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The source of cerebral insulin

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Abstract

Insulin and its receptor are found throughout the central nervous system (CNS). Insulin administered into the CNS can exert powerful effects, yet the consensus is that little or no insulin is produced in the CNS. Therefore, CNS insulin is essentially dependent on the ability of peripheral insulin to cross the blood–brain barrier (BBB). Insulin is known to cross the BBB by a saturable transport mechanism. This transporter shows some thematic similarities to other transporters for peptides or regulatory proteins. It is unevenly distributed throughout the CNS with the olfactory bulbs having the fastest transport rate of any brain region. It is partially saturated at euglycemic levels, suggesting that its main signaling function occurs at physiological blood levels, rather than as a brake to hypoglycemic events. One probable function of the BBB transporter is to allow CNS insulin to act as a counter-regulatory hormone to peripheral insulin. The transporter is regulated, with the transport rate of insulin being altered during development and by fasting, obesity, hibernation, diabetes mellitus and Alzheimer's disease. Enhancement of insulin transport by lipopolysaccharide could be the basis for the insulin resistance seen with bacterial infections. Inhibition of insulin transport across the BBB by dexamethasone could be the basis for the enhanced appetite seen with glucocorticoid treatments. Insulin itself also has effects on the BBB, altering enzymatic and transporter functions. Overall, BBB transport of insulin provides a mechanism for peripheral insulin to act within the CNS as a regulatory peptide.

Keywords: Insulin; Central nervous system; Blood–brain barrier; Transport; Leptin; Diabetes mellitus; Lipopolysaccharide

1. Introduction

A pancreatic extract capable of lowering blood glucose was isolated by Banting and Best (1922). This substance, insulin, is important not only because it is central to understanding and treating diabetes mellitus, one of the most dreaded diseases of mankind, but also because it has been the targeted molecule used in the development of many other fields Kahn (1995). It was an early and dramatic example of the power hormones play and so helped to foster the growth of the field of endocrinology. It was one of the first proteins to be extracted on mass scale for use in the treatment of human diseases and so not only fostered the field of protein chemistry but the emergence of a major pharmaceutical company. Despite some 8 decades of intense scientific interest and over 170,000 publications in medline, there is still much about insulin which is not understood.

Insulin has once again been central to pioneering work in another important area: an understanding of how the central nervous system (CNS) communicates with the peripheral tissues. An interest in insulin and the CNS can be traced back 50 years, but the first seminal observation in this regard is probably the study of Margolis and Altszuler (1967). That insulin directly affects the CNS is clear: insulin receptors are found throughout the CNS and insulin injected into the brain affects CNS functioning. Little or no insulin is produced in the CNS, which means that the insulin acting there is of pancreatic origin. This raises the question of how insulin produced by the pancreas and circulating in the blood could enter the CNS. This is a major question because lipid insoluble substances the size of insulin are largely excluded from the CNS by the blood–brain barrier (BBB).

Thus, an understanding of the source of CNS insulin is largely synonymous with an understanding of the ways the BBB and insulin interact. Because the BBB is not an inert structure, but a dynamic interface separating the blood from the fluids of the CNS, one must consider certain of its characteristics relevant to how it works with

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regards to insulin. This is particularly true because our knowledge of how the BBB interacts with protein hormones like insulin is far from complete. Therefore, an understanding of how the BBB interacts with peptides and other regulatory proteins is crucial if one is to anticipate future discoveries of how the BBB regulates levels of insulin in the CNS.

2. A general overview of BBB function

The term blood–brain barrier in its most restrictive sense refers to the vascular bed of the CNS, which is specially modified to prevent the unrestricted transfer of molecules between the blood and the extracellular fluid of the CNS. In this sense, the term BBB is distinguished from other barriers, which also regulate transfer, such as the choroid plexus and the barriers at the circumventricular organs (Johanson, 1988; Gross and Weindl, 1987). In a broader sense, the term BBB can include these barriers. In a still broader sense, the term can include other mechanisms that ultimately affect transfer, such as cerebrospinal fluid (CSF) reabsorption. Indeed, the term BBB arose from the observation that some blood-borne dyes do not stain the CNS; perhaps the purest definition of the BBB is as the concept to explain the phenomenon rather than as an anatomical structure (Davson and Segal, 1996a).

