Functional and therapeutical implications of insulin synthesized or received by neurons of the cerebral cortex

Éva Adrienn Csajbók[1] and Gábor Tamás[2]

[1]University of Szeged, 1st Department of Internal Medicine, Endocrine Unit, Korányi fasor 8-10, 6720, Szeged, Hungary

[2]MTA-SZTE Research Group for Cortical Microcircuits, Department of Physiology, Anatomy and Neuroscience, University of Szeged, Közép fasor 52. Szeged, 6726 Hungary

Corresponding author:

Gabor Tamas

MTA-SZTE Research Group for Cortical Microcircuits, Department of Physiology, Anatomy and Neuroscience, University of Szeged, Közép fasor 52. Szeged, 6726 Hungary

Tel.: +36 62 544851

Fax: +36 62 544291

e-mail: gtamas@bio.u-szeged.hu

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Abstract

Recent results suggest that insulin is synthesized by a subpopulation of neurons in the cerebral cortex and neural progenitor cells of hippocampus. Supplementing the slow supply of insulin to the brain by pancreatic beta cells, insulin locally released by neurons provides rapid means for the regulation of local microcircuits effectively modulating synaptic transmission and on demand energy homeostasis of neural networks. Modulation of insulin production by neurons of the brain by GLP-1 agonists might be useful in counteracting diabetes, obesity and neurodegenerative diseases and replacement of lost pancreatic beta cells by autologous transplants of insulin producing neural progenitor cells could be a viable therapy for diabetes.

Multiple functions of insulin the brain

A central concept in diabetology is that insulin promotes cellular glucose uptake, thus lowers the concentration of glucose in extracellular compartments of the body. Insulin mediated glucose uptake primarily requires the action of the glucose uptake transporter GLUT4, however, in the brain, the insulin independent GLUT1 and GLUT3 are predominantly

responsible for glucose uptake in glial cells and neurons, respectively [1]. Accordingly, metabolism of the brain has been considered insulin independent for decades, but the discovery of insulin receptors in the brain [2] indicated that cerebral functions of insulin are more complex. Indeed, a series of reviews on the topic highlight that insulin is an effective neuromodulatory peptide with an array of effects including control of food intake and body weight, regulation of the reproductive or hypothalamic-pituitary-gonadal axis, influencing neuronal survival and modulation of memory and cognitive processes [3–6]. Moreover, the insulin dependence of brain metabolism has been revisited by a number of human in vivo studies [7–9] suggesting that insulin might also effectively regulate glucose uptake in the brain, especially during periods of intensive neuronal activity [10]. This is of particular interest for two reasons. First, neuronal ensembles of the hippocampus and the neocortex are engaged in increased, high frequency epochs of firing during memory formation and cognitive tasks and the extra metabolic demand created by intensive action potential generation might be met by alternative routes of supply. An unorthodox pathway of glucose supply during cognitive surges in energy demand was suggested by Emmanuel et al. [10] proposing that noninsulin-dependent GLUT1 and GLUT3 transport is sufficient for resting brain activity, while sustained cognitive activity induces the addition of insulin-signaled GLUT4 transport. Second, unlike in other organs, glucose is central for the energy metabolism of the brain and temporary or sustained changes in glucose supply could be crucial in differentiating normal and pathological functions of neural circuits. Cognitive deficits are associated with insulin resistance [11, 12] and impaired insulin dependent mechanisms for glucose uptake during tasks requiring extra supply might lead to deficient energy metabolism [10]. Along the same vein, “type 3 diabetes” was suggested as an alternative term for Alzheimer’ disease [13] based on observations showing reduced insulin expression and signaling mechanisms in the sporadic form of the disease [14].

