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REVIEW ARTICLE

Understanding glucose metabolism and insulin action at the blood–brain barrier: Implications for brain health and neurodegenerative diseases

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Abstract

The blood–brain barrier (BBB) is a highly selective, semipermeable barrier critical for maintaining brain homeostasis. The BBB regulates the transport of essential nutrients, hormones, and signaling molecules between the bloodstream and the central nervous system (CNS), while simultaneously protecting the brain from potentially harmful substances and pathogens. This selective permeability ensures that the brain is nourished and shielded from toxins. An exception to this are brain regions, such as the hypothalamus and circumventricular organs, which are irrigated by fenestrated capillaries, allowing rapid and direct response to various blood components. We overview the metabolic functions of the BBB, with an emphasis on the impact of altered glucose metabolism and insulin signaling on BBB in the pathogenesis of neurodegenerative diseases. Notably, endothelial cells constituting the BBB exhibit distinct metabolic characteristics, primarily generating ATP through aerobic glycolysis. This occurs despite their direct exposure to the abundant oxygen in the bloodstream, which typically supports oxidative phosphorylation. The effects of insulin on astrocytes, which form the glial limitans component of the BBB, show a marked sexual dimorphism. BBB nutrient sensing in the hypothalamus, along with insulin signaling, regulates systemic metabolism. Insulin modifies BBB permeability by regulating the expression of tight junction proteins, angiogenesis, and vascular remodeling, as well as modulating blood flow in the brain. The disruptions in glucose and insulin signaling are particularly evident in neurodegenerative diseases, such as Alzheimer's disease and

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Parkinson's disease, where BBB breakdown accelerates cognitive decline. This review highlights the critical role of normal glucose metabolism and insulin signaling in maintaining BBB functionality and investigates how disruptions in these pathways contribute to the onset and progression of neurodegenerative diseases.

KEYWORDS

Alzheimer's disease, amyotrophic lateral sclerosis, fenestrated capillaries, glucose transporter, Huntington's disease, insulin resistance, neurodegeneration, Parkinson's disease

1 | BRAIN BARRIERS: HISTORIC PRELUDE

The concept of a selective barrier between the blood and the brain emerged from experiments performed by English physician Humphrey Ridley in 1695; when he injected quicksilver (mercury) into the circulation. Remarkably, the mercury did not penetrate nervous tissue.[1] More evidence came in the 1880s when Paul Ehrlich injected aniline dyes (alizarin blue and indophenol blue) intravenously. While these dyes stained most tissues, they did not label the nervous system. However, Ehrlich attributed this difference to varying tissue affinities rather than a distinct barrier.[2–4] By the late 19th century, pharmacological studies provided further support for the barrier concept. Bield, Kraus, and Lewandowsky, observed that certain toxins (e.g., cholic acids or sodium ferrocyanide) had no neurological effects when injected intravenously but caused significant effects when administered intraventricularly.[5,6] In 1913, Max Goldmann advanced Ehrlich's work by showing that trypan blue injected into the lumbar subarachnoid space stained only the spinal cord and central nervous system (CNS), while intravenous injections stained the body but not the nervous system. Goldmann proposed the idea of the existence of *Physiologische Grenzmembran* ("physiological boundary membrane") or the border between blood circulation and nervous tissue.[7,8] Finally, the concept of the blood–brain barrier (BBB) was formally articulated by Lina Stern and her colleague Raymond Gautier. They introduced the term *barrière hémato-encéphalique* and outlined principles of barrier selectivity and barrier resistance, which remain foundational to our understanding of the BBB today.[9–11]

2 | COMPOSITION OF BBB

The barriers fencing the brain from the rest of the body emerged early in evolution. These barriers were formed by glial cells and sealed with septate junctions.[12] Glial barriers are also present in early vertebrates, including elasmobranchii and chondrostei; starting with gnathostomata (or jawed vertebrates), the BBB is secured by tight junctions between brain endotheliocytes.[12,13] There are several cellular barriers separating the brain from biological fluids represented by the blood in circulation and by cerebrospinal fluid (CSF) in the CNS[14] (Figure 1). These barriers are fundamental for the CNS homeostatic system that dynamically limits and regulates molecular exchange between the blood and the nervous tissue. The brain tissue is separated from the blood by four barrier systems: (i) the blood–brain and blood-spinal cord barrier (BBB and BSCB, respectively) between the intracerebral and intraspinal blood vessels and the brain parenchyma; the barrier is made by tight junctions clasping together endothelial cells of the blood vessels; (ii) the arachnoid blood–CSF barrier separating the subarachnoid CSF from the blood and sealed by tight junctions between the cells of the arachnoid mater; (iii) the hypothalamic blood–cerebrospinal barrier secured by tight junctions between somata of tanycytes and (iv) the choroid plexus blood-CSF barrier in the ventricles of the brain, this barrier is sealed by tight junctions between the choroid ependymocytes. In addition, there is an ependymal barrier lining the walls of the ventricles and the central canal of the spinal cord and separating the CSF from the nervous tissue. This barrier, however, is only partial, as postnatal ependymocytes do not express tight junctions; adherent junctions and desmosomes create certain diffusion barriers, while ependymoglial cells, by changing their volume, can regulate cerebrospinal fluids flux into the nervous tissue and peripheral nerves.[15] In this review, we shall only focus on the BBB and only on the capillary section of the BBB as the latter shows a degree of heterogeneity across the vascular tree in the CNS.[16]

The BBB at the level of microvessels separates the blood from the CNS[17–20] (Figure 2). The BBB is a complex structure made by several cell types which together compose the neurovascular (also known as neurogliovascular) unit.[21] The luminal side of the BBB includes glycocalyx, the monolayer of brain endothelial cells, and the vascular basement membrane. Glycocalyx, composed of proteoglycans and glycoproteins, separates the blood from the endothelial monolayer, thus preventing direct contact of blood cells and plasma constituencies with the surface