Nevertheless, it is easy to encapsulate many of the major aspects of the BBB, even broadly defined, by considering how the capillary bed of the brain is modified (Rapoport, 1976). Whereas the capillary bed of most tissues is leaky, that of the CNS is not. Capillaries are joined together by tight junctions which eliminate intercellular spaces. Fenestrations, or intracellular pores spanning the capillary wall, are virtually absent and brain endothelial cells have a greatly reduced rate of endocytosis, pinocytosis and transcytosis in comparison to endothelial cells from most other capillary beds. These and other modifications prevent the production of an ultrafiltrate and the unrestricted entry of blood-borne molecules into the brain.

Most blood-borne substances known to enter the CNS under non-pathological conditions do so by one of two major mechanisms. Small, lipid soluble molecules are able to cross the BBB by diffusing across the capillary membranes (Bradbury, 1979). Many classic drugs, such as morphine, use this mechanism to cross the BBB (Oldendorf, 1974). The rate of entry is in proportion to lipid solubility and inversely related to molecular weight. Some peptides have been shown to cross the BBB by this mechanism in amounts sufficient to affect CNS function (Banks and Kastin, 1985). Many substances are transported across the BBB by saturable transport systems (Davson and Segal, 1996b). These include glucose, amino acids, vitamins, minerals and free fatty acids. It also includes many peptides and regulatory proteins, including insulin (Banks and Kastin, 1996).

The rate at which substances used in CNS metabolism are transported across the BBB differs markedly from the rate for substances involved in communication. Oxygen and glucose are extracted at near maximal rates. The only way that more of these substances can be transported into brain is by increasing cerebral blood flow. As such, these substances are sometimes referred to as having flow-dependent entry and their accumulation in brain can be used to measure rates of cerebral blood flow and brain metabolism. The saturable systems for peptides and regulatory proteins usually transport these substances into the CNS at rates which are far lower. As such, the rates of entry of these informational molecules are independent on cerebral blood flow.

This does not mean, however, that the transport systems for peptides and regulatory proteins are static. Several examples of different types of regulation are known. In fact, the regulation of a transporter is often related to the role the transporter plays in physiology or disease. Peptide transport system-1 (PTS-1) transports two peptides, Tyr-melanocyte-stimulating hormone inhibitory factor-1 (Tyr-MIF-1) and [Met^5]enkephalin in the brain-to-blood direction (Banks and Kastin, 1990). It is greatly attenuated in animals addicted to alcohol, but recovers within hours of the animal ceasing alcohol ingestion. This recovery of PTS-1 likely is responsible for the decrease in brain methionine enkephalin levels which, in turn, are thought to be responsible for alcohol withdrawal seizures. Transport of leptin across the BBB can be totally abolished by starvation, thus blocking an anorectic signal to brain (Kastin and Akerstrom, 2000). Therefore, the transporters for peptides and regulatory proteins are often modified in ways important to their roles of communication between the CNS and peripheral tissues.

3. Evidence that insulin crosses the BBB

3.1. Early studies and controversies

The idea that insulin could cross the BBB was first suggested by Margolis and Altszuler (1967). They used the newly available technique of radioimmunoassay to show that levels of insulin in the CSF increased with peripheral infusions of insulin. These findings contradicted previous studies, some dating back to as early as 1954, which used radioactively labeled insulin or bioassays for insulin. The authors concluded that insulin crossed the BBB, possibly by way of a saturable transport system. These results were confirmed by Woods and Porte (1977), who found correlations between the levels of insulin in CSF and serum. They concluded that CSF insulin was likely acting as a signal to the brain, perhaps acting as an adipostat.

