidence of reduced brain glucose utilization in AD (An et al., 2018; Hoyer, 1986, 2004; Mielke et al., 1992; Mosconi et al., 2008a; Mosconi et al., 2004; Rivera et al., 2005). Together, however, these initial observations, along with critical work from other labs, secured insulin resistance as a potential therapeutic target in the brains of mild cognitive impairment (MCI), AD, and perhaps even Type-2 diabetes mellitus (T2DM) patients.

Evidence for insulin resistance in the brain has been mostly derived from data using molecular approaches to quantify levels of accessory proteins in the insulin signaling pathway, from IR itself to targets further downstream in the signaling pathway, including insulin receptor substrate-1 (IRS-1) and the resulting transport of GLUTs to the plasma membrane (Barone et al., 2018; Bell and Fadool, 2017; Benedict and Grillo, 2018; Biessels and Reagan, 2015; Bomfim et al., 2012; De Felice et al., 2014; de la Monte and Wands, 2008; Grillo et al., 2015; Mao et al., 2016; Pearson-Leary and McNay, 2012; Schubert et al., 2004; Talbot et al., 2012; van der Heide et al., 2006; Willette et al., 2013; Zhao et al., 2008). However, these links are almost always based on associations, as it is difficult to directly address mechanisms and causality using CNS exposure to the ligand in clinical samples. Further, the nature of these techniques often limits experiments to transient signaling snapshots, thus preventing investigations of the physiological impact of insulin across time. Other approaches that do not introduce the ligand to the brain, but instead use molecular techniques to knockout the IR (Bruning et al., 2000; Schubert et al., 2004) or introduce an IR antisense sequence (LV-IRAS) via a lentiviral delivery system (Grillo et al., 2015), have been very informative in defining the role of IR and downstream signaling in the CNS. Similarly, introducing an IR binding peptide (i.e., affibody) has been employed to reduce insulin actions at the IR (Luckett et al., 2013; Maimaiti et al., 2017; Paranjape et al., 2010). Yet, these loss-of-function approaches only report on the impact of reducing IR activity in the brain by attempting to mimic conditions of reduced brain insulin levels or IR function, as seen in aging or AD.

Furthermore, a large body of evidence supports the hypothesis that peripheral metabolic dysregulation contributes to cognitive decline in T2DM and obesity, suggesting the presence of parallel mechanisms between neuronal and chronic peripheral insulin resistance (Arnold et al., 2018; Benedict and Grillo, 2018; Biessels and Reagan, 2015; Cholerton et al., 2011; Craft, 2005; De Felice, 2013; de la Monte, 2014; Fadel and Reagan, 2016; Gispen and Biessels, 2000; Luchsinger, 2008; Luchsinger et al., 2004). With respect to peripheral insulin levels and their potential impact on cognitive function, well-powered studies have provided evidence that elevated peripheral insulin levels are associated with poorer cognitive function in elderly patients with or without hypertension or diabetes, perhaps even more so in females than males (Kuusisto et al., 1993; Stolk et al., 1997). Indeed, higher *chronic* insulin levels, peripheral insulin resistance, and a pre-diabetic state (e.g., greater insulin area-under-the-curve (AUC) in response to an acute intravenous glucose tolerance test (GTT)) are associated with greater decline in performance over a 2-year period in healthy elderly subjects (Burns et al., 2012). Interestingly, the same group also showed that in AD patients, increased insulin AUC during GTT was associated with less brain atrophy and lower dementia severity across a similar time period (Burns et al., 2007; Burns et al., 2012). These later results align well with early work from Craft and others showing that *acute* elevations in peripheral insulin levels along with maintained euglycemia enhances memory recall in AD patients (Craft et al., 1996; Craft and Watson, 2004; Kern et al., 1999; Long et al., 1992). It remains to be determined whether the duration of elevated peripheral insulin is important in the development of brain insulin resistance. While work from several labs has demonstrated a form of reduced brain insulin sensitivity, peripheral insulin resistance status was not always provided; thus, it is difficult to directly test for associations between the periphery and the brain in these samples (Moloney et al., 2010; Steen et al., 2005; Talbot et al., 2012; Tramutola et al., 2017). Nevertheless, initial assessment suggests that elevations in peripheral insulin, whether chronic or acute, might be beneficial in individuals with AD.

