



Glucagon-Like Polypeptide-1 and Brain

Glukagon Benzeri Polipeptid-1 ve Beyin

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Abstract

Glucagon-like polypeptide-1 has specific effects on the central nervous system, including regulation of glucose metabolism, positive cardiovascular effects, slowing intestinal motility, immune modulation, and regulation of appetite and energy expenditure. Recently, positive effects of GLP-1 on brain energy utilization, inhibition and restoration of neurodegeneration, response to stress, and protection against ischemic neuron damage have been demonstrated. Herein, the effects of glucagon-like polypeptide-1 on the central nervous system will be discussed.

Keywords: Glucagon-like polypeptide-1; brain; central nervous system; neurodegeneration; stroke

Introduction

Glucagon-like polypeptide-1 (GLP-1) is mainly produced in the small intestinal entero-endocrine cells, pancreatic alpha cells, and the nucleus tractus solitari (NTS) of the central nervous system, using preproglucagon as a precursor. It can be released after conversion by prohormone convertase, and in less than 2 min, is broken down and inactivated by dipeptidyl peptidase-4 (DPP-4). In the circulation, GLP-1 can freely pass the blood-brain barrier (BBB), although only 20% reaches the brain

Özet

Glukagon benzeri peptid 1'in glukoz bağımlı insülin sekresyonu, beta hücre proliferasyonu, glukagon sekresyon inhibiyonu ile hepatik glukoz çıkışının azalması gibi metabolik etkilerinin yanında kardiyovasküler sistem üzerine olumlu etkiler, gastrointestinal motilitede yavaşlama, immunomodülasyon gibi etkileri olduğu bilinmektedir. Son zamanlarda santral sinir sistemi üzerine de belirgin etkiler gösterdiği saptanmıştır. Santral etkiler esas olarak iştah regülasyonu ve metabolizma üzerine olan etkilerin dışında beyin enerji kullanımının düzenlenmesi, nörodejenerasyonun önlenmesi ve düzeltilmesi, iskemik nöron hasarına karşı koruma ve organizmanın strese olan cevabının düzenlenmesi şeklinde sıralanabilir. Burada glukagon benzeri peptid 1'in santral sinir sistemi üzerine olan etkileri tartışılacaktır.

Anahtar kelimeler: Glukagon benzeri peptid 1; beyin; merkezi sinir sistemi; nörodejenerasyon; inme

owing its short half-life in circulation (humoral pathway) (1,2). Industrially produced GLP-1 receptor agonists (GLP-1RA) can pass the BBB, whereas DPP-4 inhibitors cannot (3).

The important effects of GLP-1 in the brain have recently been discovered. It was observed that the main sources of GLP-1 in the brain were the neurons that expressed preproglucagon in the NTS (4). Unlike the intestinal enteroendocrine cells, which are stimulated by food, these cells are activated with gastric distention, systemic cholecys-

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tokinin, lipopolysaccharide, or the application of lithium chloride, local leptin, or cholecystokinin stimuli. Also, GLP-1 in the peripheral circulation has the potential to excite the neurons that produce preproglucagon in the NTS. Moreover, vagal excitation is the main source of stimulus on the preproglucagon neurons in NTS and secretes GLP-1 locally (neural pathway). GLP-1 secreted from the intestine can generate vagal excitation by either local effect via intestinal vagal afferents, or by stimulating the GLP-1 receptors on the portal venous system. Vagal afferents can also be directly stimulated by cholecystokinin, leptin, oxytocin, peptide yy, and nesfatin (Figure 1). Injection of GLP-1 in the portal vein increases insulin secretion from the pancreas, whereas this effect vanishes after hepatic vagotomy or knockdown of GLP-1 receptors in vagal afferents. This indicates that GLP-1 generates its insulinotropic effect not only by directly acting on beta cells but also by central effect (neuroendocrine effect) (5). Also, excitatory, inhibitory, or neuromodulatory effects were observed on the feeding behavior of test animals after vagal denervation. Therefore, GLP-1 might play an important role in long-term energy homeostasis, besides its known systemic effects. This hypothesis is also supported by

the projection of neurons in the NTS that produces preproglucagon for supply to the presynaptic areas of regions such as area postrema, interpeduncular nucleus, posterodorsal and ventral tegmental nucleus, hypothalamic nuclei, thalamic nuclei, nucleus accumbens, and lateral septum, especially for feeding and energy homeostasis. The presence of GLP-1 receptors in these areas also support this hypothesis (6).

