REVIEW

Evidence for an alternative insulin transporter at the blood-brain barrier

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Abstract

Accumulating evidence suggests there is an alternative insulin transporter besides the insulin receptor at the blood-brain barrier (BBB), responsible for shuttling insulin from the circulation into the brain. In this review, we summarize key features of the BBB and what makes it unique compared to other capillary beds; summarize what we know about insulin BBB transport; provide an extensive list of diseases, physiological states, and serum factors tested in modifying insulin BBB transport; and lastly, highlight potential alternative transport systems that may be involved in or have already been tested in mediating insulin BBB transport. Identifying the transport system for insulin at the BBB would aide in controlling central nervous system (CNS) insulin levels in multiple diseases and conditions including Alzheimer's disease (AD) and obesity, where availability of insulin to the CNS is limited.

Keywords: Insulin, transport, blood-brain barrier

Introduction

The ability of insulin to act within the brain has been known since the early 20th century [1, 2]. However, as blood substrate entry into the brain is regulated by the blood-brain barrier (BBB) interface, evidence of insulin crossing the brain barriers was identified decades later [3, 4]. It is now well recognized that the majority of insulin acting within the brain crosses the BBB via a saturable, receptor-mediated transport system that is affected by various physiological states [5, 6]. Once present within the brain, it is assumed insulin must navigate the brain parenchyma to reach various cell types to act as a ligand by binding its receptors, including the insulin receptor, insulin-like growth factor 1 receptor (IGF-1R), and hybrids of the two, activating intracellular signaling cascades. Insulin signaling within the central nervous system (CNS) is important not only for regulation of metabolism but also cognition. CNS insulin signaling can become dysfunctional with age and in neurodegenerative diseases such as Alzheimer's disease [7] and insulin BBB interactions are impaired [7-9]. BBB transport of insulin could be a regulator of CNS insulin signaling since it is one of the mediators of CNS insulin levels [7]. Additionally, insulin interactions with the BBB are impaired in obesity [10, 11]. Without sufficient ligand or receptor signaling, insulin functions within the CNS become impaired. Therefore, understanding more about the transport system and interactions at the BBB for insulin will aid in combating such deficiency in aging, Alzheimer's disease, and obesity.

Blood-brain barrier (BBB)

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Components of the BBB

Conceptually, the BBB can be thought of as those structures which inhibit or otherwise regulate the exchange of substances between the CNS and blood. These barriers include the vascular BBB, the choroid plexus, the tanycytic barrier located between the circumventricular organs and adjacent brain tissue, the meningeal barrier, and the barriers of the cranial nerves such as the blood-retinal barrier [12]. Likely, all these barriers participate in insulin/CNS interactions, but it is the vascular BBB that has been most studied in this regard.

The physical wall that forms the BBB is comprised of brain endothelial cells (BECs) and occurs in the arteriolar, capillary, and venule portions of the cerebral vasculature [13]. These cells are in constant communication with other cells of CNS, forming the neurovascular unit (NVU). The NVU includes microglia, pericytes, astrocytes, neurons, and mast cells, but it is the astrocytes and pericytes which have received the most attention in regard to their interactions with the BBB. Pericytes are anatomically connected by gap junctions with the BEC. Astrocytes form a sheath around the BBB capillaries and are separated from the abluminal surface of the BEC by the basement membranes. It is the pericytes and astrocytes which induce the BECs to express BBB characteristics, including the formation of tight junctions and the loss of fenestrae and micropinocytosis [14]. The cells of the NVU also modulate other BBB functions, such as cytokine secretions and transporter functions [14].

The BBB is also in communication with the circulating immune cells and, by way of secretions into blood, with the peripheral tissues. This communication can also affect various functions of the BBB. For example, lipopolysaccharide, a fragment of the cell wall of gram-negative bacteria that is a potent stimulator of the innate immune system, increases insulin transport across the BBB by inducing nitric oxide release from immune cells [15, 16].

