Neurobiology of Disease 137 (2020) 104755

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Review

ApoE and cerebral insulin: Trafficking, receptors, and resistance☆

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ARTICLE INFO

Keywords:
Apolipoprotein E
Central nervous system insulin
Insulin receptor
Blood-brain barrier
Alzheimer's disease
Amyloid beta

ABSTRACT

Central nervous system (CNS) insulin resistance is associated with Alzheimer's disease (AD). In addition, the apolipoprotein E4 (apoE4) isoform is a risk factor for AD. The connection between these two factors in relation to AD is being actively explored. We summarize this literature with a focus on the transport of insulin and apoE across the blood-brain barrier (BBB) and into the CNS, the impact of apoE and insulin on the BBB, and the interactions between apoE, insulin, and the insulin receptor once present in the CNS. We highlight how CNS insulin resistance is apparent in AD and potential ways to overcome this resistance by repurposing currently approved drugs, with apoE genotype taken into consideration as the treatment response following most interventions is apoE isoform-dependent. This review is part of a special issue focusing on apoE in AD and neurodegeneration.

1. Introduction

Insulin has a dual role, being both a metabolic hormone and a mitogen. This multi-faceted fate of insulin is defined by the tissue in which it acts, in addition to the receptor activated. In the central nervous system (CNS), insulin contributes to the control of nutrient homeostasis, reproduction, olfaction, learning, and memory (Blazquez et al., 2014). Insulin in the CNS is primarily derived from blood insulin, which has been transported across the blood-brain barrier (BBB) (Banks et al., 1997a; Margolis and Altszuler, 1967; Woods and Porte, 1977). In the euglycemic state, insulin levels are approximately 14 fold less in the cerebrospinal fluid (CSF) levels than in the plasma (Mahley et al., 2009; Pitas et al., 1987).

Apolipoprotein E (apoE) plays an important role in the metabolism and redistribution of lipoproteins and cholesterol. In the brain, apoE is primarily produced by astrocytes (Kim et al., 2009), but also by neurons under stress (Xu et al., 1999) and is responsible for neuronal maintenance and repair (Holtzman et al., 2012). It was also recently reported that another major source of CNS apoE originates from the choroid plexus by glymphatic fluid transport (Achariyar et al., 2016). The peripheral pool of apoE is primarily produced by hepatocytes, and also by macrophages and adipocytes, each requiring tissue specific machinery for expression (Kockx et al., 2018). ApoE levels in the CSF are approximately 10-fold less than in the plasma (Chernick et al., 2019) but the correlation between apoE levels in these two pools is weak (Cruchaga et al., 2012). Regardless, apoE is important for BBB repair after injury (Bell et al., 2012; Donahue and Johanson, 2008; Fullerton et al., 2001).

Here, we review the literature supporting the link between CNS insulin and apoE. We first introduce how insulin enters and travels throughout the CNS and highlight some of the CNS-related roles. In this context, we touch on how CNS insulin resistance (IR) can lead to AD. We also present information regarding the role of apoE on the BBB and cerebrovascular blood flow and how insulin can modulate these interactions. Lastly, some recent clinical data is presented in ways to overcome CNS IR and challenges based on apoE genotype.

2. CNS access to insulin

Endogenous insulin is secreted by the pancreas and into the bloodstream where it can enter the brain via the BBB and blood-CSF barrier. Insulin is actively transported in a saturable manner across the BBB at levels below those needed to induce hypoglycemia (Banks et al., 1997a; Baura et al., 1993). It has been traditionally thought that a molecule the size of insulin would require a transcytotic transport mechanism to enter the brain. This can occur independent of the endothelial insulin receptor (Hersom et al., 2018; Rhea et al., 2018). Once insulin is transported across the BBB, efflux does not occur, even with

☆ Neurobiology of Disease Review

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https://doi.org/10.1016/j.nbd.2020.104755
Received 30 October 2019; Received in revised form 13 January 2020; Accepted 20 January 2020
Available online 21 January 2020

cerebrospinal fluid (CSF) reabsorption, and is sequestered by the CNS (Cashion et al., 1996). Therefore, the transport protein present at the BBB responsible for allowing insulin to enter the CNS is unidirectional, moving insulin from the luminal side of the brain endothelial cell to the abluminal side. The average rate of insulin transport across the mouse BBB is approximately 0.55 μL/g-m (Banks et al., 2012). For reference, amyloid β (1–42) is transported across the BBB at a slower rate of 0.39 μL/g-min (Banks et al., 1991).

Exogenous insulin can be administered by multiple different routes to increase CNS insulin as recently reviewed by us (Rhea et al., 2019b). Administrations that increase serum insulin are always dangerous for the risk of hypoglycemia. Therefore, getting insulin directly to the CNS is optimal for therapeutic purposes. Distribution of insulin throughout the brain varies based on the delivery route. For example, following intravenous delivery (which likely reflects endogenous transport), the olfactory bulb and pons/medulla contain the greatest content, while following intranasal delivery, the olfactory bulb, striatum, hypothalamus, and midbrain contain the most (Rhea et al., 2017; Rhea et al., 2018). Following intracerebroventricular injection, the hypothalamus and hippocampus contain the greatest amount (Rhea et al., 2019b).

Insulin transport across the mouse BBB is fastest into the olfactory bulb and pons/medulla (Rhea et al., 2019b). It is unknown whether humans have the same regional rates of insulin transport into the brain. As human insulin has the same saturable effect on murine insulin and vice versa in mice (Banks et al., 1997a), it is likely the two transport systems are similar. Human insulin can also lower endogenous circulating murine insulin (Banks et al., 1997b). However, the primary evolutionary role for the olfactory bulb in the rodent is different than the primary role for it in humans. The mouse olfactory bulb also has the highest rate of insulin degradation (Banks et al., 1999). How sequestration and degradation compares to the human is difficult to determine.