Pancreatic insulin reaches the brain

As outlined above, normal supply of insulin in the brain appears to be crucial for neural function including metabolism and, consequently, dynamic or persistent alterations in insulin dependent mechanisms could contribute to pathological processes. Sources of insulin found in the brain are not completely clear. It is generally accepted that insulin synthesized by pancreatic beta cells is delivered to the brain [3–6, 15, 16], but an accurate picture of this process is missing [17]. Pancreatic insulin circulating in the plasma finds two ways into the interstitial fluid immediately surrounding neurons and glial cells of the brain. The first pathway delivers relatively small amounts of plasma insulin through the choroid plexus to the cerebrospinal fluid. Plasma concentrations of insulin are an order of magnitude higher compared to those measured in the cerebrospinal fluid [18, 19]. Interestingly, this difference is increased by obesity [20] in spite of higher plasma insulin concentrations in the obese. This process is saturable [16, 21], but it is not clear whether saturation is caused by the potential involvement of insulin receptors of the choroid plexus or by the suspected contribution of megalin, a transporter known to mediate leptin transport across the choroid plexus and involved in insulin transport [22] in epithelial cells of renal tubules [23]. The second pathway takes insulin from the plasma into endothelial cells of the brain microvasculature. Based on experiments showing the ability of aortic endothelial cells outside the brain to concentrate insulin [24], the second pathway is hypothesized to transport the bulk of peripheral insulin to the brain. Mechanisms of transendothelial insulin transport in the brain were not directly studied to date, but one can speculate that a vesicular trafficking process, beginning with insulin binding to its receptor followed by the involvement of caveolae and promoted by NO signaling [24–27], could be involved according to experiments testing peripheral endothelia.

The two pathways join at the Virchow-Robin space surrounded by endothelial cells, astrocytic endfeet and pericytes, then peripheral insulin has to pass the line of astrocytic endfeet, an effective filter and movement speed limiter for larger molecules [28], before reaching the interstitial space around neurons and glia. Absolute insulin concentrations are difficult to measure reliably in the interstitial space of the brain, but the relative changes detected in response to food intake were independent of plasma insulin concentrations [29, 30] raising the possibility for pancreas independent, local insulin synthesis in the brain [4].

Evidence for local insulin synthesis in the brain

Whether insulin is produced locally in the central nervous system is not a trivial question to answer. Initial studies on the subject suggested that immunoreactive insulin is present in the the rat brain in concentrations 10 to 100 times higher compared to the plasma [31], but this was challenged by subsequent findings [32] leading to conclusions that "little or no insulin is produced in the bran" [33]. The heart of the problem is that experiments must be able to differentiate between insulin of pancreatic origin and insulin synthesized locally. Anti-insulin antibodies recognize the same epitopes on pancreatic and brain derived insulin, thus methods like anti-insulin immunocytochemistry or radioimmunoassay capable of detecting insulin in small amounts are not adequate. Increase in the resolution to cellular or subcellular localization of anti-insulin immunoreaction signals are of no unequivocal help due to receptor bound and internalized insulin pools being degraded or recycled to the plasma membrane having potentially overlapping intracellular localisation with the locally synthesized peptide [34]. Immunoreactions detecting peptides involved in steps of insulin synthesis which, to might overcome these limitations. Indeed, C-peptide, an integral part of proinsulin was localised to the same neurons as insulin [35–37] and proinsulin-like immunoreactivity was also documented in samples derived from the central nervous system [38] arguing for local synthesis in the brain.

Another strategy for detecting insulin production in the brain is to search for mRNAs of insulin coding genes represented by both *ins1* and *ins2* in mice, but only by *ins2* and *ins* in rat and human, respectively. A pioneering RT-PCR study detected widespread *ins2* expression in the rat brain throughout development [39] and the same laboratory confirmed this in rabbit showing *ins2* expression in neurons of the hippocampus and olfactory bulb [40]. More recently, hippocampal granule cells from adult rats and neuronal progenitor cells derived from the hippocampus or olfactory bulb were also found to express insulin mRNAs [37]. Furthermore, expression of the *ins2*, but not *ins1* gene was found in cortical and subcortical areas of the mouse brain [41, 42] and *ins* mRNA expression characterized human samples of the hippocampus, amygdala and temporal lobe in addition to olfactory bulb, cerebellar and pontine regions [42]. Recent methodological developments in precisely quantifying copy numbers of mRNAs in single neurons [43] provided an effective tool for determining ins2 in several rat neuron types and astrocytes in the rat cerebral cortex [44]. Interestingly, a subset on inhibitory GABAergic neurons, the so-called neurogliaform interneurons, expressed *ins2* mRNAs in the highest copy numbers tested, excitatory pyramidal neurons contained *ins2* mRNAs in small copy numbers and other GABAergic neurons and astroglia cells did not express *ins2* mRNAs above detection threshold [44]. Importantly, the authors found that mRNA numbers were raised in response to increasing extracellular glucose concentrations selectively in the cell types which expressed *ins2* [44] suggesting that neuronal production of insulin could be associated with local metabolic supply and functional demand especially in neocortical and hippocampal areas of the cerebral cortex.