Roles of the BBB

The most widely appreciated role of the BBB and for which it was named is that of limiting the unregulated leakage of substances from blood into the CNS. Unlike other capillary beds, that of the CNS has greatly reduced transcytosis, few fenestrations, and adjacent BEC cell membranes are cemented together by tight junctions [17]. Thus, paracellular (between cells) and transcellular (across a cell) leakage is essentially absent in the healthy BBB so that no ultrafiltrate is produced by the capillary bed of the CNS. This lack of an ultrafiltrate protects brain tissue from blood-borne substances, both endogenous and xenobiotic, which would be toxic to those tissues. The physical barrier is reinforced for some substances by the presence of brain-to-blood efflux systems which prevent circulating substances from entering or remaining in brain tissue. For example, the anti-helminthic ivermectin is prevented from entering the CNS by the brain-to-blood transporter p-glycoprotein (Pgp) [18]. In animals that do not express Pgp at their BBB, ivermectin is a potent neurotoxin [19].

The BBB can also be an enzymatic barrier, digesting substances such as the monoamines which could otherwise enter the brain from the circulation [20]. Insulin degrading enzyme (IDE) protein and mRNA is present in BECs, less than [21] and similar to levels present in neurons [22], respectively, regulating intracellular insulin levels.

The lack of an ultrafiltrate may protect the CNS from circulating toxins, but the ultrafiltrate is the major route by which most tissues receive their nourishment from the blood. Thus, the BBB has other mechanisms to provide the CNS with nutrients. The most prominent of these are the transport mechanisms. The BBB contains many transporters, and it is likely there are still more to be discovered. These transporters deliver to the brain the glucose, amino acids, free fatty acids, vitamins, and other nutrients needed by the brain. The transporters of the BBB also play a homeostatic role for the CNS by regulating electrolyte balance [23], bicarbonate levels [24], and as exemplified by Pgp eliminating from the CNS both endogenous and exogenous toxins [25]. The BBB also participates in brain-body communication by regulating the transport of various informational molecules, including insulin.

Types of transport systems at the BBB

Transport systems located in cell membranes can be categorized in various ways. Pharmacokinetically, transporters demonstrate saturation and biochemically, are typically transmembrane glycoproteins. Some BBB transporters, such as the glucose transporter 1 (GLUT-1) which transports glucose across the BBB, use facilitated diffusion [26, 27]. Facilitated diffusion systems are energy independent and transport substances bidirectionally from the side of higher concentration to the side of lower concentration. Active transporters require energy or an electrochemical gradient to function, can be unidirectional, and can transport substances against a concentration gradient. Facilitated diffusion systems can be channels or carriers (GLUT-1 is a carrier), but active transporters are carriers. Carriers typically open and close so that they can be open to one environment and closed to the other, whereas channels when active, are open simultaneously to the extracellular and intracellular environments. Carriers also tend to have highly selective binding sites so that they transport a specific ligand or class of ligands and follow Michaelis-Menten kinetics (GLUT-1 is specific for hexoses). Some channels can undergo conformational changes and so become inactive (closed to both environments), whereas pores are channels that are always open and active. Channels and carriers typically transport substances into or out of the cytoplasm, therefore, transcellular transport as occurs at the BBB would depend on a set of transporters located at both the luminal and abluminal cell membranes. These strict definitions from cellular biology [28, 29] are not always appropriately applied in the BBB literature. For example, the term "active" is often used to refer to any saturable transport system, whereas it should be reserved to refer only to energy-requiring carriers.

Receptor-mediated refers to a binding site for the ligand on the transporter and is a hallmark of carriers. Carrier-

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mediated and receptor-mediated transport are not generally distinguishable terms. Receptor-mediated endocytosis refers to the internalization into the cell cytoplasm of the carrier protein with its ligand within a vesicle formed by the cell membrane. Those endosomes can be routed to various cellular structures, including back to the cell membrane. The term receptor-mediated endocytosis is often, but not always, used specifically to refer to endocytosis involving clathrin [30]. Clathrin-independent endocytic mechanisms include potocytosis (internalization of caveolae), adsorptive transcytosis, pinocytosis and phagocytosis.