However, other studies, failing to find correlations between CNS and peripheral levels of insulin, concluded that

the brain must be able to synthesize insulin (Reiser et al., 1985; Havrankova et al., 1979). The case for (and against) a CNS sources of insulin was elegantly summed up in a review by Plata-Salaman (1991). If insulin is produced in brain, then findings of correlations between peripheral and CNS sources is not strong evidence for passage across the BBB. This is because such correlations can be produced by the coincident release of hormone from its peripheral and central sources. For example, correlations between the CSF and serum levels of arginine vasopressin can occur when stimuli induce simultaneous release into blood and the CNS (Szczepanska-Sadowska et al., 1983). If there is no CNS source of insulin, then just its presence in CNS is proof of passage across the BBB, even in the absence of correlations between blood and CNS levels.

Therefore, whether or not insulin is produced in the brain is critical to the interpretation of the early studies examining the ability of insulin to cross the BBB. As recently reviewed, it is now clear that little or no insulin is produced in brain (Woods et al., 2003). This means that, in retrospect, those early studies detecting insulin in the CNS were proof that insulin can cross the blood–brain barrier.

3.2. Later studies and the saturable nature of transport

Studies with radioactively labeled insulin or with species-specific immunoassays have also demonstrated that insulin crosses the BBB. For example, human insulin was shown to cross the BBB of the mouse by assaying with species-specific immunoassays (Banks et al., 1997c). This method was also used to demonstrate that essentially all insulin in the CNS is derived from blood. Blood levels of insulin are under negative feedback control, so that peripheral infusion of human insulin into mice suppresses the level of murine insulin in blood. In other words, as levels of human insulin in blood increase, the levels of murine insulin decrease. To the extent that brain insulin is derived from blood, human insulin will increase and murine insulin will decrease in brain (Fig. 1). It was found that levels of murine insulin in brain and blood fell in tandem as infusion rates of human insulin increased. Extrapolation of results indicated that all of the insulin in the CNS was likely derived from the blood (Banks et al., 1997c).

As noted by Margolis and Altszuler (1967) and Woods and Porte (Kahn, 1995), the correlation between CSF and serum levels of insulin is nonlinear. This is because the rate of increase in CSF levels becomes proportionately smaller at higher serum levels. This has been interpreted as evidence that insulin likely enters the CNS by way of a saturable transport system. Although molecules of the size and lipophilicity of insulin can gain some entry into the CNS by non-saturable mechanisms (Banks and Kastin, 1985), the rate at which insulin crosses the BBB is more consistent with a carrier-mediated process. This saturable process is easily demonstrated when radioactively labeled insulin is used (Banks et al., 1997a). Binding studies indicate that insulin is likely to enter the CNS at both the choroid plexus and the capillary bed (Baskin et al., 1986; Miller and Borchardt, 1991).