Function and therapeutic considerations – peripheral and central routes of insulin to and from neurons

The speed of the process transporting pancreatic insulin into the cerebrospinal fluid and then to interstitial space of the brain is orders of magnitude slower compared to the speed of operation of neural networks estimated according to the lowest frequency of brain oscillations. Several hours of peripheral hyperinsulinemic euglycemic clamp is required to produce insulin levels in the cerebrospinal fluid not reaching fasting levels [19, 21] and, moreover, fasting insulin levels in the cerebrospinal fluid (~7 pmol/L)[45] are insufficient for signal transduction through insulin receptors. Even if insulin concentrations in the cerebrospinal fluid are elevated to effective levels, it was estimated that the slow circulation of cerebrospinal fluid limits insulin delivery to the interstitial space of the brain at a rate of ~1/600th of skeletal muscles and at <1/30000th of the liver [17]. Alternatively, insulin might move directly from the plasma through the blood brain barrier to the Virchow-Robin space and to the interstitial fluid, but studies examining the involvement of this route measured tissue content of radiolabeled insulin in brain regions [46] not allowing determination of insulin concentration in the interstitial fluid. To date, estimations of the speed by which insulin moves across the blood brain barrier are limited due to the ability of brain microvessels to bind insulin with high affinity without significant degradation of insulin [47]. Nevertheless, insulin finds its way from the plasma to the immediate vicinity of neurons, but equilibration of the interstitial space in the brain is achieved at timescales consistent with long term homeostatic regulation outside of the frequency range (~0.1-200 Hz) of membrane potential changes in neural networks.

The limited speed by which external insulin is being distributed is also a factor to consider when delivering insulin to the brain through intranasal application [45]. This process has gained particular relevance following encouraging reports [48] and clinical trials [49] providing evidence for cognitive improvements of daily intranasal insulin administration for patients with mild cognitive impairment or mild to moderate Alzheimer's disease. Counteracting reduced levels of insulin in Alzheimer's disease [13, 50] intranasally applied insulin raises concentrations in the cerebrospinal fluid within 10 minutes of application with maximal levels after 30 minutes, while plasma insulin and glucose levels remain unaffected [45]. How intranasal insulin reaches the brain remains mechanistically unclear [51] but can be stimulated by the inhibition of protein kinase C [52]. A different strategy for increasing insulin concentrations in key areas affected by Alzheimer's disease like the hippocampus and neocortex would be to boost insulin release from neurons or neuronal progenitors expressing insulin locally.

The first experiments showing local release of insulin in the cerebral cortex followed classic ideas of mimicking the effect of externally added compounds with endogeneously released substances. In this case, Molnar et al. [44] first determined that external insulin is effective in suppressing spontaneous excitatory potentials arriving to neurons of the neocortex, then, using local delivery of glucose or glibenclamide to neurogliaform interneurons (known to express *ins2* mRNAs, see above) forced the release of an endogeneous substance which also suppressed spontaneous excitatory potentials. Finally, they blocked this effect with the specific insulin receptor antagonist S961 revealing the identity of the endogeneous substance as insulin. Thus, insulin can be released from a subpopulation of inhibitory neurons of the cerebral cortex and has an excitation suppressing effect in local neural microcircuits. Insulin is instrumental in moving additional GABAA receptors to inhibitory synapses [53] and extrasynaptic regions of the plasma membrane [54] and stimulates endocytosis of AMPA

receptors from excitatory synapses [55] providing synergistic mechanisms for shifting the balance away from excitation in neural networks. It is not yet known which combination of neural afferents elicit insulin release from neurogliaform cells, however, it is reasonable to assume that strong excitatory inputs might contribute to intracellular Ca^{2+} accumulation required for peptide release. One can speculate that insulin release could be synchronized to above average overall activity in networks around neurogliaform neurons. This way transient local energy demand could be met by insulin release driven additional glucose transport through insulin dependent GLUT4 as suggested for epochs of intense hippocampal or cortical activity during cognitive processing [10] and, at the same time, the overall excitation suppressing activity of insulin might curtail energy demand.