More investigations are needed to determine if, and/or how closely, central insulin resistance and associated molecular processes mirror peripheral elements of insulin resistance in muscle or fat cells. However, at least one study using magnetoencephalography has characterized insulin-mediated changes in cortical activity in lean and obese subjects and shows reductions in obese humans, likely through alterations in IRS-1 signaling (Tschritter et al., 2006). Interestingly, these authors also conclude that transport of insulin across the blood-brain barrier (BBB) could have an impact on cortical insulin sensitivity. Several other labs have shown that chronic hyperinsulinemia or insulin resistance may reduce insulin transport into the CNS (reviewed in Banks et al., 2012; Baura et al., 1996; reviewed in Craft, 2005; Heni et al., 2014; Israel et al., 1993; Schwartz et al., 1990), and have even

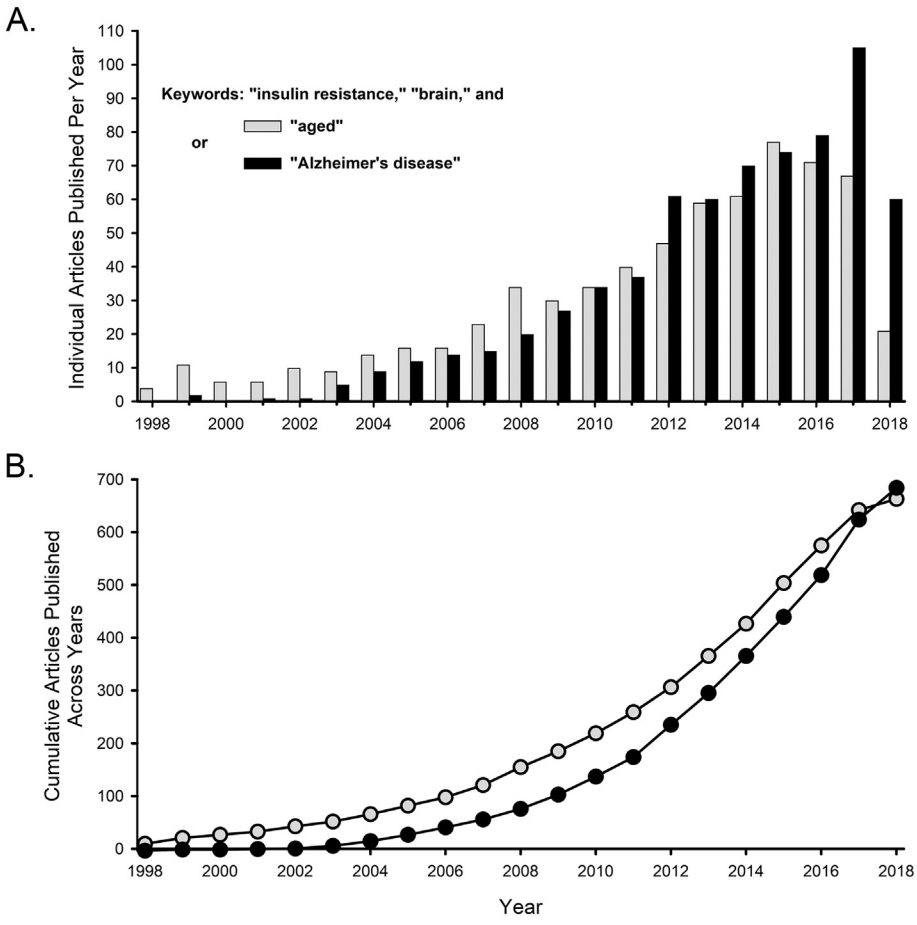

Fig. 1. Number of articles containing the keywords "insulin resistance," "brain," and either "aged" or "Alzheimer's disease" between the years 1998 and 2018. Number of articles were determined by querying the PubMed online database via the EndNote search engine (EndNote™; X8.2, build 11343). A. Number of individual articles published per year. B. Number of cumulative articles in the literature between 1998 and 2018.

raised the concept of cell-selective insulin resistance in the brain (Vogt and Bruning, 2013). Together, these results suggest that brain insulin resistance can take place through mechanisms that may depend on insulin transport, insulin levels, IRS-1 pathway sensitivity, and other yet unknown processes.