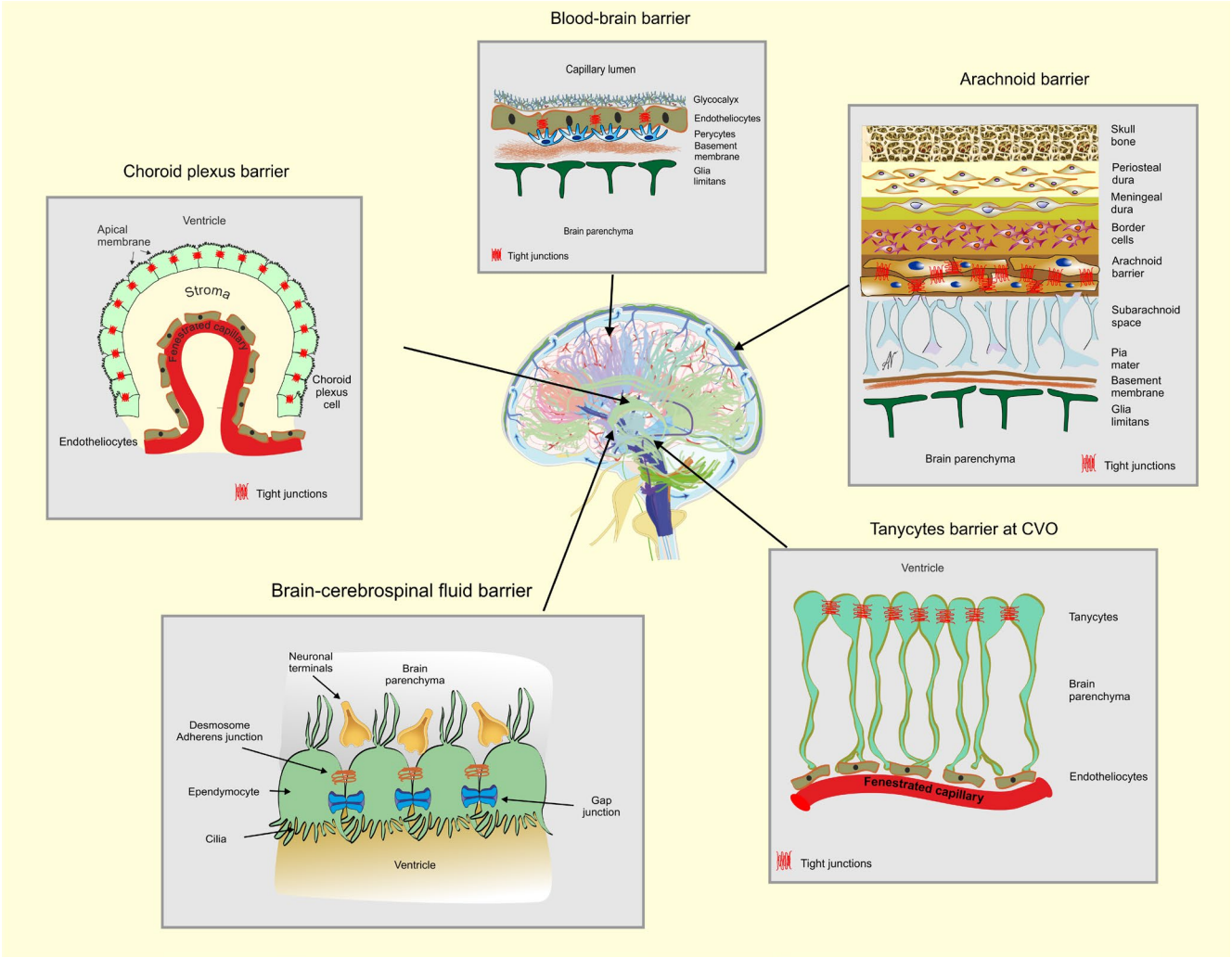

FIGURE 1 Brain barriers. The main brain barriers include (form the top clockwise): The blood brain barrier (secured by tight junctions between brain endothelial cells), the arachnoid barrier (secured by tight junctions between arachnoid epithelial cells), tanycytes barrier at the circumventricular organs (secured by tight junctions between tanycytes somata), brain-cerebrospinal barrier (partial barrier enforced by desmosomes and adherens junctions), and choroid plexus barrier (sealed by tight junctions between cuboid ependymogliocytes, also known as choroid plexus cells). Modified from Verkhratsky & Butt (2023).

of endotheliocytes.[22] Brain endothelial cells are simple squamous epithelial cells of mesodermal origin, with an average length of endotheliocyte of 30–50 μm, width of 10–50 μm, and thickness between 0.1 and 10 μm.[23] The human brain contains ~5–10 billion of endotheliocytes. The brain endothelial cells express tight and adherent junctions which clasp these cells tightly together and seal the barrier by preventing paracellular flux of hydrophilic molecules or invasion of blood cells. The adherent junctions are composed of cadherin adhesion molecules.[24] The tight junctions form electron-dense junctional plaques made from occludin and claudins together with zonula occludens proteins.[25] These tight junctions restrict paracellular diffusion and in particular limit ion fluxes, which translates into very high (up to 5000 Ω/cm^2) electrical resistance of the brain endothelial barrier that is >100 times larger than in peripheral capillaries where the transendothelial resistance varies between 2 and 20 Ω/cm^2.[26,27] In addition to tight junctions, endothelial cells express Connexin 43 (Cx43), a key connexin in brain endothelial cells that forms gap junctions and primarily enables direct intercellular communication and signal transduction. Endothelial Cx43 plays a role in regulating barrier function and contributes to responses to injury, inflammation, and metabolic homeostasis.[28,29] Endotheliocytes are responsible for the selective transport of various molecules between the circulation and brain parenchyma; this transport is mediated by membrane transporters, transcytosis, and the transcellular lipophilic route.[17,30] Endothelial cells on microvessels are closely contacted by pericytes; both endothelial cells and pericytes share the same basement membrane. The brain contains several types of pericytes