Insulin at the BBB is not just present as an effector for regulating substrate entry, but its own transport can also be influenced by other conditions. As an effector, insulin can regulate not only a variety of substrate entry across the BBB but also neurotransmitter signaling. For example, insulin can regulate leptin (Kastin and Akerstrom, 2001) and insulin-like growth factor 1 (IGF-1) (Yu et al., 2006) transport into the brain. In contrast, there is no role of insulin in electrolyte (Thurston et al., 1976), GLP-1 (Kastin et al., 2002), or estrogen (May et al., 2016) transport into brain. Insulin can also regulate amino acid transport across the BBB. Tryptophan BBB transport is regulated by insulin (Cangiano et al., 1983), which can lead to multiple changes in CNS signaling pathways, especially in the serotonin pathway. In addition, dopamine and insulin signaling systems have a cross-regulatory interaction (Nash, 2017) in which insulin can regulate neuronal dopamine uptake and dopamine can regulate pancreatic insulin secretion. Therefore, insulin action just at the BBB can lead to a multitude of changes within the CNS. There are also different physiological states, including diabetes, obesity, and aging, that can impact the transport of insulin into the brain (Banks et al., 2012; Rhea and Banks, 2019).

As the above illustrates, insulin in the CNS has many roles and these differ from its metabolic role in the periphery. In addition, the receptors for insulin in the brain have a different structure and size compared to those expressed peripherally, consistent with a different evolutionary pathway that preserved more of ancestral insulin's effects on growth (Banks et al., 2012; Chan et al., 1992). Insulin in the brain does not primarily affect glucose uptake as in the periphery since GLUT1 is the primary glucose BBB transporter and is non-responsive to insulin (Hasselbalch et al., 1999). However, there are some regions such as the hippocampus and hypothalamus that express GLUT4, an insulin-sensitive glucose transporter, localized to neurons (Alquier et al., 2006; Grillo et al., 2009; McEwen and Reagan, 2004). Here, GLUT4 translocates to the neuronal membrane downstream of insulin and plays an important role in hippocampal memory processes and brain insulin resistance (Duarte et al., 2012; McNay and Pearson-Leary, 2020). Peripheral insulin levels can regulate cerebellar GLUT4 expression (Vannucci et al., 1998). In addition, it has recently been shown that intranasal insulin can increase cerebral glucose uptake, as measured by [^{18}F]-FDG uptake following traumatic brain injury in rats, although the mechanism for increased uptake was not examined (Duarte et al., 2012).

CNS insulin can have a direct, localized effect in addition to an indirect effect that elicits a change in the periphery (i.e. change in peripheral metabolism). CNS insulin can play a major role in reward, memory, and feeding behavior by impacting signal transduction pathways involved in neuronal survival, synaptic maintenance, dendritic arbor development, cognition, neuronal circuitry formation, and BBB transporter expression/localization (Banks, 2004; Banks et al., 2012; Schulingkamp et al., 2000; Stranahan et al., 2008). Areas within the brain that have high levels of insulin receptors, such as the hippocampus and hypothalamus, play important roles in cognition and regulation of peripheral metabolism, respectively (Ghasemi et al., 2013; Porte Jr. et al., 1998). This is consistent with evidence showing that insulin can influence cognition (Infante-Garcia et al., 2015). It was recently shown that insulin in the hippocampus could affect other signaling pathways besides the typical insulin receptor pathway (Frazier et al., 2019b; Rhea et al., 2019a). As an indirect mechanism, CNS insulin can act as a neuroregulatory peptide, regulating food intake and peripheral energy stores (Filippi et al., 2013; Gray et al., 2014). The impact of CNS insulin on food intake data should be interpreted with caution, however, as supraphysiological levels of insulin are often injected into the CNS. In such studies, the IGF-1 receptor and perhaps other receptors as well could be activated (Rechler and Nissley, 1986). CNS insulin infusion decreases serum insulin levels, which appears to be independent of a change in food intake (Foster et al., 1991).

Less studied is the role of insulin receptor signaling in other cell types of the neurovascular unit (astrocytes, pericytes, brain endothelial cells) and how this signaling might affect the BBB (Rhea and Banks, 2019). Many of the functions of CNS insulin have been well described, as discussed above. However, once insulin is transported across the BBB, it is unclear how this signaling peptide navigates the extracellular space and accesses its target. Whether insulin is released into the interstitium, packaged into exosomes, or transported directly to surrounding cells of the neurovascular unit, including pericytes, astrocytes, and neurons is not known. Brain extracellular diffusion patterns of similarly sized molecules including epidermal growth factor and nerve growth factor have been investigated (Stroh et al., 2003; Thorne et al., 2004), but research on insulin diffusion is limited. Importantly, diffusion parameters are known to change with age (Sykova, 2004). It is likely that these parameters also change with disease. It is also not clear whether insulin is sequestered by these cells and released as needed or if the CNS requirement for insulin is regulated by BBB transport, sequestration, and degradation. These concepts warrant further investigation to better understand the etiology of CNS IR.

Intranasal insulin is one way to therapeutically increase CNS insulin concentrations to overcome CNS IR. Clinical studies have shown a dose dependent benefit of intranasal insulin on cognition that is modulated by apoE genotype (Reger et al., 2006; Reger et al., 2008). Cerebrovascular function is critical for cognitive performance (Ozturk and Tan, 2018). Intranasal insulin can increase cerebral blood flow in young adults (Kullmann et al., 2017), older adults (Akintola et al., 2017), and cognitively impaired rats (Rajasekar et al., 2017). This therapeutic delivery of insulin will be brought up again later. The relation between cerebrovascular function, the BBB, and apoE isoforms will be discussed below.