3. Evidence for preserved insulin sensitivity in the aged and AD brain

While the bulk of the literature supports the hypothesis that overall brain insulin signaling is reduced in aging, AD and T2DM, work from several labs has provided evidence that insulin *sensitivity* may actually be preserved. An early study measuring insulin binding across multiple brain regions in aged rats found no reduction in specific binding in the frontal cortex, hippocampus, or hypothalamus compared to young adult animals (Tchilian et al., 1990). A more recent report showed that in both younger and older APP/PS1 mice, insulin could still signal normally, provided it was delivered directly to the brain (Stanley et al., 2016). Using electrophysiological analyses of calcium-dependent processes, our lab showed that neurons of mid-age and aged rats responded robustly to exogeneous insulin following direct application of the ligand to hippocampal slices (Fig. 2 and Fig. 3). Of note, the insulin response, which reduces the calcium-dependent afterhyperpolarization (AHP), appears greater in aged compared to young animals (Maimaiti et al., 2016; Pancani et al., 2013). Our group has also reported that measures of CBF in aged animals respond more significantly to direct administration of exogenous insulin in the brain compared to younger animals (Anderson et al., 2017). Together, these results suggest that a deficiency in insulin availability might govern some parts of brain insulin resistance.

Indeed, transport of insulin across the BBB has been implicated as a potential contributor to CNS insulin resistance. In a recent study, measures of brain IR signaling in response to subcutaneous insulin administration showed reductions in aged mice; yet, in these same animals, a robust response was detected provided insulin was delivered directly to the brain (Sartorius et al., 2015). Clinical studies corroborate these findings, with the same group of investigators also showing

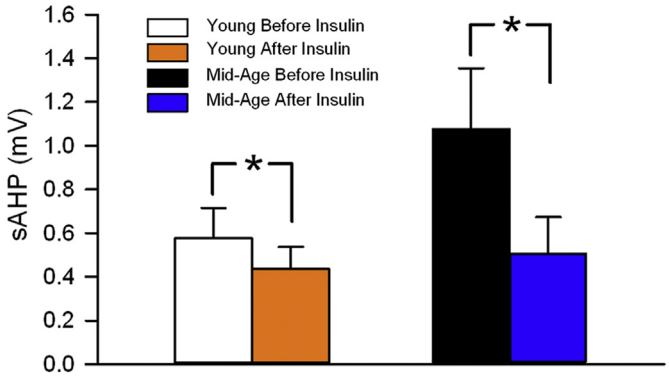

Fig. 2. Insulin's acute effect on the slow afterhyperpolarization (sAHP) in young and mid-aged F344 male rats. A. Comparison of insulin-mediated sAHP reduction between hippocampal slices obtained from young (2.5–4 mo) and mid-aged (12.5–13.5 mo) animals following 15 min perfusion of Humalog® (500 nM). A significant reduction in the sAHP was observed in both groups of animals ($F(1,10) = 7.1$; $p < .05$). Reduction in the sAHP of mid-aged animals suggests that insulin-sensitivity in the hippocampus is preserved, not diminished, in early aging. * indicates $p < .05$. All data represent means +/− SEM. *Reprinted from Neurobiology of Aging, Vol 34(8), Pancani,* et al., *"Effect of high fat diet on metabolic indices, cognition and neuronal physiology in aging F344 rats," pg. 1977–1987, Copyright (2013), with permission from Elsevier.*