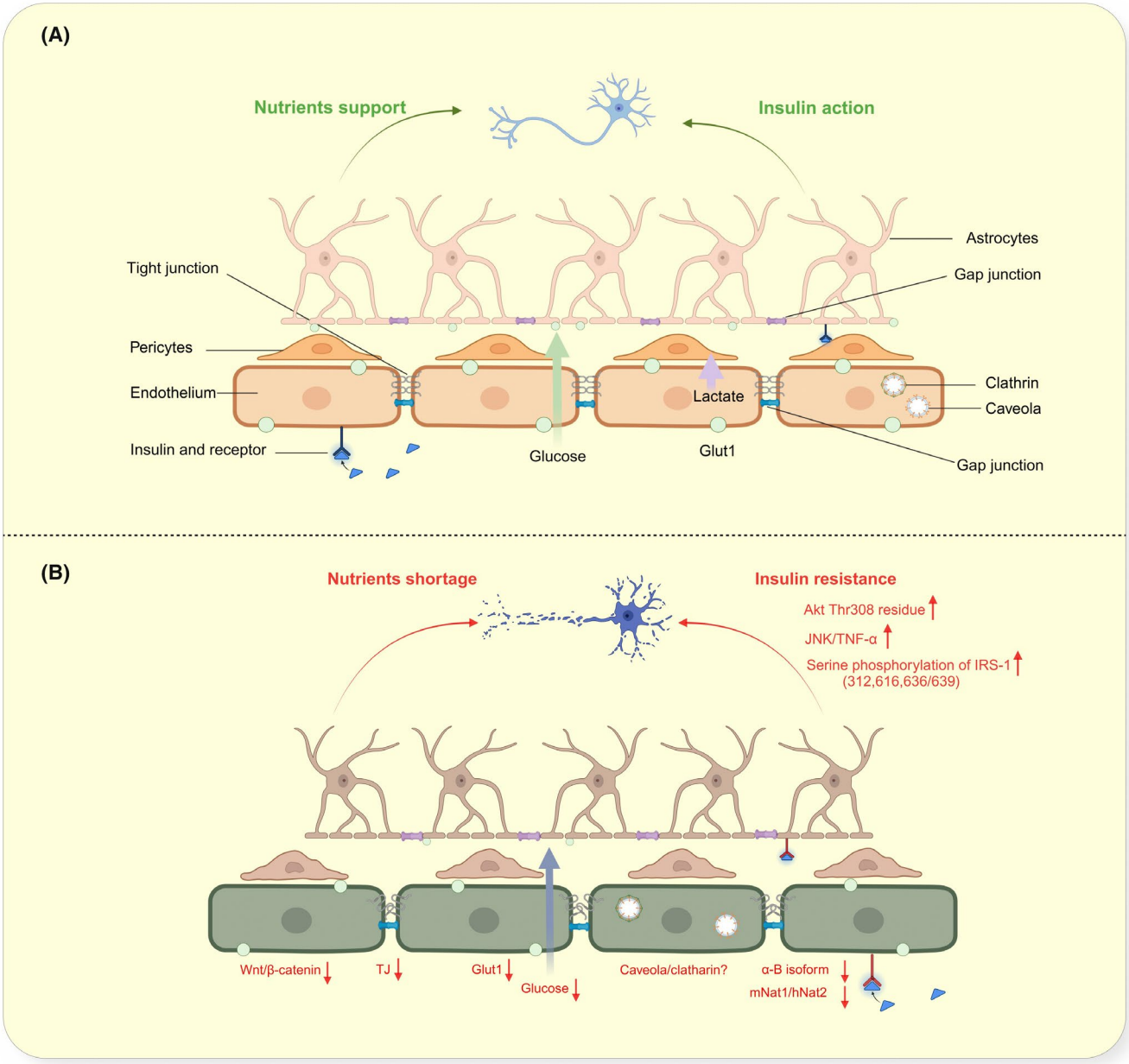

FIGURE 2 Blood–brain barrier (BBB) structure and function in physiological status and Alzheimer's disease (AD). Upper panel (A) The main components of the BBB, including endothelial cells (sealed by tight junctions), pericytes, and astrocytes (connected by gap junctions). The illustration highlights glucose transport and insulin signaling. Arrows indicate nutrient transport (glucose and lactate) and metabolic interactions at the BBB. Lower panel (B) Insulin resistance and nutrient shortage are highlighted as key factors in AD, with arrows indicating disrupted glucose transport and insulin action. Molecular changes include decreased Glut1 expression, Wnt/β-catenin signaling malfunction, reduced tight junction integrity, altered insulin signaling, and aberrant transcytosis (clathrin/caveola), leading to impaired nutrient transport and overall BBB malfunction in the AD state.

differentially covering capillaries as well as pre- and post-capillary vessels; helical pericytes associate with capillaries, while hybrid and mesh pericytes with pre- and postcapillaries.[31]

The parenchymal part of the BBB is represented by glia limitans formed by astrocyte endfeet and parenchymal basement membrane. At arterioles and venules, vascular and parenchymal membranes are separated by perivascular space, which represents the anatomical substrate of the glymphatic system[32]; at the capillary level, both basement membranes join, and the perivascular space disappears. Astrocytes exert multiple effects on the endothelial barrier, regulating its development and expression of tight junctions.[13] Complex BBB is fundamental in protecting the brain from blood-borne toxins and pathogens while allowing essential nutrients and other

molecules to pass through. Its unique structure ensures the homeostasis of the brain microenvironment, which is the key to proper cellular function and overall CNS health.

The BBB is heterogeneous throughout the brain. In several brain regions, most notable in the hypothalamus and circumventricular organs, including median eminence, the organum vasculosum of the lamina terminals, and the subfornical organ[33] capillary walls are composed of fenestrated endothelium with transcellular pores to allow the passage of small and medium-sized molecules (such as glucose [180 Da], ghrelin [3.3 kDa],[34] insulin [5.8 kDa],[35,36] and leptin [16 kDa][36,37]), and direct access of macronutrients and hormones to hypothalamic neurones, allowing chemosensing, rapid response to nutrient availability and regulation of systemic energy homeostasis.[33] The fenestrae are transcellular pores extending through the whole thickness of endotheliocytes, 50–60 nm in diameter, covered by a thin (5–6 nm) nonmembranous diaphragm composed of radial fibrils.[38,39] Molecular characteristics of the fenestrated endothelium remain largely unknown. The only reasonably well studied component of the fenestrated diaphragm is plasmalemma vesicle-associated protein (PLVAP), a type II transmembrane protein with a molecular weight of ~60 kDa. It has an extracellular domain at the C-terminus consisting of around 390 amino acids, a single transmembrane domain, and a cytoplasmic domain at the N-terminus consisting of 26 amino acids.[40] PLVAP regulates the permeability of fenestrae through its unique structural characteristics. It forms thin fibrils that originate at the inner surfaces of endothelial cells and intertwine to create a central knot-like structure within diaphragms. This configuration acts as a filter, allowing selective passage of molecules. Notably, these diaphragms extend beyond simply covering the fenestrae; they also envelop the openings of caveolae and transendothelial channels, ensuring comprehensive regulation of permeability across various endothelial structures.[41] Although PLVAP is involved in maintaining the selective barrier properties of the fenestrated BBB, it is also widely expressed in fenestrated endothelium throughout the body, including kidneys, gastrointestinal mucosa, and endocrine organs.[42] In these tissues, PLVAP regulates vascular permeability to maintain tissue-specific homeostasis. For instance, in kidneys, PLVAP contributes to the filtration barrier.[43] The loss of PLVAP compromises the selective barrier function, resulting in increased permeability and aberrant vascular homeostasis. Consequently, PLVAP knockout mice develop subcutaneous edema, hemorrhages, and cardiovascular defects.[38] Therefore, PLVAP is not merely a structural component but a critical determinant of endothelial cells and related organ function, providing a compelling rationale for further research into its role in vascular homeostasis. However, studies on PLVAP, specifically knockout at the BBB, are still lacking, leaving the neurological implications of PLVAP loss unclear.

3 | GLUCOSE METABOLISM IN THE BBB

The brain constitutes approximately 2% of the body weight, however utilises around 20% of total glucose-derived energy in the body, which equates to ~120 g of glucose per day.[44] This percentage is even higher in children, with the developing brain consuming up to 50% of the body's total energy supply during the first decade of life.[45] Notably, this energy demand in youngsters is met not only by glucose but also by other substrates, such as ketone bodies.[46] Glucose transportation and metabolism in the CNS are often independent of insulin signaling, this being distinct from the glucose deposition organs, such as the skeletal muscle and adipocytes. This difference reflects the difference in glucose transporters expressed in the cell membrane. The glucose transporter (Glut/SLC2A) family is responsible for transporting glucose and other substrates like fructose, myoinositol, and urate into the cells and consists of 14 members in humans.[47] While the specific roles of many Glut proteins remain unclear, Glut1-4 are well-characterised for their critical roles in glucose homeostasis, with distinct regulatory and kinetic properties.[47] These transporters are largely conserved between humans and rodents. Among the Glut family, Glut1, Glut2, Glut4, and Glut5 are operational in the BBB.

3.1 | Glucose transporters

Glucose transporter-1 (Glut1) has been comprehensively characterised. Glut1 has a high affinity for glucose, with a K_m value of 0.7–3.2 mM, which allows it to effectively transport glucose into cells even at low extracellular glucose concentrations.[48] It is widely expressed in cell membranes across multiple cell types, including erythrocytes, BBB endothelial cells, and astrocytes. This transporter is also critical in facilitating the transfer of glucose from the maternal circulation to the foetal blood in the placenta, which is the key to maintaining an adequate glucose supply to the developing foetus.[49] In the CNS, Glut1 is the major protein in the endothelial cells and astrocytes at the BBB mediating glucose transport from blood into the brain.[50,51] In mice, a 67% reduction of Glut1 levels in the brain endothelial cells led to 100% mortality within 4 days following tamoxifen-induced Glut1 deletion, while a 40% reduction of Glut1 levels resulted in 50% mortality over

28 days,[50] suggesting the principal role of Glut1 in maintaining brain energy supply and survival.

