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INSULIN AND THE BRAIN

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Abstract

The brain represents an important site for the action of insulin. Besides the traditionally known importance in glucoregulation, insulin has significant neurotrophic properties and influences the brain activity: insulin influences eating behavior, regulates the storage of energy and several aspects concerning memory and knowledge. Insulin resistance and hyperinsulinism could be associated with brain aging, vascular and metabolic pathologies. Elucidating the pathways and metabolism of brain insulin could have a major impact on future targeted therapies.

key words: *insulin, brain, aging, metabolism.*

Introduction

The brain as an organ is dependent on insulin [1]. Receptors for insulin and insulin itself can be found both in the brain and the choroid plexus. After its peripheral administration, insulin has been identified in the cerebrospinal fluid (CSF). In addition, there is thorough documentation on the role of insulin on energy metabolism after its intracerebroventricular (ICV) administration. These data prove that the brain is an important site for the action of insulin where the peripheral signals are integrated by means of vast interactions of neuropeptides and hypothalamic neurotransmitters with the aim of controlling homeostasis [2]. Insulin also has particular roles within the central nervous system (CNS). Being secreted almost entirely in periphery by the pancreatic beta cells, insulin then penetrates the blood brain barrier (BBB) by means of a carrier, and influences in the CNS the feeding and cognition processes.

Cerebral metabolism of glucose

Intuitively, the main mechanism through which insulin modulates neuronal functions is by regulating glucose metabolism. The uptake of fuel, mainly glucose, from the cerebral blood vessels initiates the energy metabolism of the brain. Brain accounts for 25% of total glucose consumption. Alternative substrates are ketone bodies, fatty acids, lactate and pyruvate, and rarely amino acids. Once inside the cell, glucose is irreversibly transformed into glucose-6-phosphate (G6P) by hexokinase (HK). In neurons, each G6P molecule is oxidized by glycolysis, oxidative phosphorylation, the

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tricarboxylic acid cycle (TCA) and the pathway of pentose phosphate (PPP), producing carbon dioxide, water and up to 36 adenosine triphosphate molecules (ATP) [3]. The contribution of glycolysis to oxidative phosphorylation for the total amount of ATP varies in different cells, growth and micro-media. In addition to ATP production, glucose is used to produce glycogen in astrocytes, pentoses for nucleotide synthesis and to generate intermediate metabolites required for lipid synthesis in the membranes and myelin structures, and amino acids in the structure of proteins and neurotransmitters.

The metabolic pathway of glucose in the brain depends on the type of cell and the selective expression of the enzymes involved. Neurons predominantly have an oxidative metabolism, whereas astrocytes are largely glycolic [4]. Following cytosolic reactions, energy fuels are mainly metabolised in the mitochondria. Pyruvate metabolised glucose can be actively transported into the mitochondria where it is converted to acetyl coenzyme A, which in turn is complexed with citrate produces nicotinamide adenine dinucleotides (NADH) and flavin adenine dinucleotides (FADH2) [5]. In order to maintain an effective and prompt mitochondrial network, the cell has adaptive control mechanisms for environmental changes [6]. Glycolysis and oxidative phosphorylation are closely coupled and serve as a molecular interconversion system. The balance between glycolysis and PPP rates in neurons is very important. Thus, misappropriation of glucose utilization exclusively to glycolysis may result in decreased NADPH availability, increased oxidative stress and cell death. Though negligible compared to peripheral energy deposits, glycogen is the largest energy reserve in the brain [4]. Astrocyte use of glucose is complementary to that of neurons.

There are two enzymes primarily involved in the metabolism of glycogen, one being glycogen synthase (GS) and the other glycogen phosphorylase (GP). Glycogen, a glycolic product, is stored exclusively in astrocytes because GS is in an inactive state in the neurons. Lactate is formed from glucose in the astrocytes and then transferred to the neurons, where it is vital not only for the metabolism of neurons, but also in creating synapses and dendrites and in expressing genes involved in the memory processes.