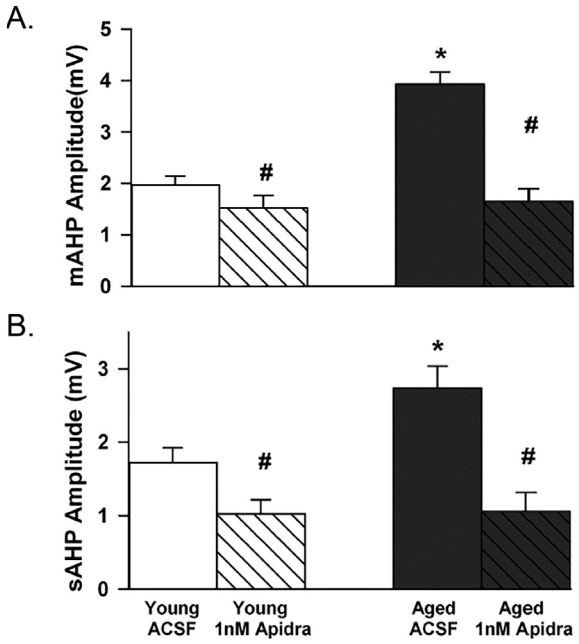

Fig. 3. Effect of a zinc-free insulin formulation (Apidra®) on the AHP ex vivo. A. Measures of the medium AHP (mAHP) in hippocampal slices obtained from young (3 mo) and aged (21 mo) F344 male rats following administration of 1 nM Apidra®. Apidra® significantly reduced the mAHP in slices from both young ($n = 8$) and aged ($n = 9$) animals, indicated by a main effect of treatment ($F(1,24) = 32$, $p < .0001$). B. Measures of the sAHP in hippocampal slices obtained from young and aged F344 male rats following administration of 1 nM Apidra®. As with the mAHP, Apidra® significantly reduced the sAHP in both young and aged animals ($F(1,24) = 29.1$, $p < .0001$). This effect was larger in aged animals compared to young, evidenced by a significant interaction term following 2-way ANOVA ([$F(1,24) = 10.9$, $p < .005$ for the mAHP). This suggests a preservation of insulin-sensitivity in aged animals. # indicates $p < .10$; * indicates $p < .05$. All data represent means +/− SEM. *Reprinted from: Maimaiti,* et al., *"Intranasal Insulin Improves Age-Related Cognitive Deficits and Reverses Electrophysiological Correlates of Brain Aging," Journal of Gerontology, 2016, Vol. 71, No. 1, 30–39, by permission of Oxford University Press.*

decreased insulin transport in the brains of human subjects with impaired peripheral glucose tolerance (Heni et al., 2014). This series of studies suggests insulin resistance in aging or AD may be mediated by mechanisms independent of decreased IR sensitivity. Indeed, previous work from the Banks lab has proposed that entry of insulin into the brain is a likely component of CNS insulin resistance associated with aging, AD, or T2DM (Banks et al., 2012). Note that a similar reduction in transport has also been seen in the context of leptin resistance in obese humans and rodents (Banks, 2001).

Irrespective of the mechanism mediating insulin resistance, the evidence for the reduction in insulin transport suggests the aged brain is starved of insulin. Additionally, these results clearly point to the importance of ligand transport into the CNS, and also bolster the rationale for the work of Craft and others, who demonstrated that INI administration may overcome the chronic hyperinsulinemia-induced down-regulation of insulin transport at the BBB by raising the ligand concentration in the brain (reviewed in Banks et al., 2012; Baura et al., 1996; reviewed in Craft, 2005; Heni et al., 2014; Israel et al., 1993; Schwartz et al., 1990). It is likely that brain insulin resistance manifests through a combination of altered pathways, from IR signaling to the presence and transport of the ligand into the brain, and it is therefore not surprising that bypassing these reduced elements is a therapeutically relevant and often beneficial approach to offset memory decline in aging and AD.

4. Combating brain insulin resistance using INI

Early work using intracerebroventricular (ICV) insulin administration in baboons, dogs, rats, and mice framed a series of experiments to introduce the ligand into the brain. Since then, alternative approaches, including INI delivery, have continued to be investigated in different animal models and have moved the field forward with much success in clinical settings. Indeed, initial work (Benedict et al., 2004; Kern et al., 1999; Reger et al., 2006) provided some evidence that INI could alter cerebral function, and in some cases, improve word recall in younger male subjects or early apolipoprotein E (ApoE)-ε4 positive AD patients. Since then, a significant number of studies have corroborated these findings (Benedict and Grillo, 2018; Chapman et al., 2018; de la Monte, 2013; Freiherr et al., 2013; Hughes and Craft, 2016) and highlighted potential mechanisms governing these beneficial effects, including alterations in CBF flow (Akintola et al., 2017; Kullmann et al., 2017) and circulating β-amyloid (Reger et al., 2008), as well as changes in peripheral corticosteroid concentrations (Thienel et al., 2017).