3. ApoE and the BBB

The importance of cerebrovascular function for cognitive performance is already evident in the developing brain (Bakker et al., 2014). The BBB is also critical for cognitive performance. Brain capillary

damage and BBB breakdown in the hippocampus are early biomarkers for cognitive dysfunction independent of amyloid β or tau (Nation et al., 2019) and reductions in microvascular length and decreased brain capillary density have been reported in several animal models of AD (Lee et al., 2005; Paris et al., 2004). BBB dysfunction can lead to microvascular impairments (Zlokovic, 2013) and is associated with cognitive decline in nondemented elders (Bowman et al., 2018). The importance of the BBB for cognitive performance is also evident in the developing brain, for example in the context of obstructive sleep apnea (Khalyfa et al., 2018). With the need to diagnose early and start long-term prevention interventions in neurodegenerative diseases such as AD (Yassine, 2017), cerebrovascular function and the BBB are attractive early biomarkers. As described below, apoE isoforms have differential effects on cerebrovascular function and the BBB. Therefore, it is important to understand these apoE isoform-dependent impacts in health and disease and in the developing and aging brain.

Transport of apoE across the BBB or blood-CSF barrier has not been reported to occur, as evident by independent studies (Liu et al., 2012; Zlokovic et al., 1994). However, apoE can affect the BBB directly in addition to altering transport of substrates, including amyloid β. Specifically, apoE4 disrupts amyloid β BBB efflux the most (Deane et al., 2008) and primarily impacts BBB integrity by contributing to BBB anomalies (Alata et al., 2015). Lastly, the diffusion of apoE throughout the CNS is isoform-dependent with diffusion being ranked as follows: apoE2 > apoE3 > apoeE4 (Achariyar et al., 2016).

In the developing brain, E4 carriers exhibit greater changes in regional activation than non-carriers in distinct regions of the temporal gyrus during a nonverbal memory task (Scarmeas et al., 2005). These effects and alterations in cerebral blood flow are not transient and are also seen in people during middle-age (Bookheimer et al., 2000). Consistent with these human data, vascular alterations are seen in mice at two weeks of age expressing E4 or mice deficient in murine apoE (KO) (Bell et al., 2012). The fact that this phenotype is also seen in apoE KO mice suggests that it is due to lack of trophic effects rather than a dominant negative effect of E4 as is seen for some other phenotypes (Raber, 2004; Raber et al., 2004), which in turn has implications for developing and testing therapeutic strategies. It also suggests that the interaction of this phenotype with age might be what contributes to increased risk of E4 carriers to develop age-related cognitive decline (Berteau-Pavy et al., 2007) and AD (Farrer et al., 1997). The BBB might play a critical role here (Zlokovic, 2011). Using a guinea-pig brain perfusion model, an anti-amyloidogenic role for the BBB involving transport of apoE and apolipoprotein J (apoJ) in AD and impairments in cerebrovascular function being an initiating event leading to neurodegeneration was proposed 23 years ago (Zlokovic, 1996). Environmental risk factors like a Western diet might also interact with this phenotype to further increase this risk. Importantly, both IR and E4 have been linked to vascular impairments. Insulin has many important actions in the brain as described above, and brain IR has been proposed to contribute to the progression of AD (de la Monte, 2012).

In the aging brain, cerebrovascular flow is lower in E4 carriers than non-carriers (Filippini et al., 2011). The aging brain of cognitively healthy E4 individuals show accelerated declines in regional cerebrovascular flow over time (Thambisetty et al., 2010). The relation between cognitive function and age-related changes in cerebrovascular flow is also modulated by apoE isoform (Wierenga et al., 2013). There are differences in blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI) signals, measures of functional brain activation, in middle-aged and aged E4 carriers (Scarmeas and Stern, 2006). E4 carriers, including cognitively healthy elderly and those at younger ages, show glucose hypometabolism (Altmann et al., 2015; Reiman et al., 2001; Reiman et al., 1996; Reiman et al., 2004; Small et al., 2000). This early onset of cerebrovascular and BBB phenotypes in E4 carriers is important as these phenotypes have been proposed as initiating events ultimately causing neurodegeneration associated with vascular disease (Snyder et al., 2015). *APOE* genotype itself has clear effects on several risk factors for dementia noted above, such as obesity (Arbones-Mainar et al., 2008; Arbones-Mainar et al., 2016; Elosua et al., 2003; Huebbe et al., 2015; Tejedor et al., 2014), hypercholesterolemia (Bennet et al., 2007; Wilson et al., 1996), and increasing in vivo and in vitro evidence also points to multiple cerebrovascular effects of *APOE* in the brain (Alata et al., 2015; Bell et al., 2012; Lin et al., 2017; Nishitsuji et al., 2011; Schilling et al., 2013; Wiesmann et al., 2016).

In general, this pattern is consistent with the proposed developmental origin of health and disease later in life (Heindel and Vandeberg, 2016; Roseboom et al., 2001). E4 isoform is associated with hyperactivity and hyperconnectivity (Koelewijn et al., 2019), increased BBB permeability, reductions in cerebral vascularization, thinner vessel walls, and reduced cerebrovascular blood flow (Alata et al., 2015; Bell et al., 2012). However, it is likely more complex since age of the E4 carriers results in lower (Filippini et al., 2011; Scarmeas et al., 2005; Thambisetty et al., 2010) and higher (Wierenga et al., 2013; Zlatar et al., 2014) cerebral blood flow. Since cerebral blood flow is decreased in people with age-related cognitive decline and AD (Celsis et al., 1997; Roher et al., 2012), it is conceivable that the complexity is partly due to the fact that cerebral hypoperfusion could be a result of diminished energy demands of the aging or AD brain or contributes more directly to neurodegeneration. So in E4 carriers with unaltered or higher cerebral blood flow, this might be part of a compensatory change and hypoperfusion might precede and even actively contribute to the risk to develop AD (Tai et al., 2016).