Glucose transporter-2 (Glut2) is a high-capacity, low-affinity glucose transporter with a K_m value of approximately 17 mM, indicating its operation at high glucose concentrations.[52] It is predominantly expressed in the liver, pancreatic β cells, kidneys, intestines, and CNS. In pancreatic β cells, Glut2 enables sensing the fluctuation of extracellular glucose levels, which is essential for regulating insulin secretion to maintain postprandial glucose levels.[52] In the CNS, Glut2 plays a vital role in ensuring the proper central regulation of peripheral glucagon secretion, a key process necessary for the prevention of hypoglycaemia.[53,54] In particular, Glut2 is expressed in the hypothalamic and brainstem neurones to regulate feeding behaviour and glucagon secretion. Specifically, Glut2-expressing neurones are activated when glucose levels fall, triggering a cascade of cellular events, including increased activity of AMP-activated protein kinase and the closure of potassium channels. This enhances parasympathetic nerve activity, effectively linking the detection of hypoglycaemia to increased vagal output and the stimulation of glucagon secretion by the pancreas.[54–56] At the BBB, Glut2 is expressed in astrocytes.[57,58] In a genetically modified mouse model lacking Glut2 expression (ripglut1:glut2$^{-/-}$ mice), glucose sensors dependent on Glut2 are predominantly located in astrocytes rather than neurones.[53] Glut2 deficiency was compensated by increased Glut1 levels to maintain insulin secretion in response to postprandial glucose increase. However, the absence of systemic Glut2 results in malfunctional glucagon secretion. The restoration of Glut2 in astrocytes, but not neurones, in ripglut1:glut2$^{-/-}$ mice restored peripheral glucagon secretion in response to physiological hypoglycaemia,[53] suggesting the importance of astrocytic Glut2 and related glucose metabolism in astrocytes.

Glucose transporter-4 (Glut4) is an insulin-responsive glucose transporter predominantly expressed in the myocytes and adipocytes.[59,60] Glut4 is also located in the vascular structures within the ventromedial nucleus of the hypothalamus, co-localising with Glut1 and the endothelial tight junction protein Zonula Occludens-1 (ZO-1).[61] Moreover, it is also found in the rat forebrain endothelium.[62] Although astrocytes appear to express the glucose transporter Glut4, insulin does not regulate glucose entry across the astrocyte plasma membrane.[63] However, the role of Glut4 in the BBB remains largely unknown, and neither are Glut4 interactions with other glucose transporters and the resulting effects on brain glucose uptake and overall energy balance.

Glucose transporter-5 (Glut5) exclusively transports fructose, which being primarily involved in fructose absorption in the enterocytes of the small intestine.[64] Glut5 is also present in the testis, kidney, muscle, adipocytes, and the brain, where it may mediate fructose intake.[65] However, when radiolabeled fructose was injected into rat arteries, little fructose accumulation was detected in the brain, indicating that Glut5 on BBB may be quiescent under physiological conditions.[66] A remarkable discovery in the African naked mole-rat demonstrates that fructose transportation in BBB may be operational under extreme circumstances.[67] These rats can endure severe hypoxic conditions and survive up to 18 minutes by shifting to anaerobic metabolism, utilising fructose as the primary energy source.[67] This metabolic switch allows fructose to substitute glucose as an energy source in the brain, enabling short-term survival in oxygen-deprived environments.[67]

3.2 | Glucose metabolism in cells of BBB

Endothelial cells in different tissues are metabolically heterogeneous.[68] Brain endothelial cells express 55-kDa Glut1 isoform and generate ATP primarily through anaerobic glycolysis despite their direct exposure to the blood and ample oxygen availability.[69,70] This conclusion is based on the analysis of key metabolic pathways in brain endothelial cells, which predominantly rely on anaerobic glycolysis for ATP production, rather than the tricarboxylic acid (TCA) cycle. This notion is supported by several key findings: (i) glycolytic enzymes are expressed at higher levels in brain endothelial cells compared with TCA cycle enzymes, indicating that glycolysis plays a dominant role; (ii) metabolite analysis using capillary electrophoresis mass spectrometry revealed high levels of key glycolytic intermediates, such as fructose-1,6-bisphosphate, glyceraldehyde-3-phosphate, and 3-phosphoglycerate, further confirming the active glycolytic state; the lactate-to-pyruvate ratio was 1.5:1, suggesting a preference for lactate production via anaerobic glycolysis; and (iii) key TCA cycle metabolites like acetyl-CoA, isocitrate, fumarate, and malate were found at low levels, again indicating that the TCA cycle is less active in these cells.[70] It is well-established that the energy yield of glycolysis (2 molecules of ATP per 1 molecule of glucose) is much lower than oxidative phosphorylation (OXPHOS, 36 molecules of ATP per 1 molecule of glucose). Why do endothelial cells thus not use the abundant oxygen resources but generate energy less efficiently? This paradoxical preference is arguably linked to their anatomical position and physiological demands: (i) endothelial cells prioritise sufficient oxygen delivery to neurones, which favour aerobic glucose metabolism that is critical for maintaining normal neuronal function.[71] (ii) endothelial cells possess a relatively

small amount of mitochondria, less than 5% of total cellular volume, compared with approximately 28% in hepatocytes.[72] (iii) glycolysis generates lower amounts of reactive oxygen species, minimising potential damage to blood vessels.[73] Anaerobic glycolysis not only supports energy production in endothelial cells but also plays a crucial role in regulating their functions. For example, inhibition of 6-phosphofructo-2-kinase/ fructose-2,6-bisphosphatase 3, a key enzyme in the regulation of glycolysis, suppresses vessel sprouting in endothelial spheroids, zebrafish embryos, and postnatal mouse retinas by impairing endotheliocytes proliferation and migration.[74] Moreover, fructose-1,6-bisphosphate, a glycolytic intermediate, alleviates LPS-induced BBB dysfunction by maintaining endothelial junctional integrity.[75] Finally, blocking glycolysis in the mouse brain through the administration of 2-deoxyglucose led to a reduction in the transcellular permeability of the BBB.[70]

The glucose utilization in pericytes remains largely unexplored. Arguably, glycolysis in the brain endothelial cells produces lactate, which is subsequently taken up by pericytes to support their energy needs.[76] The proximity between brain endothelial cells and pericytes ensures efficient lactate transfer.[76] Thus, the role of glucose metabolism in normal pericyte function may be marginal.

Astrocytic glucose uptake is partially mediated by Glut1. Astrocytes express 45-kDa Glut1 isoform[77] in the cell body and endfeet, to accumulate glucose from the extracellular space.[78] While neurones were traditionally viewed as the primary energy consumers in the brain, new evidence shows that astrocytes also have substantial energy demands. Astrocytes actively use Na^+/K^+ ATPase for potassium buffering during synaptic activity, which significantly increases ATP consumption. This process highlights the importance of astrocytic oxidative metabolism, as mitochondrial ATP production plays a larger role than previously thought (more than 75% of ATP produced by astrocytes has been demonstrated to originate from mitochondrial processes).[79] In addition to potassium, astrocytes are also influenced by other extracellular factors. For instance, extracellular ATP leads to a decrease in astrocytes glucose concentrations, while neurotransmitters like glutamate and noradrenaline increase glucose uptake.[80] Additionally, astrocytes demonstrate metabolic flexibility, using both glycolysis and oxidative phosphorylation.[79]

Glucose can be transferred to distal neurones through astroglial syncytium supported by gap junctions composed of connexin 43 and connexin 30.[81] Tracking of fluorescent glucose derivative 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino]-2-deoxyglucose (2-NBDG) demonstrated its trafficking through the astrocytic syncytium in acute hippocampal slices. Deletion of astrocytic connexin 30 reduced glucose traffic by 35% in astrocytes lacking connexin 30, while deletion of connexin 43 reduced it by 50%.[81] In double-knockout mice where both connexins 30 and 43 were deleted, glucose trafficking was completely abolished.[81] Moreover, gap junctions in astrocytes preferentially transport glucose over glucose-6-phosphate, an intermediate in glycolysis.[81] Excess glucose-6-phosphate can be stored as glycogen within astrocytes, serving as an energy reserve that can be mobilised during periods of high neuronal demand or glucose scarcity, although the pool is very small and only lasts for minutes.[82,83] During the suckling period or long-term starvation, when blood ketone levels are elevated, astrocytes can utilise ketone bodies as an alternative fuel source.[84,85] These ketone bodies are then metabolised into acetyl-CoA, which can enter the TCA cycle to produce ATP.[83]