GLP-1 in both circulation and brain show their effect by connecting with the GLP-1 receptors (GLP-1R). Excitation of GLP-1R in different centers of the central nervous system can create different responses. As a result of GLP-1 activation in the brain, various physiological processes, such as appetite modulation, regulation of nausea, inhibition of reward and motivation centers, energy homeostasis, neuroprotection, increase in neural insulin sensitivity, neural plasticity, and memory formation, and immune modulation can occur.

Experiments conducted using GLP-1R analogs have demonstrated that they influence the arcuate nucleus (ARC), paraventricular nucleus (PVN), and lateral hypothalamic area by acting like GLP-1R, mainly on the Proopiomelanocortin/Cocaine- and amphetamine-regulated transcript (POMC/CART) anorexigenic neurons and in-

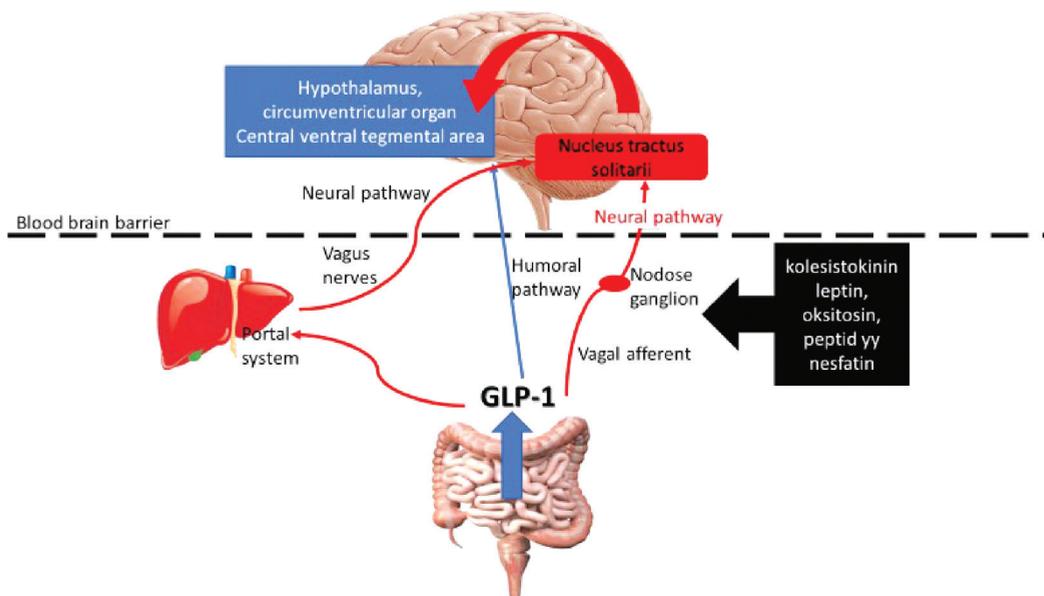


Figure 1: Humoral and neural pathways of the GLP-1 effect on the brain.

directly inhibit the neuropeptide Y/Agouti-related peptide (NPY/AgRP) orexigenic neurons through GABAergic signals. Similarly, the stimulation of GLP-1 in the ventral tegmental area, known as the reward-motivation center, has been observed to control the mesolimbic dopaminergic signals, which decreases the pleasure of eating (7). It has also been demonstrated that the effect of GLP-1 analogs on the ventromedial hypothalamic neurons increases the sympathetic activity, which increases the thermogenesis with retention of glucose and triglyceride in the brown fat tissue, and causes the transformation of the white fat tissue to brown fat tissue (8).