Consistent with the human data, aged E4 mice have a lower cerebrovascular flow than wild-type mice (Lin et al., 2017; Wiesmann et al., 2016). These changes are not necessarily associated with alterations in vessel morphology. While the cerebral blood volume was lower in in E4 than E3 mice, in one study this was observed in the absence of changes in vessel morphology (Johnson et al., 2019) while in another study they were associated with reductions in microvascular length (Bell et al., 2012).

Remarkably, one environmental factor that is front and center for the developmental origin of health and disease is nutrition and insulin sensitivity/resistance. In this context, it is extraordinary that type II diabetes, IR, and E4 carrier status are associated with a reduced ability to preserve a stable and sufficient cerebral blood flow (Jansen et al., 2016; Novak et al., 2014). This link between IR and cerebral blood flow is not surprising, as the microvasculature is sensitive to insulin and this sensitivity is impaired by IR (Belcik et al., 2015). Consistent with the human data, both E4 and a Western diet induce IR and reduce cerebral blood volume and glucose uptake, in an additive fashion, in apoE targeted replacement (TR) mice Johnson et al., 2019. However, the cognitive, metabolic, and cerebrovascular responses to an acute glucose challenge revealed apoE isoform-dependent effects. E4, but not E3, mice benefitted from an acute increase in blood glucose levels Johnson et al., 2019. Consistent with these mouse data, cognitively healthy people and cognitively impaired people carrying E4 have higher memory scores following a meal with a high glycemic index and high in saturated fat, while lower scores are seen in cognitively healthy people who do not carry E4, as compared to genotype-matched controls (Hanson et al., 2015). IR also affects cognitive performance (de la Monte, 2012) and might also contribute to the progression of AD. Administration of intranasal insulin enhances cognition and affects regional vasoreactivity in patients with IR as part of type II diabetes (Novak et al., 2014). Recently, nicotine addiction in rats was associated with a risk for type II diabetes by activation of a nicotinic acetylcholine receptor present in the habenula (Duncan et al., 2019). Nicotine consumption led to increased circulating glucagon and insulin levels and dysregulated glucose homeostasis via a direct connection from the habenula to the pancreas. An apoE isoform-dependent effect remains to be elucidated.

In E4 carriers, in addition to the central effects described above, there might also be indirect effects on cognitive function and brain

metabolism (Pendse et al., 2009). E4 is associated with peripheral vascular disease (Johnson et al., 2011; Johnson et al., 2013; Minihane et al., 2007) and the peripheral response to acute or chronic glucose or a dietary challenge in E4 carriers might affect cognitive function (Hanson et al., 2016; Hanson et al., 2013; Hanson et al., 2015).

In studies involving special diets, it is important to consider potential effects of the diet on plasma and brain apoE levels. For example, a Western diet administered for 5 weeks starting at 2–3 months of age was shown to significantly reduce apoE levels in the hippocampus of E3 but not E4 TR mice (Lane-Donovan and Herz, 2016). Conversely, plasma levels of apoE were significantly increased in E4, but not E3, TR mice on a Western diet (Lane-Donovan and Herz, 2016). A ketogenic diet revealed an apoE-isoform dependent effect in plasma but did not affect hippocampal apoE levels. Like what was seen with a Western diet, a ketogenic diet caused elevated plasma apoE levels in E4 but not E3 TR mice (Lane-Donovan and Herz, 2016). Perhaps related to increased apoE levels, a ketogenic diet restored systemic insulin sensitivity and metabolic flexibility in a diabetic E4 carrying patient (Stoykovich and Gibas, 2019).

4. Interaction of insulin/insulin receptor with apoE

The differential effects of apoE isoforms in insulin receptor function could be due to differential binding to the receptor. In AD brain samples, more insulin receptor is precipitated by extracts from E3 brains than extracts from E4 brains (Chan et al., 2018). Consistent with these human data, E3 binds stronger than E4 to insulin receptor in brain extracts of 26- and 78-week old apoE TR mice crossed with APP J20 mice carrying a mutant human APP gene containing the Swedish (K670 N/M671 L) and Indiana (V717F) mutations (Chan et al., 2015). Interestingly, using hippocampal neuronal cultures prepared from postnatal day 0, E3 and E4 pups, there was no difference in insulin sensitivity except in the presence of 500 nM of amyloid β (1–42). Here, the insulin response was impaired only in hippocampal neuronal cultures from E4 pups (Chan et al., 2015). Amyloid β peptides can reduce insulin binding to the insulin receptor which decreases insulin signaling (Xie et al., 2002). E4 also has a stronger binding affinity to amyloid β than E3 (Chan et al., 2015; Saunders et al., 1993). More on the interaction of amyloid β and apoE is covered in another review in this special issue by Dr. Thomas Wisniewski.