Deterioration of energy metabolism is characteristic to brain aging, and to Alzheimer's disease (AD) and other neurodegenerative diseases. Both age, and the decreased metabolism state found in these diseases share common risk for oxidative stress and neuroinflammation. The cognitive decline associated with age could be explained by the activation of microglia and higher expression of cytokines involved in inflammation. Also, age and AD could also have in common an impaired neurovascular activity mediated by nitric oxide (NO), leading to progression of neuronal dysfunction [7].

Insulin receptor and insulin resistance in the brain

The insulin receptor (IR) belongs to the family of protein kinase receptors. Studies in recent decades have confirmed the existence of insulin and IR in the brain and have demonstrated the role of insulin-dependent brain regulation in maintaining balanced body energy. The presence of cerebral IR has been proved in animal and human neurons, but also in glial cells. It has been shown that the structure of these central receptors and their mechanism of action is similar to peripheral insulin receptors. They have a tetramer structure, composed of four subunits: two alpha and two beta. After coupling with insulin, the tyrosine kinase is

activated and initiates a cascade of intracellular events similar to those observed in the periphery [8]. However, there are differences between the brain and peripheral IR, regarding molecular size, capacity to determine an immune response, and the way they are regulated by insulin [9]. Whereas peripheral IR show down-regulation to excess insulin, the brain IR do not, suggesting different mechanisms of regulation of the IR in the two sites. There are also differences in the concentration of cerebral IR in particular regions of the brain: the olfactory bulb, vascular plexus, hypothalamus (especially arcuate nucleus), limbic system, cerebellum, brain stem, mesencephalic structures, thalamus are the sites with most abundant in IR [10]. The presence of insulin and a high concentration of IRs have been observed in the hippocampus, a cerebral structure responsible for cognitive function, memory and learning ability (including memory-associated signals) [11]. By acting through specific receptors, cerebral insulin signaling is involved in regulating vital processes responsible for the smooth functioning of the CNS, not only for the metabolism of "brain" glucose [12]. Insulin exerts its effects by allowing control or as a mediator of several processes such as: cognitive and reproductive function, energy and weight homeostasis, neurotransmitters release, synaptic plasticity, growth, differentiation and function of neurons [8].

Insulin resistance implies a decreased ability of insulin to act on the targeted tissues. Accumulated evidence suggests that cerebral insulin resistance and decreased glucose hypometabolism at this site could be the cause, rather than the consequence of age-related or disease related neurodegeneration. CNS has an important contribution in the development of insulin resistance, obesity and type 2 diabetes mellitus (T2DM). Impaired central insulin signaling leads to hyperinsulinemia, decreased insulin sensitivity and body mass gain. Post mortem, in people with T2DM or obesity, reduced expression of neuronal insulin receptors, along with lower levels of insulin have been reported [13]. Traditionally, it is understood that the bond between insulin and its dimerized receptors activate specific transport proteins that in turn mediate the facilitated glucose uptake. However, neurons have the ability to uptake glucose by other mechanisms as well, including non-insulin-dependent transporters [12]. Because most of the cerebral absorption of glucose is not insulin dependent, the brain has long been considered insensitive to insulin.

However, the presence and activity of glucose transporter GLUT4 (which is insulin-sensitive) have been demonstrated in several nuclei. GLUT3 is the most abundant transporter of glucose in neurons. Glial and endothelial cells of the brain depend on GLUT1 activity for interstitial fluid (ISF) and plasma glucose absorption [14]. Since GLUT1 or GLUT3 are not insulin-sensitive transporters, most of the transport of glucose in brain cells does not require insulin signaling. Still, the brain as an organ has been proved to be receptive to insulin, which in particular acts as a neuroregulratory peptide [1]. GLUT4 has been found together with IR in the brain and also have been proven to decrease after T2DM has been induced. Cognitive processes are vitally regulated by the uptake of glucose in the brain, and particularly in the hippocampus, which is why insulin signaling leads to translation of GLUT4 to the cell membrane of neurons in the hippocampus. Studies have shown that administration of insulin in the hippocampus leads to a fast and steady rise in local glycolysis in normal animals, but not in animals with induced T2DM [15]. In studies showing memory enhancement, insulin has a dose-response curve with an inverse U