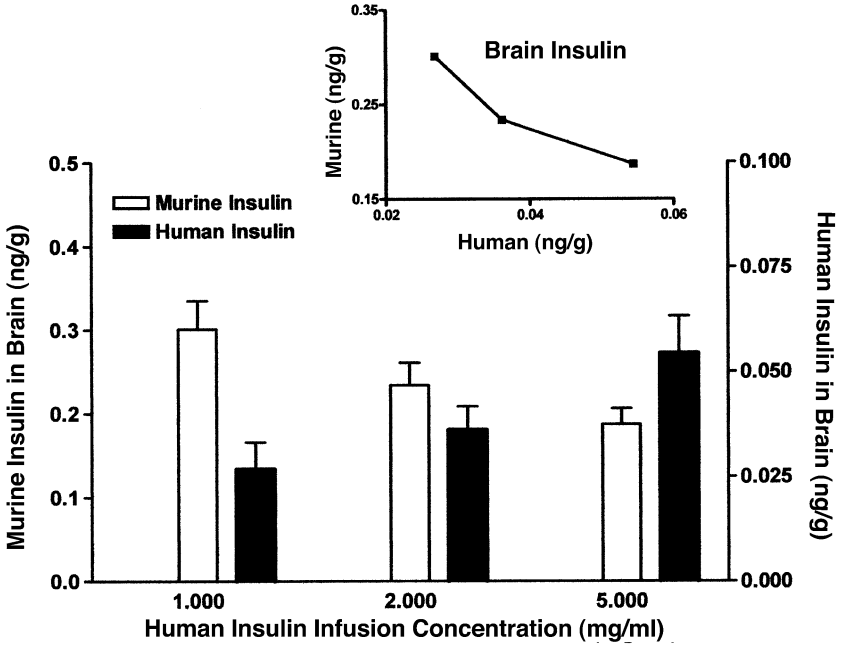

Fig. 1. Effects of peripheral infusion in mouse of human insulin on levels of insulin in brain. Alzet pumps delivering 0.98 μl/h were implanted subcutaneously containing one of three concentrations of human insulin (1, 2 or 5 mg/ml). Endogenous (murine) levels of insulin decreased as levels of exogenous (human) insulin increased. Inset shows relation between human and murine levels of insulin in brain. (Based on data from Banks et al., 1997c).

4. Physiology of the insulin transporter

4.1. Physiological roles of transport

The insulin transporter is largely saturated by serum levels of insulin which do not induce hypoglycemia (Banks et al., 1997a,c). This suggests that the signal which blood-borne insulin conveys to the brain by crossing the BBB is not related to reversal of hypoglycemia but is more likely to be conveying information about physiological parameters.

Insulin given directly into the CNS has effects which are often opposite to those when given peripherally. For example, CNS insulin results in lower levels of serum insulin, increases levels of serum glucose, suppresses appetite and decreases body weight (Florant et al., 1991b; Ajaya and Haranath, 1982; Foster et al., 1991; McGowan et al., 1992; Brief and Davis, 1984; Hatfield et al., 1974); insulin antibodies given into the CNS increase feeding and body weight (Strubbe and Mein, 1977; McGowan et al., 1992); and animals lacking neuronal insulin receptors have higher serum insulin levels, mild insulin resistance, eat more and are obese (Bruning et al., 2000). Thus, insulin appears to act as its own counter-regulatory hormone after crossing the BBB (Morley and Silver, 1991; Banks and Kastin, 1993). Consistent with this idea is the observation that removal of the olfactory bulb (the area of the brain with the highest transport rate for insulin, level of insulin and level of insulin receptors) results in a greater sensitivity to the hypoglycemic effects of peripherally administered insulin (Perassi et al., 1972). CNS insulin also enhances the anorectic properties of CCK (Figlewicz et al., 1995).

4.2. Evidence for physiological regulation of the transporter

The BBB transporter is not static, but is a dynamic system which responds to a great number of physiologic events. Binding of insulin to brain endothelial cells is much higher in the newborn than the adult, probably reflecting a greater need by the brain for insulin during maturation (Frank et al., 1985). Fasting decreases insulin transport across the BBB, and so would result in an appropriately decreased anorectic signal to the brain (Strubbe et al., 1988).The correlation between the CSF and serum levels of insulin is lost during hibernation in the marmot (Florant et al., 1991a), suggesting that the insulin transporter is not functional during hibernation (Table 1).

Table 1
Regulation of the insulin BBB transporter

Event	Proposed or demonstrated effect	Reference
Neonatal period	Increased transport	Frank et al., 1985
Fasting	Decreased transport	Strubbe et al., 1988
Hibernation	Abolishes transport	Florant et al., 1991a
Hyperglycemia	Abolishes transport	Banks et al., 1997b
Obesity	Decreased transport	Kaiyala et al., 2000; Baskin et al., 1985
Diabetes mellitus	Increased transport	Banks et al., 1997b
Lipopolysaccharide	Increased transport	Xaio et al., 2001
Dexamethasone	Decreased transport	Baura et al., 1996
Alzheimer's disease	Decreased transport	Craft et al., 1998
Aging	Decreased transport	Frolich et al., 1998

4.3. Regional variation

All regions of the BBB are not equally permeable to insulin (Fig. 2). The BBB transports insulin into the pons-medulla and the hypothalamus over twice as fast as into whole brain (Banks and Kastin, 1998). However, the transport rate into the olfactory bulb is two to eight times faster than into the whole brain (Banks et al., 1999b). This is consistent with findings that the olfactory bulb is the brain region with the highest concentration of insulin (Baskin et al., 1983; Hill et al., 1998). The olfactory bulb also contains the highest concentration of insulin receptors (Hill et al., 1998; Gupta et al., 1992; Werther et al., 1987) and this is the region most enzymatically active against insulin (Banks et al., 1999b). Removal of olfactory bulb increases sensitivity to peripherally administered insulin (Perassi et al., 1972). Overall, BBB transport of insulin provides a mechanism by which pancreatic insulin can act within the CNS as a regulatory peptide.