To date, the effects of INI have only been investigated in relatively small, albeit numerous, trials and for limited periods of time. Only one study has reported that INI could rapidly raise insulin concentrations in the brain (Born et al., 2002). This study was conducted in healthy adults and similar measures have not yet been obtained in cognitively impaired individuals. Nevertheless, as these trials continue, notable sex differences are beginning to emerge, further highlighting the complexity of this therapeutic approach (Benedict et al., 2008; Claxton et al., 2013; Hallschmid et al., 2004; Kullmann et al., 2013). With respect to the impact on feeding behavior, women appeared to gain more weight during an 8-week trial compared to men (Hallschmid et al., 2004). This result was corroborated in a later study (Benedict et al., 2008). In another report, this time focusing on short-term memory function in AD patients, sex differences were only apparent in ApoE negative individuals (Claxton et al., 2013). Furthermore, the authors provided evidence that the beneficial effects of INI were dependent on at least three factors, including sex, ApoE genotype, and dose. Finally, it is important to consider the potential impact of the different formulations of insulin, the duration of treatment, or the regimen of the interventions (reviewed in Arnold et al., 2018).

With respect to the use of INI in animal models of AD, Banks and colleagues have shown that the presence of the ligand in the brain following coadministration of INI with serum albumin is independent of age and phenotype in the SAMP8 mouse model of AD, but did not investigate downstream consequences of this application (Rhea et al., 2017). A 2014 study in female 3xTG mice exposed to INI for 7 days showed reductions in amyloid beta (Aβ) levels, restoration of insulin signaling, and enhancement of synaptic proteins (synapsin-1 and PSD-95) (Chen et al., 2014). A more recent report from the same group, this time conducted in female APPswe/PS1dE9 mice exposed to INI for 6 weeks, revealed a significant improvement in cognitive performance, neurogenesis, and insulin signaling, as well as reductions in Aβ production and amyloid plaque burden (Mao et al., 2016). The impact of INI in the 3xTg animal model again revealed an improvement in spatial memory recall, as well as increased insulin signaling (Barone et al., 2018). The authors also suggested potential mechanisms mediating this increase, specifically biliverdin reductase A enhancement, reductions in Tau phosphorylation, and increased serine phosphorylation of IRS-1.

In wildtype mice (C57Bl/6) fed a control diet, short-term INI exposure (5–7 days) was able to improve object recognition memory and olfactory discrimination while reducing anxiety and increasing the phosphorylation state of a potassium channel (Kv1.3). However, compared to older mice fed a control diet, older mice made prediabetic using a high fat diet failed to show increases in object memory following INI administration (Marks et al., 2009). This same group recently published evidence that longer INI treatment (~2 months) did not improve object recognition or olfactory memory in adult male mice. The authors attribute these differences to the onset of a brain insulin-

resistant state (Bell and Fadool, 2017). Similarly, we have also witnessed that long-term INI (~3 months) has minimal impact on spatial learning or memory performance in younger or older F344 rats (in preparation). In another study, we reported a positive impact of two formulations of insulin on spatial memory performance following intranasal delivery in aged F344 rats compared to young (Maimaiti et al., 2016). Furthermore, we have also recently shown that INI increases CBF (Anderson et al., 2017). The question still remains as to which cognitive processes and cellular functions are directly mediated by insulin signaling and targeted by therapeutic INI. One potential answer is likely to involve elements of glucose metabolism in the brain.