The altered interaction of insulin with the insulin receptor might also related to the trapping of insulin receptor in the presence of E4 in endosomes contributing to impaired insulin signaling and impaired mitochondrial respiration and glycolysis by insulin (Zhao et al., 2017). Age might be able to replace the amyloid β challenge. In cortex and hippocampus of 22-month old E3 and E4 mice, insulin signaling was impaired in E4 mice compared to E3 mice (Zhao et al., 2017). This was not seen in the cortex at 3 or 12 months of age. Therefore, in aging brains, aggregation of E4 and endosomal dysfunction might drive this effect. A Western diet might be able to replace both amyloid β and aging. When E3 and E4 TR mice were put on a Western diet for 4 months starting at 8 months of age, insulin signaling was impaired in E4 mice, while the Western diet did not affect insulin signaling in the E3 mice (Zhao et al., 2017). Remarkably and consistent with the data described above, when neuronal-enriched extracellular vesicles were isolated from plasma of patients with amnestic mild cognitive impairment or probable AD receiving intranasal insulin, positive correlations in the relationship between insulin signaling mediators and measures of cognitive performance were only seen in non-E4 carriers (Mustapic et al., 2019). These data suggest a clear overlap and interaction between amyloid β, the insulin receptor, apoE, and insulin.

5. CNS insulin resistance in Alzheimer's Disease

AD has been associated with a deficiency in insulin action within the CNS. Deficient action could be caused by either a decreased level of insulin in the brain or to a resistance to insulin actions at the level of its receptor. One of the earliest pieces of supporting evidence for defective insulin action was that there is decreased glucose utilization by the AD brain. However, neither glucose transport across the BBB nor glucose uptake by the vast majority of brain cells, with the exception of GLUT4-expressing neurons, is insulin-dependent. Four independent lines of evidence for a deficient insulin action in the AD brain are i) a decreased CSF/serum ratio, ii) insulin resistant diseases (obesity and diabetes) are risk factors for AD, iii) AD brains have impaired insulin signaling, and iv) insulin delivery to the CNS improves memory. These four lines of evidence are considered below.

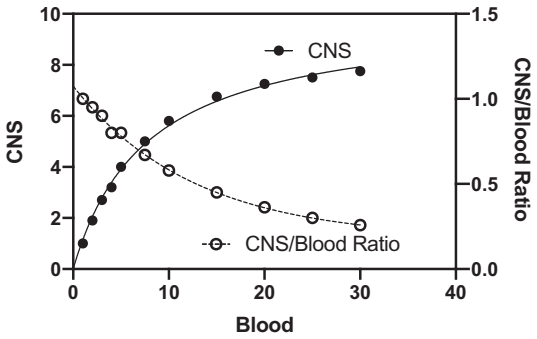

Fig. 1. Relation of CNS levels and CNS/blood ratios to blood levels: *Theoretical data*. This figure illustrates how the nonlinear relation between CNS and Blood levels can result in increasing blood levels being accompanied by both increasing CNS levels and decreasing CNS/Blood Ratios. A less efficient transporter would shift the CNS curve downward/to the right so that CNS levels will be lower for any given Blood level.

5.1. Decreased CSF/serum ratio

A decreased CSF/serum ratio for insulin has been reported in aged and AD subjects (Craft et al., 1998; Sartorius et al., 2015). The reasoning that this is powerful presumptive evidence for a defect in insulin transport across the BBB relies on an understanding of the nonlinear pharmacokinetics of insulin transport across the BBB. As insulin transport across the BBB is saturable, the relation between CSF insulin and serum insulin is hyperbolic (Fig. 1). That is, it is linear only at "physiologic" levels of insulin. As blood levels of insulin rise, CSF levels also rise, although to an ever lesser degree. In other words, the numerator (CSF insulin levels) rises less robustly than the denominator (blood insulin levels) and so the ratio (CSF/serum insulin ratios) declines as the serum insulin level rises.

This decrease in CSF/serum ratios could occur in two basic ways that have fundamentally different implications for the relation between central and peripheral IR, as well as the role of the BBB. First, serum levels of insulin could increase, as for example happens with peripheral IR. In this case, CSF insulin levels would be paradoxically elevated while CSF/serum insulin ratios would be decreased. Thus, a decrease in CNS insulin action would only result if there were an accompanying CNS IR; and the resistance would need to be greater than could be overcome by the increased CNS level of insulin. In this case, IR at both the CNS and peripheral levels could occur simultaneously while the insulin transporter at the BBB is functioning normally. A shifting to the right of the curve relating CSF and serum insulin levels is a second way that a decreased CSF/serum insulin ratio could occur. In this case, the amount of insulin transported across the BBB is lower than normal for any given level of insulin in the blood. As the insulin transporter is modulated by various factors (Banks et al., 2008; Urayama and Banks, 2008), such a shift is possible. CSF insulin levels are not necessarily increased and could even be decreased. In this case, CSF insulin levels could be lower than normal, CNS IR can occur independently of peripheral IR, and the BBB insulin transporter is impaired (Fig. 2). Which