shape like that noticed when glucose is directly delivered [11]. The two main sites where insulin plays a role in neural metabolism by promoting the absorption of glucose are the hippocampus and the medial temporal lobe. These data offer an explanation for the role of insulin in the cognitive processes in the hippocampus and create a starting point when addressing the cognitive decline noticed in diabetes [15]. Administration of insulin into cerebral ventricles significantly decreases the intake of food and reduced weight in primates, while also increasing cognitive function in rats [16,17]. In humans, the hyperinsulinemic euglycemic clamp was not found to have an effect on the food intake in the short-term [18]. Because basal plasma insulin level correlates with adiposity, it has been argued that insulin provides CNS with a regulating or reporting signal regarding energy reserves, and, even more, a satiety signal [19].

The roles of peripheral insulin on the brain

The intranasal administration of insulin facilitates its penetration into cerebrospinal fluid (CSF) more than the subcutaneous or intravenous injections. It induces a reduction of food intake in men, less desire for tasty foods in women, with no risk of hypoglycemia, and improved memory in the elderly with cognitive impairment [20-22]. Many studies have used CSF as a surrogate for brain interstitial fluid (ISF). However, recent findings acknowledge differences in the CSF composition compared with ISF, when CSF is assessed directly (in the cerebral ventricles or in the lumbar spine). CSF is a contributor to the cerebral ISF, but there are also other solutes carried through the BBB which create a different composition. The relative contribution of each of these two pathways makes it difficult to know the percentage of peripheral insulin found in ISF. Animal and human studies consistently show a high gradient of plasma insulin to insulin in CSF in healthy subjects; plasma insulin is increased up to 10 or 20 times in those with insulin resistance [23,24]. This gradient is even greater in obese people [25].

The consensus is that insulin is produced in the CNS in little or no amount [26]. Therefore, insulin from the CNS essentially depends on the peripheral insulin capacity to cross the BBB. The role of BBB is to restrict the passage of proteins and peptides between the two compartments and is an important interface in the mediation of intestinal-brain axes. However, there are peptides and regulatory proteins that pass through the BBB using either saturable or unsaturable mechanisms. The hormones insulin and leptin have specific carriers for passing the BBB, and the mechanism used by insulin is saturable. The conveyor is unevenly distributed in the CNS. Being partially saturated in euglycemia implies that the main function of signaling takes place at normal blood levels, and it's not to prevent hypoglycaemic events [27]. The insulin transporter through BBB might exist in order to facilitate the role of insulin in the CNS as a regulatory peptide [28]. A number of conditions, such as growth and development, but also fasting, hibernation, obesity and diabetes, AD are characterized by a modified insulin transport rate through the BBB carrier. Administrating lipopolysaccharides to mice increases insulin transport through BBB approximately three-fold, indicating a mechanism that would promote insulin resistance in sepsis. Dexamethasone inhibits the transport of insulin through the BBB which could explain the improved appetite noticed during treatment with corticosteroids [29].

Endothelial cells of the brain (BEC) found in the BBB produce different substances, among which cytokines, different for each part of the barrier. As such, adiponectin apparently inhibits

the production of interleukin-6 from BEC. Insulin itself influences BBB, altering its roles in transport and as enzyme system (e.g. in BEC). The integrity of the BBB and its capacity to transport insulin is maintained by the pericytes, which are pluripotent cells in close contact with BEC [30,31].