Transcytosis occurs when the endosome moves from one membrane of a polarized cell to the other (*e.g.*, from apical to basal or luminal to abluminal). Transcytosis, therefore, requires that a cell have distinctive regions to its membrane as in the case of barrier cells. The BBB field tends to label any transport of a large molecule as receptor-mediated transcytosis, even when there is no evidence of involvement of vesicles or clathrin. The assumption is that vesicles are required to move larger substances, but enzymes and cytokines can be exported via carriers, as exemplified by interleukin 2 (IL-2) and Pgp [31]. Transport of insulin across the BBB is assumed to be clathrin-dependent but has not been directly tested *in vivo*. Insulin endocytosis is clathrin dependent for most cells [32], including peripheral endothelial cells [33] but caveolin-1 has also been shown to be involved in insulin uptake [34]. Insulin transcytosis across the retinal vascular endothelial cells is clathrin-dependent [35]. We recently showed insulin binding to isolated brain microvessels is clathrin-dependent [36]. We further went on to show transport across the BBB may be regionally mediated, involving caveolin-1 in the hypothalamus. This shows insulin transport across the various vascular beds can involve different processes.

Insulin BBB Transport

As established above, it is now well acknowledged that insulin can directly cross the BBB. This occurs in a saturable, specific, receptor-mediated process [37]. Saturability has been demonstrated by the nonlinear relation between CSF and blood levels of insulin [38-41], brain tissue and blood levels of insulin [41, 42], and by the inhibition of the rate of radioactive insulin transport across the BBB by unlabeled insulin [37, 43]. The transporter for insulin seems to be specific for it as no other ligands have to date been found, although substances have been found that modulate transporter activity (Table 1). The saturable, specific nature of the transport system which follows Michaelis-Menton kinetics suggests that it is receptor-mediated. The transport system is similar across species as human and rat insulin both cross the murine BBB [44], unlabeled human insulin is able to inhibit the blood to brain passage of radioactive rat insulin [37], and insulin BBB transport has been observed in dogs [38, 40, 41] and humans [45]. There is great variability in the insulin trans-

port system throughout the brain as some regions have extremely fast transport [46]. Lastly, inactive insulin, either via freeze/thaw or heat-denatured methods, is unable to cross the BBB [10, 47]. Therefore, structural changes of insulin, such as deamidation, are likely necessary for recognition by the insulin transporter.

Over the years, insulin BBB transport has been investigated using various techniques. However, due to the small amounts of insulin transported across the BBB, there can be technical difficulties in some of the techniques. The ability to measure low amounts of immunoactive insulin present in brain compared to blood, and the need to inject high insulin concentrations of immunofluorescent tracer are some examples. Therefore, radioactivity is a great alternative to measuring pharmacokinetics of insulin transport. By investigating insulin BBB transport, independent researchers have identified this transport system is impacted by metabolic changes, during development and pregnancy, and even by exercise, Alzheimer's disease, and inflammation. There have also been factors and/or states that have had no effect on insulin transport. Most surprisingly, the loss or inhibition of the BEC insulin receptor had no effect on insulin transport [43, 48-50]. We have summarized this literature in Table 1 and refer readers to the specific references mentioned for each factor/disease state/intervention investigated.

The insulin receptor was long thought to serve as the protein responsible for insulin transport across the BBB, shuttling insulin from the circulation, across the BEC, and releasing it into the brain parenchyma. S961 is a potent, selective antagonist for the insulin receptor, but not IGF-1R or hybrid receptors [70], and has regularly been used to investigate the role of the insulin receptor in various processes, including transport. However, we recently showed that loss or inhibition of the insulin receptor in BECs in mice did not affect the transport rate of insulin across the BBB [43]. Since then, others have supported this finding, showing inhibition of the BEC insulin receptor did not impact insulin transport in an *in vitro* model [50] and in mice [48]. In an exciting new paper describing a novel, *in vivo* insulin PET tracer, co-administration of S961 also had no effect on brain insulin uptake in mice [49]. These data suggest there is another protein(s) responsible for transporting insulin across the BEC. However, to date, identification of this protein is unknown.

Alternatives for the insulin transporter

The involvement of another protein besides the insulin receptor for transporting insulin across the BBB makes evolutionary and physiological sense. Receptors and transporters are not static but modulated and regulated by a variety of factors. Separate receptor and transporter proteins would permit independent regulation of the effects of insulin on BEC functions and on brain activities. As the receptor is involved in many intracellular signaling cascades and acts as a tyrosine kinase, it further supports the primary role to be a signaling protein rather than a trans-

Table 1. Impact of disease, physiological states, and serum factors on insulin BBB transport.