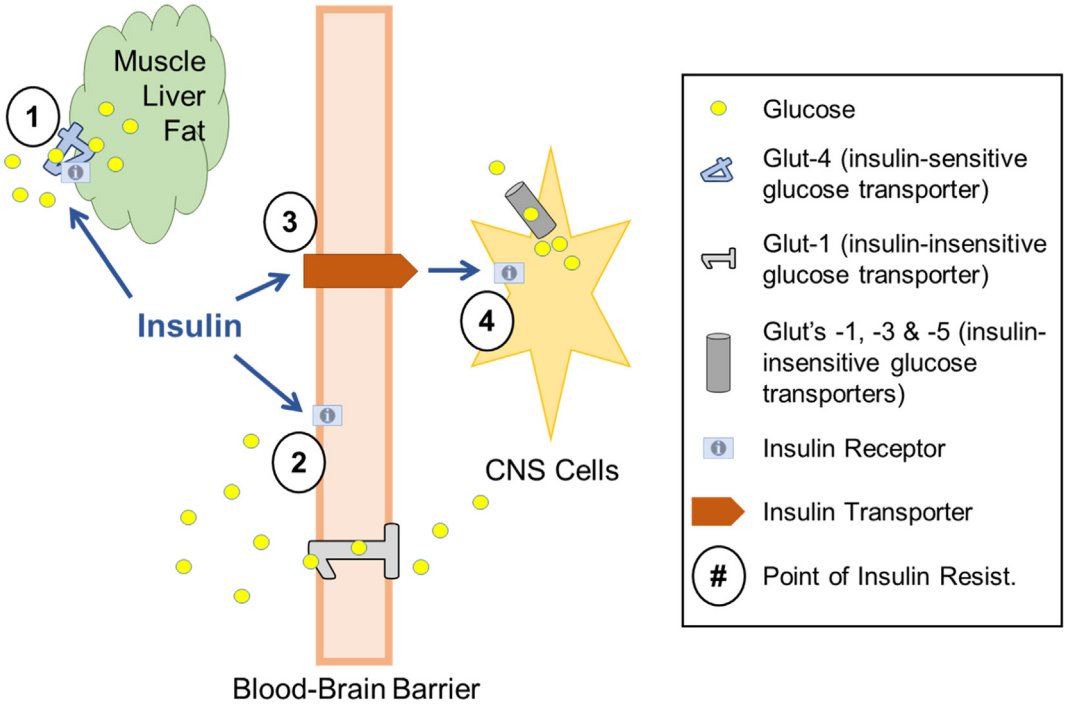

Fig. 2. Levels of insulin resistance. Glucose uptake is stimulated by tissues containing GLUT-4 (primarily muscle, liver, and adipose tissue), but not by those containing GLUT-1, 3, or 5 (such as brain endothelial cells, astrocytes, neurons, and microglia). Points of Insulin Resistance: 1) Insulin resistance at GLUT-4 dependent peripheral tissues results in hyperglycemia, unless countered by an elevation in insulin levels. 2) Brain endothelial cells respond to insulin in ways other than glucose uptake. 3) The insulin transporter at the BBB has a rate that can be modified; a decreased transport rate can result in less insulin action in the brain. 4) Insulin receptor resistance can occur on CNS cells.

of these dominate is unclear as CSF insulin levels in AD have been reported to be increased, decreased, and unchanged (Craft et al., 1998; de la Monte et al., 2019; Fugisawa et al., 1991; Gil-Bea et al., 2010; Molina et al., 2002).

Other explanations for a decrease in the CSF level or CSF/serum ratio of insulin are increased enzymatic activity in the CNS or increased efflux from the CNS.

5.2. Insulin resistant diseases (obesity and diabetes) are risk factors for AD

This line of evidence involves several strengths and weaknesses in its support. First, diabetes mellitus and obesity can increase the incidence of cognitive deficits through a number of mechanisms that don't depend on CNS insulin actions. Stroke and ischemia, multi-infarct dementia, inflammation, hypertension, dyslipidemia, and episodes of iatrogenic hypoglycemia all place the diabetic at risk of cognitive impairment (Elias et al., 2003; Manschot et al., 2006; Ott et al., 1999; Perlmuter et al., 1988; Rogers et al., 1989). Diabetes itself is associated with its own form of cognitive impairment, characterized by a decline in executive function (Elias et al., 2003; Manschot et al., 2006). On the one hand, these associations show how diabetes/obesity can be related to cognitive impairment without invoking CNS IR. On the other hand, peripheral IR is associated with factors that can affect CNS insulin actions. Hypertriglyceridemia is the signature dyslipidemia of peripheral IR, diabetes mellitus, and the metabolic syndrome. Triglycerides can cross the BBB to induce resistance at the CNS insulin receptor (Banks et al., 2018). A single high-fat meal can immediately induce cognitive impairment in subjects who are apoE2/3 positive and, paradoxically, cognitive improvement in those who carry apoE4 (Hanson et al., 2015).

Another early line of argument relied on the then untested assumption that IR is global; that is, if IR occurs in peripheral tissues, then it is also occurring in CNS tissues. However, for other receptor resistance syndromes such as resistance to thyroid hormone, resistance can occur in one tissue, such as the pituitary, but not in others (Refetoff, 1982). Furthermore, as the thyroid hormone transporter at the BBB is not the same as the thyroid hormone receptor, defects in transport of thyroid hormone across the BBB occur independently of thyroid receptor resistance (Bernal et al., 2015). An analogous situation is emerging for IR. The classic study of Talbot et al. found that CNS IR can occur in AD patients that have no evidence of peripheral IR (Talbot et al., 2012). As the insulin transporter at the BBB is not the same protein as the insulin receptor (Rhea et al., 2018), it is probable that defects in CNS insulin receptor function do not necessarily imply defects in transport as well.

5.3. Direct evidence that AD brains have impaired insulin signaling

Several studies involving post mortem tissue have revealed that the levels of protein or message for the insulin receptor and components of the insulin signaling pathway are decreased in AD brains (Chan et al., 2018; Liu et al., 2011; Rickle et al., 2004; Rivera et al., 2005; Steen et al., 2005). Talbot et al. confirmed these changes and further showed that signaling as induced by insulin was impaired in post mortem brain tissue from individuals without diabetes (Talbot et al., 2012). In addition, alterations in insulin signaling do not appear to occur simultaneously in different brain areas. Instead, recent data suggest a progression from the hippocampus to the frontal cortex (Barone et al., 2019).