GLP-1Rs are located in the G protein-coupled receptor group, and their stimulation activates some intracellular pathways (2). Stimulation of the receptor by GLP-1 activates some pathways which can lead to acute or chronic responses. Examples of acute responses include insulin secretion, exocytosis, and an increase in the intracellular calcium ion concentration. The acute response mainly occurs with the activation of phosphoryl kinase via the cyclic adenosine monophosphate (cAMP) pathway. Examples of chronic effects include modulation of the gene expression, cell growth, cell proliferation, cell differentiation, and anti-apoptotic activity. These effects occur through the phosphoinositide 3-kinase (PI3K) pathway, which is similar to the insulin downstream pathway. Activation of PI3K triggers the activation of protein kinase B (AKT). This molecule is responsible for protein synthesis, an increase in anti-apoptotic factors, and inhibition of intracellular apoptotic factors. Another important pathway responsible for the chronic effect is the mitogen-associated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway. The activation of this pathway is responsible for important cytoprotective effects, such as inhibition of apoptosis, a decrease in oxidative stress, and a decrease in the inflammatory response.

Metabolic Basis of Neurodegenerative Diseases

Neurodegenerative diseases with dementia can affect memory, learning, cognitive functions, and behavior. There are approxi-

mately 50 million dementia patients worldwide, and it is the 7th leading cause of death. Alzheimer's disease (AD) and Parkinson's disease (PD) account for a major proportion of neurodegenerative diseases. AD is characterized mainly by the extracellular accumulation of amyloid β protein, with the intracellular accumulation of hyperphosphorylated τ protein in filamentous form, neuroinflammation, and decrease in the utilization of glucose by the brain. On the other hand, PD is characterized by the accumulation of neuronal α -synuclein and Lewy fibrils, with the loss of dopaminergic neurons due to neuronal mitochondrial dysfunction. The common features of neurodegenerative diseases include the formation of neurotoxic aggregates, increased oxidative stress, activation of apoptotic pathways, neurotransmitter insufficiency, and neural differentiation failure (9). A decrease in neuronal glucose utilization due to cerebral insulin resistance is an important feature of AD (10). Initially, the brain was thought to be insensitive to insulin, but the eventual discovery of the presence of insulin receptors in certain parts of the brain has indicated that this molecule plays an important role in the metabolic, neurotropic, neuromodulatory, and neuroendocrine regulation in the brain, as well as memory and learning (11). Glucose utilization in the neurons occurs via insulin-independent glucose transporter-3 (GLUT-3) protein, whereas the forebrain, cerebral cortex, and hippocampus show coexpression of the insulin-dependent glucose transporter-4 (GLUT-4) protein (12). It is suggested that activities such as learning and remembering, which increase metabolic needs, can cause the expression of the translocated GLUT-4. Similarly, expression of GLUT-4 has also been shown in the hypothalamus, which plays an important role in metabolic control. Besides the utilization of cerebral glucose, insulin plays an important role in synaptic plasticity, neurite multiplication, neuroprotection, memory formation, cognitive arrangement, multiplication, differentiation and myelination of oligodendrocytes, and determination of the levels of neurotransmitters such as acetylcholine and norepinephrine (13,14). It has been demonstrated that insulin is synthesized in the cerebral cortex and hip-

pocampus in the central nervous system, and that small amounts of pancreatic insulin can pass the BBB via selective transport and affect brain functions (15). Insulin levels in the cerebrospinal fluid (CSF) are correlated with those in plasma, and its transport through the BBB may be affected by obesity, diabetes, inflammation, and high plasma triglyceride levels. It was demonstrated that the presence of peripheral insulin resistance decreased the CSF/peripheral insulin ratio and flow of insulin from blood to the CSF (16). In diabetic animal models, the effect of insulin in the brain decreases and is associated with dementia (17). The Rotterdam study is among the pioneering research that highlighted the risk of increased dementia in type-2 diabetic population (18). Following this, the idea of an association between AD, insulin resistance, and brain glucose utilization was first hypothesized 22 years ago (19). The risk of dementia increases by 65% in type-2 diabetics compared to non-diabetics (20). Also, peripheral insulin resistance is associated with decreased cerebral glucose utilization, decreased cerebral perfusion, and brain atrophy (11). Obesity and type 2 diabetes, which are risk factors for AD, are associated with an increase in the fat tissue-originated pro-inflammatory adipokines and plasma free fatty acids. These cytokines can pass the BBB in AD and other conditions where the cerebral circulation is decreased. In the neurons, it can cause the phosphorylation of insulin receptor substrate 1 (IRS-1) via the activation of serine kinase. Thus, the PI3K pathway is blocked, and insulin resistance occurs (21). On the other hand, insulin increases the hepatic clearance of neuronal amyloid β . In the case of peripheral insulin resistance, the accumulation of cerebral amyloid β is increased due to diminished hepatic clearance of amyloid β (22). However, some studies advocate that the type of insulin resistance in the pathogenesis of AD is not the peripheral insulin resistance, but the neuronal one, which is present in the central nervous system (23). The fact that tight glycemic control cannot help dementia regression in diabetic patients, and that the regression is even more aggravated due to frequent hypoglycemia, also indicates that intranasal in-