4 | INSULIN ACTION ON THE BBB

Human insulin is a 51-amino acid peptide hormone produced by pancreatic β-cells. Insulin is primarily recognised for its role in lowering plasma glucose levels through facilitating glucose uptake by insulin-responsive glucose transporters, particularly in skeletal muscle and adipocytes, and by suppressing glucose production (gluconeogenesis) and glucose release in the liver.[86] Insulin also acts as an anabolic hormone, promoting the uptake of fatty acids and amino acids, as well as supporting energy storage and cellular growth.[86] Insulin exerts its biological functions by interacting with the insulin receptors located on the membrane of target cells. The insulin receptor is a transmembrane tyrosine kinase composed of two extracellular α subunits that bind insulin and two β subunits that contain the intracellular tyrosine kinase catalytic domain. When insulin binds to the α subunits, it induces a conformational change in the receptor, leading to autophosphorylation and activation of the β subunits.[87] This further activates signaling pathways mediating mitogenic and metabolic activities.[88] The phosphoinositide 3-kinase/protein kinase B (PI3K-PKB/Akt) pathway, activating downstream of the insulin receptor, regulates most metabolic effects. Upon activation, Akt facilitates the translocation of Glut4 from intracellular vesicles to the cell membrane, particularly in insulin-responsive cells, such as skeletal muscle and adipocytes. This translocation of Glut4 is a key step that allows glucose to be effectively taken up into the cell for glycolysis and/or glycogen synthesis.[88]

On the contrary, the mitogenic or mitogen-activated protein kinase (MAPK) pathway regulates cell growth and differentiation.[88] It was shown that blood insulin level determines the activation of these pathways, with the metabolic pathway being activated at lower insulin levels compared with the mitogenic pathway.[89]

It is generally accepted that the BBB, in addition to protecting the brain, acts as an endocrine organ.[90] BBB responds to systemic endocrine signals by regulating hormone transport across the BBB (e.g., insulin,[35] leptin,[37] and ghrelin[34]) and actively participates in secretion. Brain endothelial cells secrete various substances, such as nitric oxide and cytokines, such as IL-6, which play a role in modulating CNS function and maintaining homeostasis.[90] Dysregulation of hormone signaling pathways at the BBB can lead to metabolic disturbances in the brain, contributing to neurodegeneration. For instance, impaired insulin signaling at the BBB is linked to brain insulin resistance, a feature commonly observed in AD, which exacerbates neuronal malfunction and cognitive decline.[91,92] As such, BBB malfunction is involved in various endocrine and neurodegenerative diseases.

4.1 | Insulin transport through BBB

The mechanism by which insulin crosses the BBB remains a topic of ongoing debate. Insulin is transported into the CNS through a saturable process (with transport rates reported to average around 0.5–0.6 μL/g·min, although values vary across different studies, ranging from 0.2 to 1.7 μL/g·min[93]). The rate of transport reaches a maximum capacity when all the transporter proteins are fully occupied, indicating a limit of the substance can be moved despite increasing blood concentrations.[94] Insulin receptors in endothelial cells may facilitate receptor-mediated transcytosis of insulin,[95,96] which, however, was challenged by more recent research. Mice with either the loss or inhibition of insulin receptor signaling demonstrated that insulin transport can occur without insulin receptor.[94] Interrogation of an in vitro BBB model confirmed that although insulin receptors are expressed and functional in endothelial cells, insulin transport across the BBB does not primarily occur through receptor-mediated transcytosis.[97] The clathrin- or caveolin-mediated endocytosis was shown to provide an insulin receptor independent mechanism for insulin transport across the BBB.[98] Clathrin-mediated endocytosis is responsible for insulin surface binding in brain microvessels, while caveolin-mediated endocytosis for insulin transport has been observed in the hypothalamus.[98] However, in addition to the transport of insulin through fenestrae, as previously stated, other possible saturable mechanisms may also contribute to insulin transport. The difference in insulin concentrations between the circulation and the CNS is significant, with the CNS and CSF levels being ~10%–25% of those in the blood.[99]

4.2 | Insulin action in brain endothelial cells

Insulin regulates brain endothelial cell function, influencing various aspects of vascular homeostasis and BBB integrity. In 1985, insulin receptors were first identified in brain endothelial cells.[95] Endothelial-specific knockout of the insulin receptor driven by the manipulation of specific endothelial promoters results in distinct phenotypes.[96,100,101] Although the endothelial-specific knockout of insulin receptors using tyrosine kinase with immunoglobulin-like and EGF-like domains 2 promoter results in a significant reduction in key vasoactive mediators, such as endothelial nitric oxide synthase (which promotes vasodilation) and endothelin-1 (a potent vasoconstrictor), it fails to impact overall vascular development or baseline glucose homeostasis.[100] However, insulin resistance developed when the mice were fed a low-salt diet.[100] This condition-specific response indicates that insulin signaling in endothelial cells may interact with molecular pathways responding to a low-salt diet, such as the activation of the renin-angiotensin system (RAS),[102,103] which in turn contributes to the development of insulin resistance.[104] In line with this finding, another study investigated the impact of endothelial-specific insulin resistance by using a mouse model with an endothelium-specific overexpressing mutated human insulin receptor under the control of the Tie2 promoter.[101] The mutation (Ala-Thr1134) occurs in the tyrosine kinase domain that disrupts insulin signaling, resulting in insulin resistance.[105] However, baseline glucose homeostasis remained unchanged. In a mouse model of endothelial-specific insulin receptor knockout driven by the VE-cadherin promoter, activation of insulin signaling was delayed in peripheral tissues, such as skeletal muscle and brown adipose tissue, as well as in specific brain regions, including the hypothalamus, hippocampus, and cortex.[96] Impaired insulin signaling in BBB endotheliocytes has been also associated with reduced hypothalamic levels of pro-opiomelanocortin, resulting in increased food consumption and subsequent obesity.[96] Thus, insulin signaling in BBB endothelial cells contributes to regulating systemic insulin sensitivity and metabolic homeostasis.

Furthermore, endothelial insulin signaling can influence BBB permeability,[96,106] by regulating tight junction

protein expression in the endothelial cells. Insulin treatment in vitro caused a dose-dependent increase in transepithelial electrical resistance and restricted the passage of 4 and 70 kDa fluorescent dyes across the monolayer of human cerebral microvascular endothelial cells.[106] In mice, the loss of endothelial insulin receptors resulted in a 40% reduction in tight junction protein ZO-1 expression in the hypothalamus with impaired BBB integrity, as well as increased BBB permeability in the olfactory bulb and median eminence.[96]

4.3 | Insulin action in pericytes

Expression of functional insulin receptors in pericytes was found in 1983.[107] Subsequently, the expression of insulin receptors was confirmed in the brain and retinal pericytes.[108,109] There is a notably higher sensitivity to insulin-induced DNA synthesis in retinal pericytes compared with vascular smooth muscle cells.[107] Another study on pericyte insulin receptor knockout mice demonstrated that the absence of insulin receptors leads to significant retinal vascular remodeling and changes in endothelial angiopoietin signaling, emphasising the importance of insulin signaling in maintaining vascular anatomy.[109] While the role of insulin in retinal pericytes was intensively studied because pericyte loss is a hallmark of diabetic retinopathy, research on pericyte insulin action in other brain regions remains relatively sparse due to the lack of understanding of the physiological function and implication in neurovascular disease pathogenesis that can give a strong rationale to investigate insulin action in brain pericytes, in relation to BBB integrity.