Study	Disease/Intervention	Model	Model	Summary	Reference
Metabolic factors					
1	Diabetes- induced	streptozotocin (ip)	Mouse	↑	[51]
2	Diabetes- induced	alloxin (iv)	Mouse	↑	[51]
3	Hyperglycemia (non-diabetic)	D-glucose (ip)	Mouse	↔	[51]
4	Obesity	high-fat diet	Dog	↓	[11]
5	Obesity	retired breeders	Mouse	↓	[10]
6	Starvation in obesity	fasting (48 hr)	Mouse	↑	[10]
7	Triglycerides	cardiac perfusion	Mouse	↑	[10]
Developmental factors					
8	Newborn/Infancy	newborn, 3 wks	Rabbit	↑	[52]
9	Pregnancy	late pregnancy, BCSFB	Mouse	↓	[53]
10	Pregnancy	late pregnancy	Rat	↑	[54]
11	Age	C57B/6J (12, 24 mo)	Mouse	↓	[55]
12	Aging	SAMP8 (12 mo)	Mouse	↔	[8]
13	Alzheimer's	APP/PS1 (6 mo)	Mouse	↑	[56]
14	Alzheimer's	APP/PSN1 (6 mo)	Mouse	↑	[49]
15	Alzheimer's	moderate/severe AD, BCSFB	Human	↓	[57]
Physiological states					
16	Iron Deficiency	nutritional iron-deficiency	Rat	↑	[58]
17	Exercise	voluntary running wheel (24 hrs)	Mouse	↑	[59]
Insulin receptor loss					
18	Insulin receptor inhibition	S961	Mouse, BECs	↓	[47]
19	Insulin receptor loss/inhibition	EndoIRKO; S961	Mouse	↔	[43]
20	Insulin receptor inhibition	S961	BECs	↔	[50]
21	Insulin receptor inhibition	S961	Mouse	↔	[49]
Genetics					
22	Young ApoE mice	apoE3/apoE4, male/female	Mouse	↔	[60]
23	Aged ApoE mice	apoE3/apoE4, male/female, HFD	Mouse	↓	[61]
Factors/Drugs/Other					
24	IGFs	IGF-1, IGF-II (perfusion)	Mouse	↓	[62]
25	Leptin	iv, co-injection	Mouse	↔	[37]
26	Aluminum	ip	Rat	↑	[63]
27	Aluminum	ip	Mouse	↑	[37]
28	Pgp inhibitor	Verapamil iv, co-injection	Mouse	↔	[37]
29	Amino Acid	Tyrosine, iv, co-injection	Mouse	↔	[37]
30	Norepinephrine	iv, co-injeciton	Mouse	↔	[64]
31	Rapamycin	rapamycin (ip, 2 wks)	Mouse	↔	[65]
32	Rosiglitazone	iv, pre-treatment	Mouse	↔	[66]
33	CCK (Cholecystokinin)	ip, fasted (16 h)	Rat	↑	[67]
34	Acute estrogen	OVX female, male, ip (48 hr)	Rat	↔	[68]
35	Chronic estrogen	Male, ip (5 wks)	Rat	↔	[68]
36	Inflammation	LPS, ip (16, 24 h)	Mouse	↑	[16]
37	nNOS	3x ip LPS, inhibitor (4 h post)	Mouse	↓	[15]
38	iNOS, eNOS	3x ip LPS, inhibitor (4 h post)	Mouse	↑	[15]
39	Dexamethasone	oral (7 d)	Dog	↓	[69]

apoE: apolipoprotein E, BCSFB: blood-cerebrospinal fluid barrier, CCK: Cholecystokinin, EndoIRKO: endothelial insulin receptor knock-out, eNOS: endothelial nitric oxide synthase, HFD: high-fat diet, IGF: insulin-growth factor, iNOS: inducible nitric oxide synthase, ip: intraperitoneal, iv: intravenous, LPS: lipopolysaccharide, nNOS: neuronal nitric oxide synthase, OVX: ovariectomized, Pgp: p-glycoprotein.

porter. Recent proteomic studies of immortalized BECs support how critical the insulin receptor is in BECs, necessary for a multitude of functions, including regulation of a variety of BBB transporters, the transferrin receptor, and the tight junction protein claudin-5 [71]. A separate transporter would allow for insulin transport across the BEC, while also allowing critical intracellular signaling events via the receptor. Endothelial cell intracellular insulin signaling is a critical metabolic event. There is evidence in other receptor/transport systems supporting different proteins to accomplish these two independent events, as described next. We also discuss other alternatives for the insulin transporter that have been hypothesized.