sulin should improve cognitive functions and memory, in doses that do not cause peripheral hypoglycemia. These findings confirm that central insulin resistance should be differentiated from peripheral resistance. Accordingly, some researchers have named the association between central insulin resistance and dementia as type-3 diabetes (24-27).

In cerebral insulin resistance, inhibition of the AKT pathway after phosphorylation of IRS-1 results in an increase in the levels of Glycogen Synthase Kinase-3 (GSK-3). Furthermore, the pathway causes an increase in Protein Kinase A (PKA), and both these conditions contribute to the pathogenesis of AD by causing the phosphorylation of τ -protein. Increasing GSK-3 levels cause an increase in the production of amyloid- β proteins. Neurotoxic amyloid oligomers and plaques are created from amyloid- β proteins. Amyloid- β oligomers may further contribute to neuronal insulin resistance by directly causing an increase in the phosphorylation of IRS-1, activation of TNF- α signal, or activating the glial cells to increase cytokine (IL-1 β , IL-6, TNF- α) secretion. Finally, decreased intracellular entry of glucose due to insulin resistance causes a decrease in neuronal ATP production and deterioration of synaptic activity. Thus, cognitive dysfunction occurs (28).

Effects of GLP-1 on Alzheimer Disease

GLP-1 affects the brain through basic anti-apoptotic, antioxidant, and neurotrophic impact on GLP-1R. PKA and PI3K pathways are activated upon increasing cAMP, which occurs due to the stimulation of GLP-1R. Activation of PKA activation increases ERK, while the activation of PI3K increases AKT. The increase in both ERK and AKT triggers the transcription of genes responsible for antioxidant, anti-apoptotic, neurotropic, and anti-inflammatory effects (Figure 2) (29).

GLP-1 can decrease insulin resistance in the brain and reverse it. It was demonstrated that liraglutide normalized the phosphorylation of neuronal IRS-1 serine and the levels of AKT and GSK-3 β , as well as decreased the cerebral insulin levels (30). GLP-1 was also demonstrated to improve cognitive functions by increasing the utilization of glucose in the brain (31).