4.4 | Insulin action in astrocytes

Although the widespread distribution of insulin receptors in the brain was first identified in 1978,[110] their specific presence in astrocytes was not recognised until 1986.[111,112] When compared to neurones, neuroglial cells exhibit significant structural and functional differences in their insulin receptors. Structurally, the β subunit of the insulin receptor in astrocytes has a molecular weight of approximately 97 kDa, which is similar to that of the insulin receptor in peripheral tissues, such as the liver (which also has a β subunit of 97 kDa). In contrast, the β subunit in neurons is smaller, with a molecular weight of about 92 kDa. This difference is largely due to variations in N-linked glycosylation between the two cell types. Functionally, the activation of insulin receptors in astrocytes leads to more pronounced effects compared with neurons. For instance, astrocytes exhibit higher sensitivity to insulin, particularly in regulating glucose uptake and metabolic activities. In contrast, the insulin receptor in neurons shows a weaker response to insulin binding.[111,113] This matter remains controversial. It has also been shown that insulin does not affect glucose uptake via Glut4 across the astrocyte plasma membrane. Instead, insulin treatment results in a reduction of cytosolic glucose levels, likely due to increased glucose utilisation for glycogen synthesis.[63] Insulin receptors on astrocytes contribute to the regulation of energy homeostasis, including eating behaviour.[114] In mice with postnatal deletion of insulin receptors in astrocytes, an abnormal increase in food intake after fasting was observed.[115] In a glucose-induced feeding suppression test, these mice showed an impaired ability to suppress fasting-induced hyperphagia in response to peripheral glucose administration. This phenomenon is likely due to insufficient or delayed glucose access to the brain, evidenced by a lower CSF/peripheral glucose ratio and reduced brain glucose uptake shown by PET imaging.[115]

Insulin receptors in astrocytes are also essential for proper neurovascular coupling.[116] Ablation of insulin receptor in astrocytes leads to reduced brain glucose uptake, probably due to concurrent reduction in Glut1 expression. Moreover, using single-photon emission computed tomography, it was found that astrocytic insulin receptor deletion in 3 months old mice led to significantly increased brain blood perfusion. However, as these mice aged (>1 year old), the brain perfusion was progressively and significantly decreased. The age-dependent alteration in brain blood perfusion was suggested to be associated with dysregulation of the hypoxia-inducible factor-1α and vascular endothelial growth factor (VEGF) pathways, resulting in abnormal angiogenic signaling. These findings suggest that insulin receptors in astrocytes contribute to age-dependent changes in brain blood flow.[116]

Astrocytes, particularly in the hypothalamus, impact systemic metabolism by influencing neuroendocrine signaling, modulating neural circuits involved in appetite and energy expenditure, and affecting autonomic functions like thermogenesis and glucose regulation.[114,117,118] A marked sexual dimorphism was also observed in mice with the deletion of astrocytic insulin receptors, albeit similarly decreased energy expenditure and body temperature at 7–9 months of age in both sexes.[119] This is because of an altered thermogenesis function of the brown adipose tissue in males reflected by significantly reduced uncoupling protein-1 and β3-adrenergic receptors; these changes were more pronounced in females.[119] Furthermore, male mice showed early-onset systemic insulin resistance at 2 months of age, while female

littermates did not develop glucose intolerance until 7 months of age.[119] Insulin synchronises the molecular clock in astrocytes by regulating key clock genes, such as *Per2, Cry1, Bmal1*, and *Dbp*; therefore, the deletion of astrocytic insulin receptors also affected circadian rhythms, again in a sex-dependent manner.[120] Female mice showed a significantly prolonged free-running period during the locomotor activity test, while male mice showed disrupted food entrainment. However, both males and females were resistant to high-fat diet-induced obesity, but through different adaptation mechanisms. Females reduced their intake of the high-fat diet and also increased energy expenditure, while males only had increased energy expenditure via thermogenesis.[120] Also, only female mice exhibited significantly improved glucose tolerance, although the underlying mechanism was not investigated.[120] Arguably, insulin signaling in astrocytes may be activated by other molecules which emerge in response to high-fat diet consumption in a sex-dependent manner.[121] This hypothesis requires further investigation, which may hold a key for effectively managing type 2 diabetes.

Loss of astrocytic insulin receptor signaling affects cognition and results in increased anxiety- and depressive-like behaviours in mice.[122] ATP exocytosis has been identified as a mediator in intercellular communication among neural cells.[123] Insulin can increase the tyrosine phosphorylation of Munc18c, which is critical for ATP exocytosis from astrocytes to regulate purinergic signaling to dopaminergic neurones.[122] The lack of insulin signaling activation in astrocytes due to insulin receptor knockout diminished the ability of Munc18c activation, resulting in reduced ATP release and dopamine secretion and consequently heightened anxiety and depression.[122]

5 | ALTERED GLUCOSE METABOLISM AND INSULIN ACTION IN NEURODEGENERATIVE DISEASES

Glucose metabolism and insulin signaling are disrupted in neurodegenerative diseases, including Alzheimer's disease (AD) and Parkinson's disease (PD). Huntington's disease (HD) has traditionally been viewed as a genetic neurodegenerative disorder. The metabolic changes in HD are often subtle and progress gradually, making them difficult to detect and study.[124] Hypermetabolism is a hallmark of many patients with amyotrophic lateral sclerosis (ALS), and interestingly, type 2 diabetes mellitus may paradoxically confer neuroprotection against ALS, potentially due to compensatory metabolic adaptations.[125] Overall, these metabolic dysfunctions highlight the interconnectedness of glucose metabolism and insulin signaling across various neurodegenerative diseases.

5.1 | Alzheimer's disease

BBB impairment plays a significant role in the progression of AD by disrupting the delicate environment in the brain, leading to increased vulnerability to neurodegenerative processes. In AD, the integrity of the BBB is compromised, allowing toxic molecules to enter brain tissue and disrupting normal energy supply. BBB leakage exacerbates inflammation, oxidative stress, and neuronal damage, contributing to the worsening of AD pathology.[126–129]

The reduction in brain Glut1 levels correlates with AD aetiology. Post-mortem analyses of brains from AD patients revealed a reduction in Glut1 level across various brain regions, including cortex, hippocampus, and caudate nucleus,[130,131] which is closely associated with the severity of AD-related pathologies.[132] An animal study showed that Glut1 deficiency in brain endothelial cells, but not in astrocytes, can exacerbate AD due to BBB breakdown, which subsequently accelerates Aβ accumulation and cognitive impairment.[133] Furthermore, Glut1 expression in brain endothelial cells can rapidly respond to short-term high-fat diet feeding by reducing mRNA expression and protein levels, and subsequently decreasing endothelial glucose uptake in mice.[50] A compensatory mechanism, involving the upregulation of VEGF in perivascular macrophages, restores brain endothelial Glut1 expression and maintains brain glucose uptake.[50] In the APP/PS1 mouse model of AD, the absence of myeloid-cell-derived VEGF was linked to accelerated neurodegeneration, marked by increased neuroinflammation and cognitive decline, independent of amyloid plaque burden,[50] suggestive of the concurrent presence of vascular dementia. We found that the suppression of Wnt/β-catenin signaling in brain endothelial cells by Aβ oligomers exacerbates BBB dysfunction and impairs Glut1 expression. It has been shown that targeting LRP6, an upstream regulator of Wnt/β-catenin signaling, can reverse these pathological changes, and restore Glut1 levels and BBB integrity in AD models (Figure 3).[131] Additionally, death receptor 6 (DR6) downregulation in brain endothelial cells has been linked to decreased Glut1 expression, mediated through JNK signaling. By upregulating DR6 expression in brain endothelial cells, it is possible to counteract Aβ-induced BBB breakdown, which may protect against Glut1 loss and improve glucose uptake, potentially slowing AD progression (Figure 3).[134] A recent study revealed that astrocytes, but not neurons, express