How IR could arise in the CNS in AD is not clear. That peripheral and central IRs interact is strongly suggested by the finding that the deficiency in insulin signaling in brain is more severe in patients who also have insulin resistant diabetes (Liu et al., 2011). Various studies have implicated amyloid β, tau, iron, and other factors (Bloom et al., 2018; Wan et al., 2019). In recent years, the role of the protein biliverdin reductase-A, a unique kinase involved in the regulation of insulin signaling (Lerner-Marmarosh et al., 2005), has been proposed to be involved in CNS insulin sensitivity (Barone et al., 2016; Barone et al., 2011; Sharma et al., 2019; Triani et al., 2018). Other conditions

associated with CNS IR could offer clues. As mentioned above, hypertriglyceridemia, which is the classic dyslipidemia of diabetes mellitus and the metabolic syndrome, can induce CNS IR (Banks et al., 2018). Other conditions can affect CNS IR. Pregnancy is associated with resistance to the sympathoexcitatory effects of CNS insulin (Shi et al., 2019b), exercise can reverse the CNS IR associated with a high fat diet (Park et al., 2019), and antipsychotic drugs affect insulin signaling at various levels (Kowalchuk et al., 2019).

Another interesting question is whether CNS insulin levels are part of a negative feedback loop. In the periphery, blood insulin levels are locked into a negative feedback loop that tightly controls both of their levels: high blood glucose induces increased blood levels of insulin and high blood levels of insulin lower blood glucose. Hence, when IR occurs in the periphery, that is, when blood insulin is less potent in its ability to decrease blood glucose levels, the body responds by simply allowing serum insulin levels to rise until glucose levels return to normal. A question then is whether CNS insulin is part of a negative feedback loop, that is, can CNS insulin levels rise to overcome CNS IR? It is unlikely that any such feedback loop would involve an increase in peripheral insulin levels as this would risk hypoglycemia. Other control points would be at the BBB (this time shifting the CNS-serum relation to the left so that more insulin crossed into the brain at any given blood level), enzymatic degradation, and CNS-to-blood efflux rates of insulin. These control points could also keep CNS insulin levels from rising in the face of increased blood levels of insulin caused from peripheral resistance. Finally, although few cells in the CNS have been shown to be capable of secreting insulin, the question arises as to whether such secretion might be more widely inducible in brain.

5.4. Insulin delivery to CNS improves cognition

Several studies have shown that insulin improves some aspects of cognition. An early study revealed an effect when the insulin was administered systemically (Craft et al., 1996). However, giving insulin by this route can induce hypoglycemia. Subsequently, a series of studies involved the intranasal route to deliver insulin to the brain as briefly touched on above. Substances delivered through the nares above the turbinates to the level of the cribriform plate show absorption by various routes into the brain (Lochhead and Thorne, 2012). Insulin given by this route shows a distribution pattern that differs from that of intravenous or intracerebroventricular injections (Rhea et al., 2019b). Cognitive improvement has been found after intranasal insulin in patients with mild cognitive impairment and AD as well as young healthy volunteers (Benedict et al., 2004; Craft et al., 1999a; Craft et al., 1999b; Craft et al., 2012; Reger et al., 2006; Reger et al., 2008; Schmid et al., 2018). Animal studies have found improvements with intranasal insulin in cognitively impaired rats (Sukhov et al., 2013) and mice (Marks et al., 2009; Salameh et al., 2015) by altering neuronal after-hyperpolarization (Maimaiti et al., 2016), cerebral blood flow (Anderson et al., 2017), amyloid β production (Mao et al., 2016), and tau hyperphosphorylation (Zhang et al., 2016). Most recently, results of animal studies investigating the molecular mechanism of intranasal insulin suggest that the beneficial effects may be independent of changes in insulin signaling and rather due to changes in inflammation and cell migration (Frazier et al., 2019b; Rhea et al., 2019a). This topic of alternative sources of insulin dysregulation has recently been reviewed by Frazier et al. (Frazier et al., 2019a).

If high levels of insulin are therapeutic, why doesn't the hyperinsulinemia of insulin resistant diabetes protect persons from AD? Interestingly, an early study investigating the relation between diabetes mellitus and AD concluded that these two conditions could not co-exist (Bucht et al., 1983). However, subsequently, diabetes mellitus has been established as a risk factor for AD. One reason the high blood insulin levels are not protective may have to do with the saturable nature of insulin transport across the BBB. As discussed above, diabetes mellitus is associated with many factors that can impact brain health, including association with other risk factors (e.g., peripheral IR, hyperglycemia, hypertension) and life styles (high-fat diets leading to hypothalamic inflammation (Thaler et al., 2013), for example). It may be that the risk factors associated with diabetes mellitus outweigh any therapeutic benefit of increased brain insulin levels.

6. Repurposing diabetes drugs for memory improvement

For the development of therapeutic strategies to improve cognitive performance, it is important to realize that there are apoE isoform differences in metabolic markers at baseline and in response to treatment. The relationship of these biomarkers with IR is also apoE isoform-dependent. When a dietary fatty acid (FA) intervention was used in patients with metabolic syndrome as part of a 12-week randomized trial, E4 carriers had higher plasma concentrations of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and apolipoprotein B (apo B) than E2 carriers and higher TC, LDL-C and apo B than E3 homozygotic carriers (Fallaize et al., 2017). In addition, while elevated plasma n-3 polyunsaturated FA (PUFA) levels were associated with a beneficially lower concentration of apo CIII in E2 carriers, a high proportion of plasma C16:0 was associated with IR in E4 carriers. Following the FA intervention, an increase in the proportion of plasma long chain (LC) n-3 PUFA was associated with an increase in triacylglycerol-rich lipoprotein (TRL)-C concentrations in E2 carriers, while a reduction in TRL-C was observed in the E3 homozygotic carriers. In E2 carriers, apo CII concentrations were reduced in E2 carriers following a reduction in plasma LC n-3 PUFA and increased when plasma LC n-3 PUFA was raised. In E4 carriers, the apo CII concentration was more increased in response to a reduction in the proportion of plasma LC n-3 PUFA than what was seen in E2 carriers. ApoE levels were also differentially affected by the intervention. Following an increase in plasma LC n-3 PUFA, E2 carriers showed increased apoE levels while E3/E3 and E4 carriers showed a decrease (Fallaize et al., 2017). Considering that the apoE levels in humans (Rezeli et al., 2015) and mice (Johnson et al., 2015), especially those expressing the human low-density lipoprotein receptor (LDL) receptor (Johnson et al., 2014) differ depending on apoE genotype and in apoE heterozygotic carriers the levels of the two apoE isoforms are not comparable either highlights the complex relationship between apoE isoform, apoE levels, and biomarkers of brain metabolism.