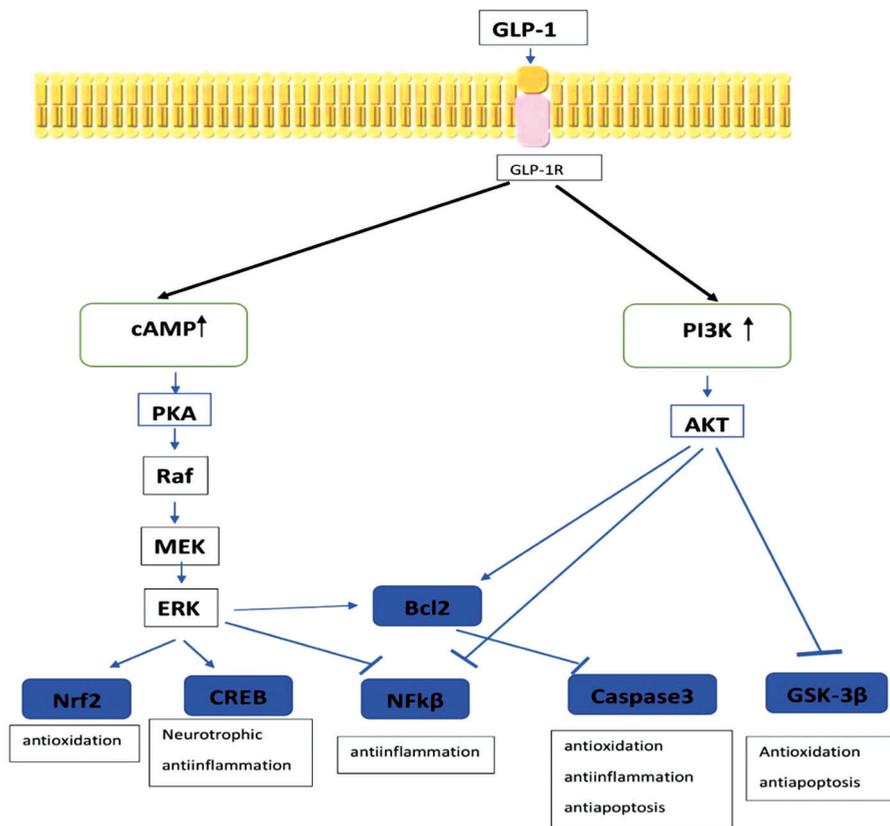


Figure 2: The neuroprotective signaling pathways of GLP-1.
cAMP: Cyclic Adenosine.

GLP-1 modulates the activation of microglia. It causes immunomodulation by inhibiting specific caspases and NF- κ B (Nuclear Factor- κ B) with PI3K/AKT pathway activity and decreases the secretion of pro-inflammatory cytokines (32).

GLP-1 protects the neurons from oxidative stress and reduces oxidative stress. GSK-3 β is the main responsible factor for oxidative stress in neurons. An increase in levels of AKT decreases in the levels of GSK-3 β and reduces oxidative stress (33). GLP-1 reduces the accumulation of intracellular ROS (Reactive oxygen species) in microglia, increases the production of nitric oxide, and increases the levels of antioxidant glutathione peroxidase and superoxide dismutase-1. These activities protect the neurons from oxidative stress.

GLP-1, via decreased caspase 3/7 activity, also inhibits microglial apoptosis and decreases the secretion of cytokines such as TNF α , IL1 β , and IL-6, that trigger insulin re-

sistance (34). When administered intraperitoneally, exenatide was observed to decrease TNF-alpha levels and neuroinflammation (35).

GLP-1 enhances synaptic plasticity in the brain. Rats treated with lixisenatide were observed to show an increase in the synaptic plasticity and learning (36). Neurites play a key role in the formation of inter-neuronal functional synapses, as well as between neurons and the surrounding microenvironment. Experimentally, exenatide has been shown to increase the levels of active neurites in cells (37). It has been observed that in rat models, lixisenatide inhibits synaptic damage due to amyloid accumulation and strengthens spatial memory (38).

GLP-1 regulates neurogenesis in the brain. It facilitates the development of new neurons and their differentiation from neuronal stem cells, rather than damaged neurons via the MAPK pathway. It also facilitates the maturation of neurites (39). Liraglutide has

been experimentally shown to increase the differentiation of neural progenitor cells to mature neurons. It inhibits neuron apoptosis by increasing the levels of the survival factors Bcl-2 (B-cell lymphoma 2) and Bcl-XL (B-cell lymphoma-extra-large) through the PI3K/AKT pathway. It increases cell proliferation through the same pathways and stimulates differentiation of neurons and growth of neurites by increasing growth factors such as brain-derived neurotrophic factor (BDNF) and glial cell-derived neurotrophic factor (GDNF) (31,40,41). Liraglutide has been shown to increase memory in experimental animals by preventing neurodegeneration and synaptic damage (42).

GLP-1 decreases the phosphorylation of τ -protein and accumulation of amyloid- β protein. It also decreases the production of GSK-3 β in the neurons via the PI3K pathway. GSK-3 β is responsible for the phosphorylation of neuronal τ -protein. Liraglutide has been observed to prevent the hyperphosphorylation of τ -protein in diabetic db/db rats (43). Besides, GLP-1 reduces the levels of endogenous amyloid- β protein in the brain and prevents the accumulation of amyloid plaques (42). The formation of amyloid plaques in experimentally created AD in female rat models has been shown to reduce by treatment with lixisenatide (44). DPP-4 (dipeptidyl peptidase) inhibitors are used as incretinergic agents for the treatment of diabetes, as are GLP-1 receptor analogs. These drugs inhibit the DPP-4 enzyme and inactivate it. They cannot pass the BBB, due to which their effect on the central nervous system is associated with increasing endogenous GLP-1 levels. Moreover, inhibition of the DPP-4 enzyme reduces the breakdown of stromal cell-derived factor-1 (SDF-1). SDF-1 binds to the chemokine receptor-4 (CXCR-4) on the surface of the neurons and activates the PI3K/AKT pathway, which causes cell proliferation and survival. DPP-4 inhibitors also positively influence vascularization in the brain by increasing the expression of vascular endothelial growth factor (VEGF) through SDF-1 (45).