Evidence for alternative transporters to canonical receptors at the BBB

Insulin binding to the luminal surface of BECs has two fates. It may activate the intracellular machinery that affects cellular functions (here termed the signaling receptor) or it may be transported across the BBB (here termed the transporter binding site). Binding to either the signaling receptor or the transporter binding site results in insulin endocytosis and exocytosis. In the case of signaling receptor binding, the exocytosis is at the luminal membrane of the BEC and in the case of transporter binding site, the exocytosis is to the abluminal membrane of the BEC. The question arises as to whether the protein forming the signaling receptor is the same protein as the transporter binding site. As we have previously reviewed [72], it seems that the usual situation is that the signaling receptor protein and the BBB transporter binding site are usually different proteins, as exemplified by prolactin [73], epidermal growth factor, Tyr-MIF-1, the enkephalins, pituitary adenylate cyclase activating polypeptide, and thyroid hormones. Our data argues that a similar dichotomy exists for insulin. We found that the insulin antagonist S961 binds avidly to the BEC, but is not transported across the BBB [43]. This means that the transporter binding site differs sufficiently from the signaling receptor as to not recognize S961 as a ligand. We also found that S961 largely blocked the ability of radioactive insulin to bind to BECs, but not its ability to cross the BBB. Finally, mice with loss of the signaling receptor in BECs demonstrated poor binding to BECs, but unimpaired transport activity. These studies are consistent with the insulin signaling receptor and the transporter binding site being different proteins.

Insulin-like growth factor-1 receptor (IGF-1R)

Could IGF-1R be insulin's transporter binding site? Insulin and IGF-1 each bind to the other's receptors, although much less avidly. IGF-1R is expressed at the BBB and choroid plexus [74]. IGF-1 crosses the BBB and inhibits the transport of radioactive insulin transport across the BBB, just as insulin inhibits the transport of radioactive IGF-1 [62, 75]. Both insulin and IGF-1 transport are reduced in obese animals and affected by triglycerides [10, 76]. However, cross inhibition studies suggest that there is a separate insulin-favoring transporter and an IGF-1-favoring transporter [62, 77]. Regulation of the two

transporters also differ, as triglycerides increase insulin transport across the BBB but inhibit transport of IGF-1 [10, 76]. Furthermore, evidence suggests that IGF-1R does not transport IGF-1 across the BBB [78], but that low-density lipoprotein receptor-related protein (LRP)-1 is involved at the vascular BBB and LRP-2 at the choroid plexus [76, 79]. Therefore, IGF-1R is not a candidate for being the BBB insulin transport protein.

Low-density lipoprotein receptor-related proteins (LRP)

The LRP family of proteins are structurally similar but participate in a wide range of physiological processes including lipid metabolism, neurodevelopment, and transport of nutrients [80]. Megalin, also known as LRP-2, is the largest sized protein in the family and can bind a wide variety of ligands. While it can play a role in reabsorption of various molecules in the proximal renal tubule, including insulin [81], it can also act as a cell signaling transducer within the CNS [80, 82]. LRP-8, also referred to as apolipoprotein E receptor 2 (apoER2), has been recognized as a signal transducer critical in brain development [83]. Both of these have been suggested to play a role in insulin transport in peripheral systems.

LRP-2/Megalin can regulate insulin transport in kidney proximal tubule cells [81] and can take up other hormones as well, including leptin [84] and IGF-1 [85]. Receptor-associated protein (RAP) is a 39 kDa protein that is a natural inhibitor of ligand binding to LRP-2. We used this non-specific inhibitor of LRP-2, RAP, and reported insulin BBB transport was unchanged [43]. However, Orlando et al has also reported that RAP does not affect insulin binding to proximal tubule cells, compared to excess, unlabeled insulin [81]. Therefore, a more specific inhibitor of LRP-2 would aid in fully identifying a role for LRP-2 in insulin BBB transport. Further evidence suggests leptin is also not transported across the BBB via LRP-2 [86], despite its role in transport at the choroid plexus [87]. IGF-1 is also transported across the choroid plexus by LRP-2 [85].