Saturable transport of insulin into the spinal cord is also higher than into whole brain, but lower than into the olfactory bulb (Banks et al., 1999b). Some regions of the brain transport little or no insulin. For example, neither the standard white laboratory mouse (ICR) nor the senescence accelerated mouse (SAMP8) transport insulin into the occipital cortex (Banks and Kastin, 1998; Banks et al., 2000b). This pattern is different from that for amylin, another pancreatic peptide co-secreted with insulin (Edwards and Morley, 1992; Butler et al., 1990), which is transported into all regions of the brain (Banks and Kastin, 1998).

This widespread transport of insulin into so many regions of the CNS with its variations in rate of transport will likely aid in explaining one of the major mysteries of CNS insulin. How can insulin derived from a single source, the blood, have so many divergent functions within the CNS, including effects on feeding, glucose regulation, cognition, neurotrophic effects, regulation of amyloid β levels, etc.? The answer in part may be these various activities are controlled by different regions of the brain. For example, insulin transported into the olfactory bulb may have different effects on the CNS than insulin transported into the spinal cord. If the kinetics of the BBB transporters varies by region and with pathophysiologic state as is the case for the leptin transporter (Banks et al., 1999a, 2000a), it may be that not all regions of the brain are maximally stimulated by the same level of serum insulin. Regional and state-dependent differences in transport across the BBB could

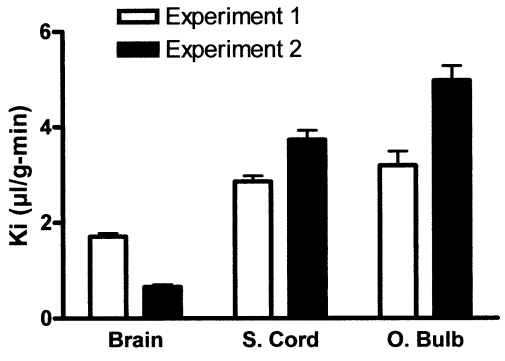

Fig. 2. Comparison of rate of uptake (Ki) of insulin by different regions of the CNS. The olfactory bulb (O. Bulb) took up insulin more rapidly than the whole brain (Brain) or spinal cord (S. Cord). Results from two separate experiments are shown. Adapted from Banks et al. (1999b).

be one factor explaining how CNS insulin can have so many diverse effects.

4.4. Identification of the insulin transporter

The question of the nature of the protein which transports insulin across the BBB is not established. It has been assumed that it is the insulin receptor, co-opted to serve the function of transporter across the BBB. However, this assumption remains untested. In the strictest sense, the term receptor refers to those proteins coupled with intracellular machinery with the function of transducing signal across the cell membrane (Flower, 2002). Transporters, on the other hand, are involved in the physical transfer of the ligand across the membrane and, in the case of the BBB, completely across the cell. It is not necessarily true that the receptor and BBB transporter are the same protein or even derived from the same gene. For example, in vitro and in vivo studies have shown that the binding site for interleukin-1 differs between the BBB transporter for interleukin-1 and the interleukin-1 type I receptor (Banks, 1999; Banks et al., 1991). Other studies have shown that BBB transporters for epidermal growth factor (Pan and Kastin, 1999), and leptin (Banks et al., 2002) differ from their receptors. In contrast, both the p55 and p75 receptors for tumor necrosis factor α are involved in its transport across the BBB (Pan and Kastin, 2002). The relation of the insulin BBB transporter to that of the insulin receptor remains unresolved.

5. Pathophysiology of the insulin transporter

As expected of a transporter which can be regulated by physiological events, the insulin transporter is also altered in disease states. In some cases, these alterations may be adaptive to the disease state, whereas in others the changes may be causal of the disease.