5. The impact of brain insulin on glucose utilization in humans

With respect to brain glucose metabolism in the context of insulin resistance, a significant number of advances have been made through measures of glucose utilization in vivo. 2-[18F]fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET) imaging has been used to infer rates of brain glucose metabolism in patients expressing a wide range of disease phenotypes, including prediabetes and T2DM, MCI, AD, non-AD dementia types, and cognitively normal, as well as across age. An FDG-PET study comparing young and aged individuals showed that aging in the absence of cognitive impairment is associated with reduced CNS aerobic glycolysis with no apparent change in glucose uptake (Goyal et al., 2017). It is important to note that in this study, age-related reductions in overall brain glucose metabolism are observed only when measures of glucose uptake are combined with oxygen utilization rates, suggesting that cellular glucose utilization and metabolism could be modulated by processes independent of uptake alone. In another FDG-PET study of 548 individuals displaying a spectrum of cognitive impairment, patterns of hypometabolism were seen across various brain regions compared to age-matched controls (Mosconi et al., 2008b). Additional work in AD patients has emphasized the potential use of FDG-PET to predict the progression of AD severity, further refine the initial clinical diagnosis of AD, and even identify individuals at risk for developing the disease (Mosconi, 2013; Swerdlow, 2007).

Using similar techniques, Craft and colleagues showed that aged, cognitively normal individuals with a higher level of peripheral insulin resistance (i.e. prediabetes or T2DM) had a marked reduction in cerebral glucose metabolic rate across several brain regions compared to age-matched controls under baseline conditions (Baker et al., 2011). However, in a study using a euglycemic-hyperinsulinemic clamp to induce a transient increase in peripheral insulin, this time performed in middle-age, cognitively intact individuals, those with impaired peripheral glucose tolerance showed increased, not decreased, brain glucose utilization during peripheral hyperinsulinemia (Hirvonen et al., 2011). As FDG-PET signals in the brains of subjects with impaired peripheral glucose tolerance were found to respond more robustly to the hyperinsulinemic condition compared to healthy controls, these results suggest that peripheral insulin resistance does not necessarily translate to reductions in IR sensitivity. While contradictory, the results presented by Baker and Hirvonen nevertheless suggest that peripheral insulin is capable of influencing central glucose utilization, albeit differently across age and phenotype. Finally, a small study of 8 healthy, middle-aged volunteers revealed that decreasing basal peripheral insulin levels significantly reduced FDG-PET measures of global brain glucose metabolism (Bingham et al., 2002). This same study also showed that a low-dose insulin infusion in the periphery restored both glucose uptake and rate of utilization in the brain.

Clearly, brain glucose metabolism has an important role in the development of cognitive impairment and is associated with CNS hypometabolism in several pathological conditions. Importantly, it is also highly sensitive to both exogenous and peripheral insulin. However, clinical studies are limited in their ability to characterize the cellular mechanisms targeted by the ligand. In the next section, we provide a review of techniques used in animal models to characterize the potential cellular targets, receptors, and pathways that mediate this process.

6. Insulin and glucose in animal models of aging and AD

Work performed in animal models of traumatic brain injury (TBI) and diabetes suggests a role of insulin in central glucose metabolism similar to that seen in the clinic. One manuscript reported a decrease in brain glucose metabolism measured by FDG-PET following TBI (Brabazon et al., 2017). In this same study, daily INI administration to adult male rats for 14 days post-TBI lead to increased glucose uptake in the hippocampus, reduced hippocampal lesion volume and microglial reactivity, and even significantly improved memory and motor function. A similar report recently highlighted INI-mediated increases in regional cerebral glucose metabolism in an ICV streptozotocin-induced model of T2DM (Chen et al., 2018). The processes underlying alterations in CNS glucose metabolism, as well as the physiological impact of insulin in the brain, depend, at least in part, on the availability of the ligands, the presence of functional receptors and their respective signaling cascades, and the transport of the ligands into the brain. Once fully characterized, these mechanisms may help provide a more complete definition of central insulin resistance. One such mechanism that has been studied extensively at the intersection of glucose metabolism and insulin resistance is the transport of glucose via GLUTs at the plasma membrane.