LRP-8/ApoER2 is not only a receptor for apoE but also acts as the primary receptor for the critical brain development protein Reelin [83]. ApoER2 is involved in long-term potentiation, learning, and memory. In the last few years, due to the AD risk gene allele, ApoE4, the role for ApoER2 in AD has begun to be explored. Post-translational proteolytic cleavage of ApoER2 [88] and pre-translational splicing [89] is dysfunctional in AD. Additionally, the risk allele ApoE4 impairs the trafficking of the insulin receptor, resulting in decreased insulin signaling [90]. How ApoER2 may fit into this pathway remains to be determined.

Amino Acid Transporter Involvement

Amino acids are transported across the BBB involving both facilitative systems and active transporters. Some of these transporters are selective for a single substrate or group of substrates while others are non-selective [91]. Recently, it was identified in a high-throughput screen that

the amino acid transporter, SLC7A1, also known as CAT-1, could regulate leptin transport across an iPSC- derived BEC model [92]. This raises as a possibility that the same, or another amino acid transporter, could regulate insulin transport across the BBB. Transport of amino acids could modify transporter expression, activity, and cellular distribution. Additionally, it is possible that the amino acid itself could aid as a co-factor for the insulin transporter. While insulin is known to impact amino acid transport, either directly or indirectly [5], the converse is less well established. The amino acid-derived hormone norepinephrine did not affect insulin BBB transport contrary to a 2-3 fold increase of leptin BBB transport [64]. In an *in vitro* co-culture model of astrocytes and brain endothelial cells, L-glutamate enhances insulin transcytosis [47]. L-arginine, in the presence of LPS, also enhances insulin BBB transport [15]. L-arginine is a nitric oxide precursor and nitric oxide has been shown to regulate insulin BBB transport, as discussed next.

Involvement of Nitric Oxide Synthase (NOS)

Nitric oxide is a common secondary messenger that helps orchestrate multiple signaling pathways. Synthesis of nitric oxide from L-arginine is primarily converted by NOS, present in multiple different cell types. One of the more common roles is to act as a vasodilator, relaxing the smooth muscle cells around the blood vessels. In the brain, there are three main NOS enzymes: endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS). NOS and nitric oxide have an important role at the BBB, regulating its structure and function. Under inflammatory-stimulated conditions *in vivo*, NOS inhibitors enhance insulin BBB transport, specifically nitric oxide coming from nNOS [15]. In an *in vitro* co-culture model, astrocytic inhibition of NOS decreases insulin transcytosis [47]. This suggests the source of the nitric oxide stimulation can regulate insulin transport. The transport system is also suspected to involve calcium signaling as pre-treating astrocytes with a calcium donor enhanced insulin transport across a BEC model [47]. As these second messengers are complex, further investigation on the interaction and the NVU cell types involved in regulating insulin BBB transport are warranted.

Conclusions

We have presented evidence that the insulin transport system at the BBB involves a protein other than the insulin receptor. Identification of this transport system will be critical in treating diseases with deficient CNS insulin signaling, such as Alzheimer's disease or dysregulated metabolism, as insulin availability could be a contributing factor to such a deficiency. While there are ways to deliver exogenous insulin to the CNS, such as via intranasal insulin [93], that have proven to be beneficial, preventing and/or restoring the endogenous insulin BBB transport system would likely be more effective and potentially even prevent a deficiency in the first place. Insulin clearly

has multiple impacts not only within the CNS but also in regulating BBB function, that any slight modification of this signaling has downstream detrimental effects. Whether this transport system is unique to the BBB or is similar to other peripheral endothelial beds remains to be determined. Leveraging multiple genetic data sets could hopefully shed light on potential targets for the transport system, but proteomic data will also be necessary. In a recent proteomics study, protein levels of the insulin receptor were detected at similar levels between rat microvessels isolated from various regions including white matter, cortical grey matter, and spinal cord [94]. While protein expression level does not necessarily translate to activity of a transporter, equivalent expression of the insulin receptor across brain regions does not support the high variability of insulin transport rate across brain regions. Additionally, if the transport system involves co-factors, the identification of the transporter could prove to be even more difficult. It is likely the abundance of the transporter(s) within BECs is low, given the limited entry of insulin into the CNS, which will further add to the difficulty. Despite these difficulties, recent technological advances in microvessel isolation, omics-based discovery approaches, and cell culture screening tools will help elucidate the transport system for insulin.

Declarations

Conflicts of interest: The authors declare there are no conflicts of interest.

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