The effect of glibenclamide in triggering neuronal release of insulin [44] also suggests that delivery of substances known to enhance insulin release from pancreatic beta cells to the brain might have therapeutic implications. A yet to be tried strategy for increasing insulin concentrations in key areas affected by Alzheimer's disease like the hippocampus and neocortex would be to boost insulin release from neurons or neuronal progenitors expressing insulin locally. Apart from sulfonylureas, incretins might represent a promising group of molecules to be tested for several reasons. GLP-1 receptors are expressed in neurons of the hippocampus and the neocortex [56], although the expression of GLP-1 receptors has not been documented on insulin expressing neurons or neural progenitor cells. Interestingly, however, GLP-1 agonists have similar effects on tonic inhibitory GABAergic currents as reported for insulin arguing for a hypothetical contribution of GLP-1 receptor mediated insulin release [54, 57]. GLP-1 is produced in the brainstem [58] suggesting that centrally synthesized GLP-1 could be effective within the brain in the mechanisms outlined above. However, GLP-1 produced by L-cells of the intestine crosses the blood brain barrier [58] and thus incretins arriving from the periphery have the possibility to enhance insulin release from neurons in the brain. Importantly, these peripheral incretins include GLP-1analogues prescribed in type II diabetes mellitus. We suggest that the weight loss caused by GLP-1 receptor analogue based therapy (attributed primarily to the inhibition of gastric emptying [59]) might have an additional, synergistic component through GLP-1 receptor mediated insulin release from neurons of the brain. Human imaging studies suggest that the prefrontal cortex is crucial in the inhibitory control of food intake [60–62] and hypothetical expression of GLP-1 receptors on insulin releasing neurogliaform neurons of the prefrontal cortex could provide mechanistic support for this process. Moreover, GLP-1 receptor agonists promise therapeutic effectiveness against neurodegeneration in models of Alzheimer's, Huntington's and Parkinson's disease [63, 64] and scenario of GLP-1 receptor mediated insulin synthesis in the brain could be extended to the therapy of these diseases.

The evidence for insulin synthesis in the brain raises the question whether brain derived insulin could be used for replacement of insulin in the periphery in type I diabetes mellitus. Insulin synthesized in the brain is unlikely to cross the blood-brain barrier in the brain to blood direction in quantities required for euglycemic control of plasma glucose concentrations [5] and intranasal insulin delivery fails to increase plasma insulin levels significantly [45]. An alternative approach might use autologous grafts of insulin expressing neurons or neural progenitor cells as a potential replacement for lost pancreatic beta cells. Such neuron or neural stem cell based therapy of diabetes is suggested by spectacular results by Kuwabara et al. [37] raising the possibility that neural stem cells isolated from the adult brain can functionally replace beta cells in diabetic patients [65, 66]. The suggested workflow for autologous neural stem cell based therapy for diabetes is critically based on the observation that insulin expressing neural stem cells of the dentate gyrus or the olfactory bulb

might find similar molecular niches for their survival and insulin expressing ability in the brain as well as in the pancreas involving Wnt3 and NeuroD [37, 66]. Neural stem cells can be isolated from rodent and human olfactory bulbs [37, 67] and rat cells can be transplanted directly into the pancreas of diabetic rats [37] where the pancreatic niche reprograms neuronal stem cells via Wnt signaling to express insulin. Isolating neural stem cells from streptozotocin induced type I diabetic or from type II diabetic Goto-Kakizaki rats followed by transplantation to the pancreas of animals of the corresponding model confirmed that grafted cells survive and produce insulin for long periods (>10 weeks) and dramatically reduce blood glucose levels [37]. The therapeutic potential of this study for human diabetic patients is immense because no genetic manipulation is necessary and the procedure bypasses tumorigenic pluripotent stem cells and concerns inherent to chronic immunosuppression.

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