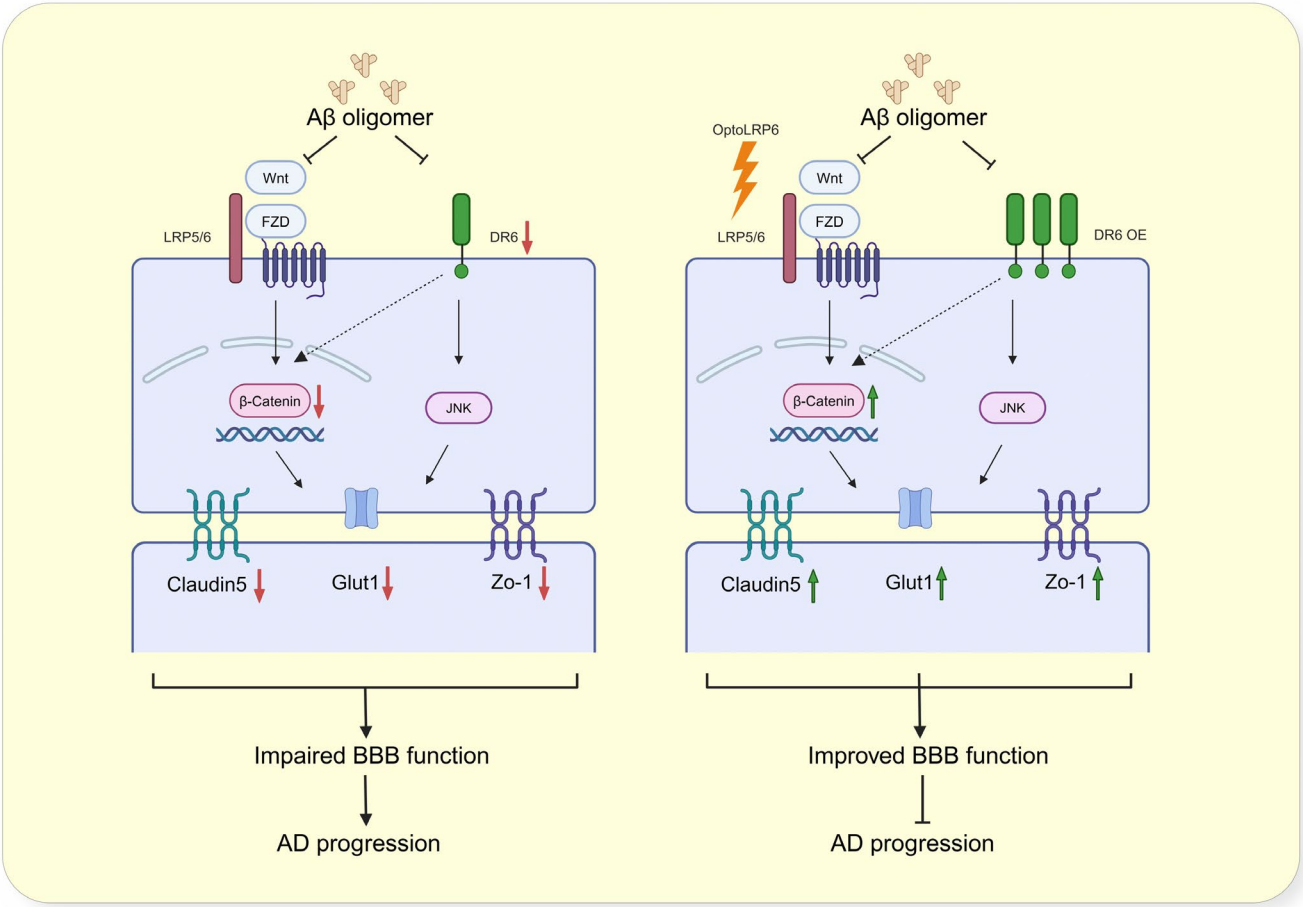

FIGURE 3 Mechanisms of blood–brain barrier (BBB) malfunction in Alzheimer's disease (AD). Targeting LRP6 in the Wnt/β-catenin pathway in brain endothelial cells can alleviate BBB dysfunction in AD. Aβ oligomers suppress this pathway in brain endothelial cells, reducing the expression of functional endothelial proteins and compromising BBB integrity. Newly developed optogenetic tool enables the precise control of lipoprotein receptor-related protein 6 (LRP6), the upstream regulator of Wnt/β-catenin signaling. Activating this pathway restores brain endothelial cells function by counteracting and preventing Aβ-induced pathological changes. (ii) Targeting death receptor 6 (DR6) to prevent BBB dysfunction in AD. Aβ oligomers suppress DR6 in brain endothelial cells, diminishing the expression of endothelial functional proteins (Cldn-5, Zo-1, and Glut1), primarily via JNK signaling. Additionally, Aβ oligomers inhibit the Wnt/β-catenin pathway, which interacts with DR6, further accelerating AD progression. Enhancing DR6 expression in brain endothelial cells can counteract Aβ-induced BBB dysfunction by simultaneously activating JNK and Wnt/β-catenin signaling.

the enzyme indoleamine-2,3-dioxygenase 1 (IDO1), which metabolises tryptophan into kynurenine (KYN). The increased production of KYN by astrocytic IDO1 disrupts the balance between aryl hydrocarbon receptor and hypoxia-inducible factor 1-α nuclear signaling, thus reducing astrocytic glycolysis, lactate production, and metabolic support to the neurons. Inhibiting IDO1 improves hippocampal glucose metabolism and rescues cognitive function in mouse models of AD. These findings suggest that IDO1 inhibitors, initially developed for cancer treatment, can be potential therapeutic agents for AD.[135]

Type 2 diabetes mellitus is recognised as a risk factor for the initiation and development of AD driven by brain insulin resistance.[136] Observations of reduced insulin receptors and signaling elements in the AD brains led to the term "type 3 diabetes," highlighting brain insulin resistance in neurodegeneration[137] (Figure 2). The reasons for impaired brain insulin signaling during type 2 diabetes were comprehensively reviewed,[137] including reduced brain endogenous insulin production (although whether insulin is synthesised in the brain remains controversial), decreased insulin transport across the BBB, reduced insulin receptors on BBB components or neurons, or insulin resistance on BBB components or neurons. Human post-mortem studies also showed significant abnormalities in the levels of insulin signaling elements in AD brains.[138–140] In AD brains, particularly in the hippocampus and entorhinal cortex, the insulin signaling pathway is disrupted, with PKB being abnormally overactivated at the Thr308 residue (p-Thr308) in neurones. Activated PKB (p-Thr308) is found, especially in neurons that are known

to later develop neurofibrillary tangles, closely linked to tau hyperphosphorylation.[139] Furthermore, $A\beta_{1-42}$ activates the JNK/TNF-α pathway, leading to increased serine phosphorylation of insulin receptor substrate-1 (IRS-1) to inhibit insulin signaling activities, akin to peripheral insulin resistance in type 2 diabetes.[141] Serine phosphorylation of IRS-1 is markedly elevated at specific residues in AD brains, including serine residues 312,[142] 616,[138,142] 636/639.[138] These observations suggest that impaired brain insulin signaling contributes to the pathogenesis of AD, especially in the setting of type 2 diabetes, mirroring the insulin resistance observed in peripheral insulin-responsive organs. Restoring insulin sensitivity in the brain can possess the potential therapeutic value in mitigating AD progression.