To date, no randomized prospective studies have been conducted on enough humans to study the neurocognitive effect, which is different from the antidiabetic effect, using

DPP-4 inhibitors or GLP-1 agonists. According to a meta-analysis, seven studies have fulfilled the inclusion criteria, five of which are interventional, while two are observational (46). An observational study on diabetic patients has revealed that increased plasma DPP-4 levels are associated with mild loss in cognitive functions (47). In a study on 240 cases, DPP-4 inhibitors have been shown to improve cognitive function compared to sulfonylurea (48). A six-month-long, randomized, placebo-controlled, and double-blind study has shown liraglutide to significantly increase brain glucose metabolism compared to placebo (49). Similar results have been reported in a two-year-long, randomized study, conducted with exenatide (50). On the other hand, a six-month-long randomized double-blind study comparing liraglutide with placebo reported no difference in the formation or regression of amyloid plaque in the brain (51). Another study has demonstrated that liraglutide did not show a significantly improved effect on cognitive functions over a relatively short period of 17 days, compared to the placebo (52). The clinical studies on this area are summarized in Table 1.

Therefore, the benefits of GLP-1 analogs and DPP-4 inhibitors on neurodegenerative diseases, independent of glycemia, have not been proven completely. The previous studies have relatively small sample sizes, and some individual phenotypic differences such as apo E4 carriage may alter neurodegenerative-diabetes and associated treatment (53).

Effects of GLP-1 on Parkinson's Disease

In preclinical studies on different experimental models of simulated Parkinson's disease, GLP-1 receptor agonists have been shown to reduce the loss of dopaminergic neurons, and regulate brain energy metabolism and motor activity with neuroprotective effect. These effects are similar to those described in AD by the activation of PKA and PI3K/AKT molecular pathways through GLP-1 receptors. Exenatide treatment in rats with experimentally induced dopaminergic neuron loss using toxic substances completely decreased the toxicity and increased dopaminergic neuron viability (54). Similarly, another preclinical study using a neu-

Table 1. Human clinical trials describing the associations between GLP-1 bases therapies and dementia risk.

Investigator, year, earlier studies	Design	Participants	Sample characteristics	Results
Zheng, 2016 (47)	Cross-sectional, observational	Evaluated plasma DPP4 activity, inflammatory markers, and oxidative stress parameters in a cross-sectional sample of 1,160 patients with type 2 diabetes aged 60 years or older in China		Increased DPP4 activities are independently associated with MCI in elderly patients with type 2 diabetes
Tasci, 2013 (48)	prospective and observational	Investigation conducted in 10 elderly type 2 diabetes mellitus patients who were started treatment with vildagliptin 50 mg twice daily to ongoing metformin.	Mean follow-up time was 10.9 ±3.7 months.	Addition of vildagliptin to ongoing metformin therapy in elderly with diabetes was accompanied by stable cognitive and functional performance after almost one year of follow-up.
Gejl, 2016, (49)	Randomized, Placebo-Controlled, Double-Blind Clinical Trial	Randomized 38 patients with AD to treatment with the GLP-1 analog liraglutide (n = 18), or placebo (n = 20)	26-week trial	GLP-1 analog treatment prevented the decline of glucose metabolism in brain that signifies cognitive impairment, synaptic dysfunction, and disease evolution.
Danielle, 2015, (50)	Double-blind, randomized The study aim was to evaluate the effects of a single injection of the GLP-1RA exenatide on cerebral and peripheral glucose metabolism in response to a glucose load.	In 15 male subjects with HbA1c of 5.7 ±0.1%, fasting glucose of 114 ±3 mg/dL, and 2-h glucose of 177 ±11 mg/dL, exenatide (5 µg) or placebo was injected		Results demonstrate that, a major effect of a GLP-1RA on regulation of brain glucose metabolism in the absorptive state.
Egefjord, 2012, (51)	Randomized, controlled, double-blinded intervention study with AD patients	AD patients treated for six months with liraglutide (n = 20) or placebo (n = 20).	The primary outcome is change in the deposition of Aβ in the central nervous system	No registered drug affects the deposition of Aβ in the brain of AD patients
Farr, 2016, (52)	Crossover, randomized, double-blind, placebo-controlled trial	21 individuals with type 2 diabetes were treated with placebo and liraglutide	For a total of 17 days, studied metabolic changes as well as neurocognitive and neuroimaging (functional MRI) of responses to food cues	Liraglutide alters brain activity related to highly desirable food cues.
Athauda, 2017, (60)	Randomized, double-blind, placebo-controlled trial	Patients were aged 25–75 years, n:32 to exenatide and n:30 to placebo.	Patients with moderate Parkinson's disease were randomly assigned (1:1) to receive subcutaneous injections of exenatide 2 mg or placebo once weekly for 48 weeks in addition to their regular medication	Exenatide had positive effects on rationally defined off-medication motor scores in Parkinson's disease