Transport across the BBB of insulin (Stein et al., 1987; Kaiyala et al., 2000), like that of leptin (Banks et al., 1999a), is reduced with obesity. Consistent with such a decreased transport, the obese Zucker rat has lower levels of insulin in the brain (Baskin et al., 1985). However, the insulin level does not correlate with the degree of obesity and the non-obese heterozygotes also have decreased levels of insulin in the brain. This differs from the impairment in leptin transport with obesity, which seems to be closely correlated with the level of obesity (Banks et al., 2002; Banks and Farrell, 2003; Hileman et al., 2002).

In contrast to the inhibition of insulin transport seen in the obese Zucker rat, animals with diabetes mellitus induced with alloxan or streptozotocin (Fig. 3) have an increased saturable transport of insulin across the BBB (Banks et al., 1997b). This paradox between these models of diabetes can in part be explained by differences in the levels of insulin in the blood. Whereas the Zucker rat is insulin resistant and has elevated levels of serum insulin, animals with diabetes induced with alloxan and streptozotocin are insulinopenic. Since the studies assessing the insulinopenic models measured the transport of radioactive insulin across the BBB, one cause of the increased transport could have been that there was less self-inhibition by endogenous serum insulin in the diabetic mice. However, brain perfusion studies were

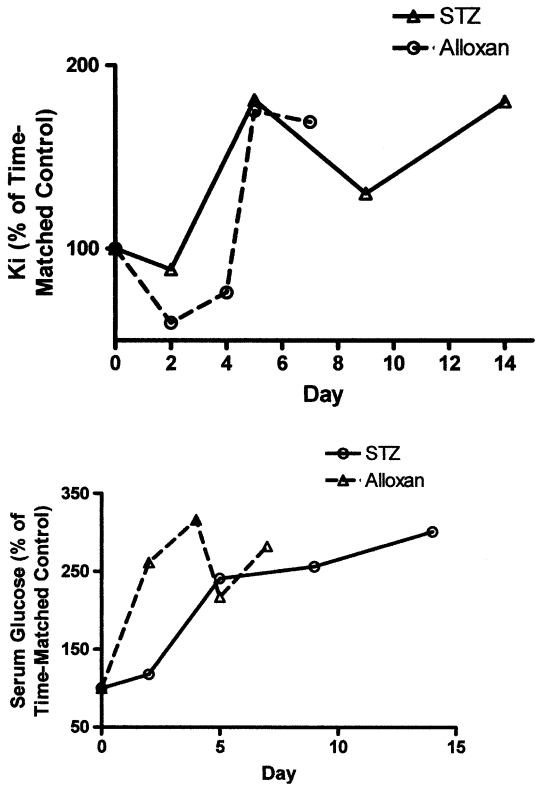

Fig. 3. Effect of diabetes mellitus induced by streptozotocin (STZ) or alloxan on transport of insulin across the BBB. Upper panel shows that both treatments result in increased transport of insulin across the BBB, although alloxan shows an early decline in transport rate. Lower panel shows that these animals were diabetic, including the alloxan-treated mice at the time when their transport rate of insulin was both decreased and increased. Adapted from data from Banks et al. (1997b).

performed which abolish the influence of serum factors (Smith et al., 1987). Insulin transport was still increased when assessed by brain perfusion, showing that endogenous serum factors, including insulin, could not explain the effect of the diabetic state on transport. The brain perfusion studies also rule out an acute effect of hyperglycemia as the cause of increased insulin transport. In fact, acute hyperglycemia induced in non-diabetic mice by administration of glucose abolished insulin transport. Fasting the diabetic mice lowered their serum glucose but did not reverse the increased transport of insulin. Alloxan-induced diabetes mellitus showed a more complex pattern than streptozotocin-induced diabetes mellitus as an early decrease in insulin transport preceded the increase. Leptin transport was unaffected by induction of diabetes mellitus, showing a selective effect of diabetes mellitus on insulin transport. Diabetes was also associated with the loss of the ability of glucose and insulin to increase leptin transport across the BBB (Kastin and Akerstrom, 2001).