Cerebrovascular insulin receptors, particularly the insulin receptor α-B isoform, are significantly reduced in both human AD and 3xTg-AD mice brains, especially in the microvessels of the parietal cortex.[91] This reduction correlates with cognitive decline and increased Aβ plaques and β-site APP cleaving enzyme 1 levels.[91] Diminished insulin receptor α-B isoform in the microvessels is also accompanied by impaired insulin receptors responding to circulating insulin in the 3xTg-AD mouse model, contributing to BBB insulin resistance.[91] Knockout of insulin receptors in astrocytes exacerbates AD-like phenotypes, characterised by increased phosphorylation of tau protein, enlarged Aβ plaque size, and impaired Aβ uptake in the 5xFAD mouse model of AD.[92] In addition, the intracellular trafficking of insulin receptors also impacts insulin signaling. Caveolin-1 is essential for stabilising insulin receptors in lipid rafts, and the ablation of Caveolin-1 in endothelial cells resulted in reduced insulin responsiveness and decreased insulin uptake.[143] Caveolin-1 expression is upregulated in the brains of AD patients, particularly in the hippocampus, compared with age-matched controls, suggesting an unsuccessful adaptation of Caveolin-1 to rescue reduced insulin signaling to AD pathology.[144]

A nonsynonymous variant (803A > G, K268R) in human N-acetyltransferase 2 (hNat2), the ortholog of murine N-acetyltransferase 1 (mNat1) linked to insulin resistance plays a significant role in AD pathology.[145,146] Reduced mNat1/hNat2 in brain endothelial cells was observed in both APP/PS1 mice and AD patients and is closely linked to endothelial cell necroptosis, which compromises BBB integrity and exacerbates Aβ accumulation.[146] Selective restoration of mNat1 expression in brain endothelial cells can inhibit endothelial necroptosis, preserve BBB integrity, and reduce Aβ deposition, resulting in improved cognitive function in AD mice.[146] Therefore, mNat1 may represent a potential new target for managing AD.

5.2 | Parkinson's disease

PD is marked by the loss of dopaminergic neurones in the substantia nigra, resulting in uninhibited motor symptoms, such as tremors, rigidity, bradykinesia, and postural instability.[147] PD is increasingly linked to glucose metabolic disorders,[148–150] while insulin resistance is also common in PD patients.[151–155]

Using ^{18}F-FDG PET imaging across three European cohorts of PD patients, glucose hypermetabolism was detected in the thalamus, putamen/pallidum, pons, cerebellum, and motor cortex, while glucose hypometabolism was observed in the posterior parietal, occipital, and frontal cortices.[156] Significant cortical glucose hypometabolism was observed in the parietal and occipital cortices of the PD patients with basal forebrain atrophy, a condition associated with severe cognitive impairment.[157] This finding highlights a direct link between reduced glucose metabolism and cognitive decline, possibly due to cellular energy deficiency. Using machine learning, FDG-PET imaging of glucose metabolic profile was employed to predict cognitive decline in PD among patients with mild cognitive impairment who are at risk of progressing to PD dementia.[158] Treatment with levodopa leads to a noticeable decrease in overall brain glucose consumption, which is reversible upon withdrawal.[159] These observations suggest that abnormalities in glucose metabolism in PD are more complex and exhibit greater region-specificity in the brain compared with AD.

Although disruptions in insulin signaling were found in PD, insulin contribution to PD pathogenesis remains unclear. Peripheral insulin resistance may not be the primary driver in PD,[160] as medication-free PD patients exhibited comparable insulin sensitivity to age-, sex-, fat-, and lean body mass-matched healthy controls. Insulin malfunction within the regions critical for motor and cognitive functions may play a significant role in PD pathogenesis. Increased serine phosphorylation of IRS-2 in rats with severe dopamine deficiency was reported in a rat model of PD.[161] A significant increase in phosphorylation of S312 on IRS-1 was also observed in substantia nigra and putamen, the key to the development of PD, which is closely associated with the presence of Lewy bodies, suggesting a potential link between insulin resistance and the onset of PD.[162]

BBB permeability is significantly higher in PD patients compared with healthy controls, particularly in the substantia nigra, white matter regardless of being healthy or lesioned, and posterior cortex.[163] Another study demonstrated increased BBB permeability in the striatum of PD patients, evidenced by erythrocyte extravasation, hemosiderin deposits, and serum protein leakage, especially in the post-commissural putamen.

This compromised barrier may allow blood-borne toxins to reach the striatum, potentially triggering α-synuclein aggregation.[164] VEGF upregulation, as seen in the substantia nigra of PD patients, could reflect an adaptive response to support neurogenesis and vascular repair, although it may also inadvertently promote BBB permeability.[165] However, there remains a notable lack of research on the role of insulin action, specifically at the BBB level in PD brains. Understanding how insulin signaling interacts with BBB in PD is crucial. This gap in knowledge represents an important area for future studies, as insights into insulin's impact on BBB dynamics in PD may open new therapeutic avenues and enhance our understanding of disease progression.

6 | CONCLUSIONS

Glucose transporters, especially Glut1, facilitate glucose entry into the brain, supporting neuronal function and overall brain health. BBB has unique characteristics in glucose metabolism, while insulin signaling mirrors that of peripheral tissues. Thus, the actions of glucose and insulin at the BBB influence systemic energy balance and play a significant role in the initiation and progression of neurodegenerative diseases. In the CNS, insulin activity can regulate cognitive functions, synaptic plasticity, and overall brain health, while central insulin resistance both at BBB and in the brain has been also increasingly recognised as a contributing factor to neurodegenerative diseases. The BBB malfunction is a critical aspect of the pathophysiology in neurodegenerative diseases, as it can lead to disrupted nutrient transport, increased neuroinflammation, and compromised protective barriers that normally shield the brain from harmful agents. In AD and PD, BBB disruption has been linked to the accumulation of toxic proteins and impaired waste clearance, further aggravating neuronal damage. All the above highlights the need for further research to unravel the complex interactions between glucose metabolism, insulin signaling, and BBB function in neurodegenerative diseases.

There are still several knowledge gaps in our understanding. (i) Although the downregulation of Glut1 has been observed in AD, whether rescuing Glut1 expression in AD brain can benefit BBB integrity, brain glucose metabolism, and neuronal function is unclear. Answering this question may offer a potential therapeutic target. (ii) While brain insulin resistance seems to parallel peripheral insulin resistance, the local specific characteristics and mechanisms in the brain are poorly defined. The precise pathways, cellular targets, and regional variations of insulin resistance in the brain, especially at the BBB and among different cell types, remain unclear. (iii) Current drugs targeting peripheral insulin resistance often fail to effectively address brain insulin resistance due to the selective permeability of the BBB, and the need for innovative therapeutic strategies that can cross the BBB and specifically target insulin signaling within the brain.

AUTHOR CONTRIBUTIONS

Yiyi Zhu: Writing – original draft. **Alexei Verkhratsky:** Conceptualisation; writing – review and editing. **Hui Chen:** Writing – original draft; writing – review and editing. **Chenju Yi:** Conceptualisation; writing – original draft.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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