Long-term hyperinsulinism exacerbates the chronic inflammatory response and increases oxidative stress. The explanation would be the intervention of ceramide resulting from alteration of peripheral lipid metabolism ("liver-brain axis") that passes the BBB with neurotoxic effects through proinflammatory cytokines [32]. Insulin acts in the CNS mainly as a metabolic regulatory hormone, where its actions are mediated by the phosphoinositide-3 kinase (PI3)/Akt pathway and the Ras/mitogen activated kinase (MAPK) cascade. The literature has focused on PI3K as a peripheral and central insulin-like signaling pathway, including in the hippocampus. However, there are other important mechanisms like opening the potassium ATP channel (K_{ATP}), thus modulating the neural metabolism. Animal studies on insulin resistance have shown that modulation of K_{ATP} channels in the hippocampus affects memory performance, proving the ability of insulin to interfere with activity in this area and through pathways independent of PI3K interaction. Significant evidence indicates that the role of insulin in the brain involves also MAPK [33].

Insulin may also modify synaptic plasticity, meaning long-term membrane potential (LTP) and long-term membrane depression (LTD). In the first situation, insulin acts on the synaptic membrane by removing AMPA channels or on the LTP induction by modifying the stimulation-response frequency curve. Insulin is probably directly responsible for acute neurotransmission (both excitatory and inhibitory), and for the long-term plasticity through GABA-ergic modulation (GABA receptors are translocated to the plasma membrane). Several studies propose that systemic insulin resistance associates itself with central resistance to insulin and that a cause for cognitive decline is impaired insulin signaling. T2DM characterized by insulin resistance is associated with mnestic disorders and blockage of insulin signaling in the hippocampus, producing major cognitive deficits in these patients [15].

Insulin signaling induces the uptake of glucose in the brain but also the production of the insulin degradation enzyme (IDE) as to prevent hypoglycaemia. IDE degrades both insulin and beta-amyloid (Aβ), which is why in hyperinsulinemia, insulin could compete with Aβ for degradation, leading to Aβ accumulation [34,35]. Insulin protects neurons against oxidative stress, associated with conditions such as diabetes mellitus, chronic ischemia or age-related neurodegenerative diseases. Oxidative stress results in insulin stimulating the uptake of glucose into neurons where it is metabolized into pyruvate; this restores the intracellular ATP and phosphocreatine [36]. Insulin also influences the concentration of adenosine (increasing it inside the cell, decreasing it outside the cell) using the PI3 kinase pathway and the one involving extracellular signal-regulated kinase [37].

There are two theories which confirm the link between T2DM and AD, even if the subject is still under debate: insulin resistance and inflammatory signaling pathways [38]. Insulin resistance and hyperinsulinemia, which by all classifications define T2DM, have also been linked to cognitive impairment in the elderly [39,40]. While acute insulin administration may improve some areas of memory, chronic insulin administration could be linked to memory impairment [38].

Age-related cognitive decline is linked to the activation of microglia and increased

inflammatory cytokines [41]. There is a theory (still in need for confirmation) which stipulates that the first modification leading to cognitive impairment in aging and AD is metabolic deficiency resulting in dysfunction of the mitochondria or neuroinflammation resulting in the activation of microglia and increasing cytokines. The impairment of mitochondria and chronic inflammation stimulate each other in a metabolic-inflammatory axis in both T2DM and AD [42]. Proinflammatory cytokines are viewed as an indirect sign of the immunological impairment which can result in insulin resistance [43]. Several studies on animals and humans have shown the anti-inflammatory properties of insulin which is able to suppress pro-inflammatory cytokines and to induce anti-inflammatory mediators [44].

Conclusions

The deterioration of energy metabolism characterizes brain aging but also many neurodegenerative diseases such as AD, but is also related to other severe mental illnesses. The decreased brain metabolism found in aged populations or some diseases is most often related to selective brain insulin resistance, microglia activation and neuroinflammation. We can assert that insulin is a neuroprotective factor against hypometabolism, oxidative stress, inflammation and apoptosis, with neuromodulatory and neurotrophic effects.

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