There is an increased interest in repurposing drugs (Pushpakom et al., 2018), including diabetes drugs, to improve cognitive function. Especially as there is an increased focus towards individualized medicine to improve medical treatments and strategies (Toplol, 2014), it is important to consider apoE genotypes in developing individual treatment strategies. Preclinical research underlines the importance of this consideration. When 13-month-old apoE TR mice were administered metformin (300 mg/kg/day) for 5 months, spatial memory of E3, but not E4, mice was improved and this was associated with enhanced insulin signaling in E3, but not, E4 mice (Zhang et al., 2019). Interestingly, tau phosphorylation was seen following metformin treatment in both E3 and E4 mice (Zhang et al., 2019), and recently apoE was shown to be critical in mediating tau pathology (Shi et al., 2019a). Interestingly, as described earlier, we found that E4 mice were benefitting from effects of an acute glucose administration on spatial memory Johnson et al., 2019. Similarly, in a different apoE mouse model in which E3 or E4 was expressed at comparable levels in brains of mice deficient in murine apoE, E4 female mice benefitted from effects of testosterone and dihydrotestosterone on memory while E3 female mice did not (Raber et al., 2002). In addition, male E4 mice were more susceptible to effects of androgen receptor blockade than E3 mice and untreated E4 mice had lower cytosolic androgen receptor binding in cortex than E4 mice (Raber et al., 2002). We recognize that in all these studies a single dose was used for the treatments and it cannot be excluded yet that E3 and E4 carriers might need to receive a different dose based on apoE isoform-dependent dose-response curves. This is unlikely though as the

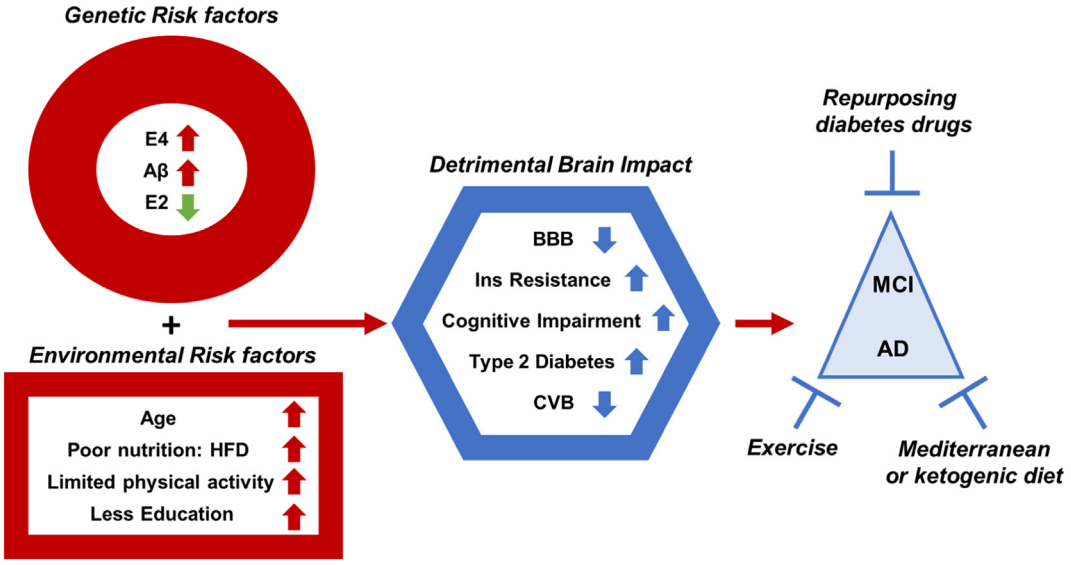

Fig. 3. Developmental origin of insulin resistance, Mild Cognitive Impairment (MCI), and AD. Genetic and environmental risk factors contribute to the detrimental brain impact that can lead to development of MCI and AD. Efficacy of intervention through either medication or life-style changes is also often apoE isoform-dependent.

protective effects of a healthy lifestyle on reducing dementia risk seems also apoE isoform-dependent and evident in non-E4 carriers only (Licher et al., 2019).

7. Conclusions

The purpose of this review was to present the evidence between the connection of apoE and cerebral insulin, implicated in the developmental origin of IR and AD (Fig. 3). The majority of this evidence has been generated within the last decade, highlighting that this research area is growing and its importance. We know a lot more about the transport of insulin into the CNS than we do about the transport of apoE. Yet we do know quite a bit about how apoE might alter cerebrovascular blood flow and BBB function. We hope to uncover more about how these two might regulate BBB transport of one another. Increased understanding of how apoE and cerebral insulin might work together to impact CNS IR will facilitate the development of therapeutic strategies to overcome this deficit.

Funding

This work was supported by the University of Washington Diabetes Research Center (EMR), National Institutes of Health [AG059088] (JR, WAB), and the Veterans Affairs Research and Development (EMR, WAB).

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