Monophosphate; PKA: Protein kinase A; MEK: Mitogen-activated protein kinase; ERK: Extracellular regulated protein kinase; P13K: Phosphoinositide 3 kinase, AKT: Protein kinase B; CREB: cAMP response element-binding protein; NFκB: Nuclear factor kappa beta; Bcl2: B-cell lymphoma; GSK-3β: Glycogen synthase kinase 3 beta.

rotoxin observed higher levels of dopamine in animals administered Exendin-4. This effect was associated with an increase in the viability of dopaminergic neurons, and an increase in the levels of tyrosine hydroxylase enzyme, which produces dopamine from its precursor, L-DOPA (55). Cell culture studies have demonstrated that liraglutide and lixisenatide also increase the activity of tyrosine hydroxylase, and trigger the anti-apoptotic mechanism, compared to exendin (56). Long-term use of liraglutide has been shown to inhibit dopaminergic neuron loss in db-/db- diabetic mice models and prevent the impairment of motor functions and development of PD (57). Experimentally, liraglutide has been shown to reduce dyskinesia, which is an important complication of L-DOPA treatment (58). Semaglutide has been shown to reduce the accumulation of α -synuclein, in addition to the beneficial effects of other GLP-1R agonists (59). The first randomized double-blind placebo-controlled clinical study conducted on humans was published in 2017 on 62 cases with PD, wherein 2 mg/week exenatide LAR sc was compared to placebo. Exenatide was observed to show positive effects on clinically monitored motor functions (60). Based on these findings, GLP-1 agonists appear to have positive effects on the survival and functions of dopaminergic cells. The field is open for additional clinical studies for the development of an appropriate treatment strategy.

Physiological Effects of Central GLP-1 on Stress

Recent studies have focused on the effect of GLP-1 on the stress response of the organism. NTS has central GLP-1-producing cells and receives vagal stimulus from the periphery, as well as a stimulus for general homeostasis. Stress activates two parallel systems in the body, hypothalamo-hypophysoadrenal (HPA) axis and the sympathetic nervous system (SNS). NTS is projected to hypothalamic and autonomic control centers, which are effective in both these systems. GLP-1 produced by NTS neurons can activate both the HPA axis and SNS. There is no GLP-1R in the adrenal cortex, and the isolated adrenal cortex cells do not release corticosterone upon GLP-1 stim-

ulus. Therefore, the effect is known to have a central origin. When administered centrally, exendin-4 was observed to increase corticosterone levels in rodents. This increase is generated through CRH levels. The central blockade of the CRH receptors eliminates this effect (61). On the other hand, the activation of GLP-1 receptors in the central autonomic system may stimulate sympathetic neuronal cells in the spinal cord and adrenal medulla (62). As a result, GLP-1 may regulate the stress response of the body. Studies related to the clinical benefits of this condition and GLP-1RA practices are ongoing.