GLUT3 has long been established as the primary neuronal GLUT (Vannucci et al., 1997). Other GLUTs, such as GLUT1, GLUT2, and the insulin-sensitive GLUT4, have also been identified in various parts of the brain (Choeiri et al., 2002; Kobayashi et al., 1996; Leino et al., 1997; McEwen and Reagan, 2004; Pearson-Leary et al., 2017; Reagan, 2005). An early study found that in the rat brain, GLUT4 was primarily expressed in areas that also possess high levels of IR, suggesting a potential role of circulating brain insulin and IR signaling in the regulation of neuronal GLUT4 function (El Messari et al., 1998). This same study found that regions with the highest level of GLUT4 expression corresponded with areas primarily involved in motor function. However, while a more recent study corroborated these initial findings, these investigators also discovered novel locations for GLUT4-expression in different subfields of the hippocampus (Choeiri et al., 2002). As the hippocampus expresses high levels of IR (Zhao et al., 1999), the localization of GLUT4 in this structure further supports the hypothesis that insulin signaling mediates brain glucose metabolism, and that this relationship may play a role in cognitive function.

Evidence for a reduction in brain glucose utilization with age, together with evidence for decreased IR numbers or function in animal model of aging (Zaia and Piantanelli, 2000; Zhao et al., 2004), strengthens the notion that these altered processes may be somehow connected. It has been reported that the permissive action of insulin during a spatial alternation task requires GLUT4 translocation and signaling, and that insulin increases *glucos*e utilization in neurons via a GLUT4 mechanism (Grillo et al., 2009; McEwen and Reagan, 2004; Pearson-Leary et al., 2018; Reagan, 2005). Unfortunately, very few studies have directly investigated the impact of insulin on glucose utilization in the brain of aged animals, although evidence is present that aged F344 rats running a cognitively demanding task display significantly reduced hippocampal glucose levels (McNay and Gold, 2001). Considering this, the authors have argued that the enhanced depletion of glucose during demanding tasks may be due to reduced glucose supply (McNay, 2005). Importantly, McNay and colleagues have since reported that acute delivery of insulin to the hippocampus 10 min prior to a cognitive task enhances performance, increases glucose uptake and glycolytic metabolism, and reduces extracellular glucose concentration (McNay et al., 2010), again providing evidence that the hippocampus, at least, is highly sensitive to insulin.

In animal models of AD, marked decreases in brain insulin signaling and sensitivity, together with deteriorations in cognitive or spatial

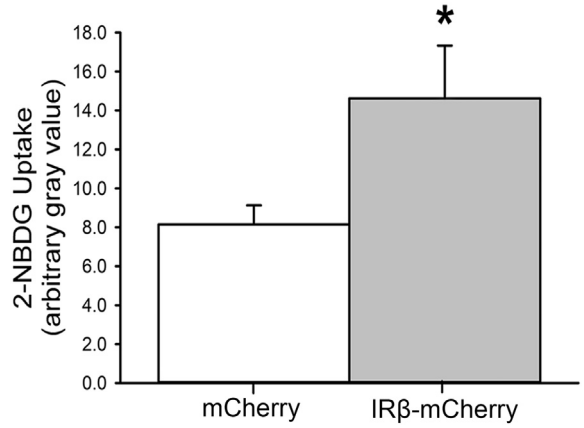

Fig. 4. 2-NBDG imaging of primary hippocampal neurons with or without expression of a constitutively active form of the human insulin receptor (IRβ). A. Analysis of indirect measures of glucose uptake using initial 2-NBDG fluorescent signal. Images were taken 3 min post-wash. All data were normalized prior to statistical analysis using background subtraction. Results indicate a significant increase in initial 2-NBDG signal in IRβ-expressing neurons (IRβ-mCherry, $n = 27$) compared to controls (mCherry, $n = 25$). * indicates $p < .05$. All data represent means +/− SEM *(for Methods, see*Pancani et al., 2013*)*.