Lipopolysaccharides are derived from the cell wall of gram negative bacteria and are widely used to induce cytokine release (Larson and Dunn, 2001; Laye et al., 1994). Lipopolysaccharides increase the saturable transport of insulin across the BBB by a mechanism that is prostaglandin-independent and opposed by nitric oxide (Xaio et al., 2001). The lipopolysaccharide-induced increase in transporter activity would mean that more insulin is entering the brain. Given that CNS insulin tends to oppose the actions of peripheral insulin, this increase in brain insulin would be expected to induce a relative insulin resistance. This line of reasoning suggests that the insulin resistance occurring with sepsis may be caused by an enhanced transport of insulin across the BBB.

Dexamethasone inhibits insulin transport into the brain (Baura et al., 1996). This would reduce the anorectic signal of CNS insulin and could be a mechanism by which glucocorticoids enhance appetite and increase body weight.

A number of interesting observations connect the insulin transporter to Alzheimer's disease. Alzheimer's patients have lower levels of insulin in the CSF and lower CSF/serum ratios of insulin even in the face of elevated levels of insulin in the plasma (Craft et al., 1998). This suggests an impaired transport of insulin across the BBB in Alzheimer's disease. Insulin concentrations in CSF also decrease with age (Frolich et al., 1998) and infusions of insulin while keeping glucose levels constant has been reported to improve memory in Alzheimer's patients (Craft et al., 1996).

6. Effects of insulin on BBB function

Insulin has effects on BBB function, including an ability to affect the transport of other substances. Binding sites for insulin have been described at both the choroid plexus and on brain endothelial cells (Baskin et al., 1986; Miller and Borchardt, 1991). Whereas some of these binding sites may represent transporters, others may represent transmembrane-signaling receptors. This would provide a mechanism for insulin's actions on BBB function.

An example of insulin's effects on the BBB is the ability of insulin to enhance the transport of tyrosine and tryptophan across the BBB (Tagliamonte et al., 1976). Since both of these amino acids are transported into the brain by the large neutral amino acid transporter, it is likely this system is affected by insulin. Whether insulin is acting directly on the amino acid transporter or by an indirect mechanism (such as by lowering the blood level of other competing ligands for the amino acid transporter) is not clear. Increasing transport of these amino acids should result in increased brain levels of serotonin and catecholamines (Fernstrom and Wurtman, 1971; Fernstrom, 1983). Insulin also enhances the transport of leptin across the BBB, an effect lost in diabetic mice (Kastin and Akerstrom, 2001).

Insulin acts as a noncompetitive inhibitor of alkaline phosphatase on brain microvessels (Catalan et al., 1988). Insulin-degrading enzymes are also present on brain microvessels (Keller and Borchardt, 1987; Miller and Borchardt, 1991). Such enzymes can impede the transport of the ligand across the BBB. In the case of insulin enzymes, however, it may be another substrate whose entry is affected: amyloid β protein (Kurochkin and Goto, 1994).

7. Conclusions

Insulin is transported across the BBB by a saturable transporter. Little or no insulin is produced in the CNS, so that CNS insulin is largely derivative from peripheral insulin. As such, CNS insulin is dependent on the BBB transport of peripheral insulin. This transporter is not static but has been shown to alter the transport rate of insulin into the CNS under a variety of circumstances. For example, insulin transport is likely faster in neonates than in adults and slower in Alzheimer's disease than in normal aging. In adults, the transporter is partially saturated at euglycemic levels, suggesting that its role is not to signal hypoglycemic events to the brain. CNS insulin likely acts in part as a counter-regulatory hormone to peripheral insulin by increasing insulin resistance at the peripheral receptor. The ability of lipopolysaccharide to enhance insulin transport across the BBB could be a mechanism accounting for insulin resistance during bacterial infection. The ability of dexamethasone to inhibit insulin transport could explain the anti-anorectic effects of glucocorticoids. Insulin transport is also affected by fasting, obesity, hibernation and with induction of diabetes mellitus. Insulin also has direct effects on BBB enzymes and on other BBB transporters. Overall, BBB transport of insulin provides a mechanism by which peripheral insulin can act within the CNS as a regulatory peptide.

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