memory performance, have been detected (reviewed in Griffith et al., 2018; Lee et al., 2018). Studies in multiple mouse models have characterized the impact of peripheral and central metabolic dysregulation with respect to the onset of AD pathology and cognitive decline (Ho et al., 2004; Macklin et al., 2017; Rodriguez-Rivera et al., 2011; Velazquez et al., 2017). Further evidence that glucose- and insulin-dependent cellular processes are dysregulated in other AD models have also been reported, particularly in relation to peripheral and central metabolism or the use of high fat diets or insulin-sensitizers (Lee et al., 2018). It is interesting to note that one study in 3xTG mice exposed to INI for 7 days did not show alterations in GLUT levels (Chen et al., 2014). While this has helped further solidify associations between peripheral insulin sensitivity and the AD phenotype, the direct connection between insulin and glucose in the brain of these models is better addressed by using exogenous insulin delivery methods. Together, this body of evidence strongly supports the proposal that utilizing insulin as means to improve glucose availability in neuronal cells could be a viable therapeutic approach. Specifically, the current literature highlights the need for continued explorations into novel therapeutic strategies to increase insulin signaling by either elevating ligand availability (i.e., INI), increasing IR trafficking to the plasma membrane, or even using molecular techniques to increase IR signaling in neurons.

7. Raising insulin signaling and glucose uptake in neurons through a molecular approach

Recently, we have designed a constitutively active form of the human IR comprised of an intact catalytic β-subunit and a heavily truncated α-subunit, referred to as IRβ (Frazier et al., 2018; Lebwohl et al., 1991), to directly test the impact of elevated IR signaling on neuronal metabolism. A lentiviral delivery system was used to express this IRβ construct in mixed primary hippocampal cultures and to test for alterations in voltage-gated calcium currents. Our first study showed no change in channel activity but did reveal robust expression and constitutive activity of the receptor (Frazier et al., 2018). To measure glucose uptake, we loaded hippocampal neurons with 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose (2-NBDG), a fluorescent glucose analogue. This analogue is metabolized to a non-fluorescent derivative at a rate shown to be dependent on glycolysis, therefore providing an indirect measure of glucose metabolism (Pancani et al., 2011). For this reason, 2-NBDG provides a powerful tool to observe not only measures of initial glucose uptake, but also the rates of glucose utilization.

Preliminary data show a robust and statistically significant increase in 2-NBDG uptake in IRβ-expressing cells compared to cells transfected with the empty vector control (Fig. 4). In addition to elevated uptake, it also appears that measures of glucose utilization rates are slightly increased following IRβ-expression (data not shown). If 2-NBDG uptake and utilization are elevated following sustained IR signaling through this molecular approach, this may provide a new tool to combat reductions in glucose uptake in the brain of aged animals. Ongoing studies in our lab are addressing which GLUT isoform is responsible for this increased glucose uptake. Studies on GLUT trafficking recently showed that treatment with insulin induced GLUT4 translocation to the plasma membrane in both a human neuronal cell line (Benomar et al., 2006) and in the rat hippocampus (Grillo et al., 2009) through a phosphatidylinositol 3-kinase-dependent pathway. Additionally, some work suggests that translocation of GLUT3, largely considered to be an insulin-insensitive isoform, may also be mediated through insulin (Uemura and Greenlee, 2006). Given the importance of neuronal glucose utilization and the impaired glycolysis as fundamental features of AD, it is imperative to identify which isoform might represent key features of the disease (An et al., 2018). Overall, the current body of evidence strongly supports the proposal that utilizing molecular strategies (i.e., adeno-associated viral delivery of IRβ) to improve glucose availability in neurons could lead to the identification of disease-modifying interventions.

8. Conclusion

Although not all studies align with the notion that insulin insensitivity is present in the aged or AD brain, the bulk of the current literature does indeed support the proposal that dysfunctional IR may be responsible, at least in part, for the memory decline associated with these disease states. Events that help give rise to brain insulin resistance include a reduction in IR signaling and/or a reduction in the ligand or its transport at the BBB. In this review, we attempted to highlight alternative sources of insulin dysregulation that could mediate the well-characterized reduction in glucose utilization seen in aging or AD and presented evidence for new approaches, from INI to IRβ, aimed at offsetting this decline. Future work is needed to continue investigations using these two approaches as well as research into other, less characterized cellular processes that could also be involved in insulin-mediated brain dysfunction.

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Statement of competing interests

The authors declare that no financial or personal competing interests exist.

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