Ischemic Stroke and Glp-1

The stroke-damaged brain region can be divided into two parts, the ischemic core and the penumbra around the ischemic core. In the ischemic core, the damage takes place rapidly and is generally irreversible. The surrounding penumbra provides blood flow, and therefore, the neurons in this area can be protected by well-planned, timely interventions. These interventions are aimed at the chemical or surgical removal of the thrombotic clot. These treatments are generally beneficial within the first few hours after the onset of stroke, and unfortunately, may not be suitable for several patients due to late admission, difficulties in diagnosis, or contraindications such as hypertension.

In a stroke, the mechanisms of action of GLP-1-based therapies have been studied thoroughly in animal models, but studies on human stroke patients have begun recently (ClinicalTrials.gov NCT02829502). In the LEADER (Liraglutide Effect and Action in Diabetes) trial, a decrease in the rates of nonfatal stroke was observed in the liraglutide group, although the difference was non-significant (63). In the late SUSTAIN-6 (Trial to Evaluate Cardiovascular and Other Long-Term Outcomes with Semaglutide in Subjects with Type-2 Diabetes) trial, semaglutide was observed to significantly reduce the risk of the same primary composite endpoint as liraglutide. Both the trials demonstrated a decrease in major cardiovascular outcomes; however, there was no reduction in cardiovascular mortality in the SUSTAIN-6 trial, as observed in the LEADER trial. There was another im-

portant difference between the studies. In the SUSTAIN-6 trial, the reduction in the major CV outcome appeared to be the result of a significant (39%) decrease in the rate of nonfatal stroke (64). In large cardiovascular safety studies using DPP-4 inhibitors, no decrease in the rates of nonfatal stroke or other major CV outcomes was reported (65). GLP-1RA can cross the BBB and enter the hypothalamic neurons and circumventricular organs, whereas DPP-4 inhibitors cannot. Therefore, GLP-1R agonists and DPP-4 inhibitors appear to have different effects on the ischemic brain tissue due to different pharmacological specialties. In mice lacking GLP-1R, DPP-4 inhibitors were observed to be neuroprotective (66). DPP-4 inhibitors may affect targets independent of GLP-1R. In experimental models, Rohnert et al. showed that non-selective DPP-4 inhibitors were related to a reduction in infarct volume, which was not true for selective DPP-4 inhibitors (67). The mechanisms of these actions are yet to be fully clear. The DPP-4 enzyme targets more active peptides, including the glucose-dependent insulinotropic polypeptide (GIP) and the pituitary adenylate-cyclase-activating polypeptide (PACAP), than the GLP-1. Han et al. have demonstrated that a dual agonist targeting both GLP-1 and GIP receptors generated stronger neuroprotection against ischemic stroke than GLP-1 analog alone, indicating the neuroprotective effects of GIP against stroke (68). Moreover, it was reported that DPP-4 inhibition could ameliorate endothelium-dependent relaxation, and cerebrovascular dysfunction and remodeling, independent of glucose regulation (69).

Previous studies on animals have highlighted the neuroprotective effect of GLP-1RAs in both diabetic and non-diabetic animals. Inflammation, oxidative stress, and neuronal apoptosis due to stroke are particularly affected by GLP-1-based therapies.

Neuroprotective Mechanisms of GLP-1-Based Therapies in Ischemic Stroke

After an ischemic stroke, GLP-1R is upregulated in astrocytes, GABAergic interneurons, and microglia (70). The molecules PI3K,

cyclic adenosine monophosphate (cAMP), protein kinase-B (Akt), protein kinase-A (PKA), and cAMP response element-binding protein (CREB) are stimulated after the administration of GLP-1RAs in experimentally-induced stroke.

Inflammation, endothelial leakage, and excitotoxicity: The effect of GLP-1-based therapies on inflammatory response after a stroke has been studied by several researchers. Both exenatide and sitagliptin reduced the production of the pro-inflammatory cytokine tumor necrosis factor- α (TNF- α) (71). Sitagliptin also reduced the expression of interleukin-6 (IL-6) and increased the production of the anti-inflammatory cytokine interleukin-10 (IL-10) (72). Therapies based on GLP-1 also influence the BBB permeability. Exendin-4 significantly reduced the expression of aquaporin-4 (AQ-4) and glial fibrillary acidic protein (GFAP), which are involved in neuroinflammation, the formation of edema, and astrogliosis. Moreover, exendin-4 decreases the intercellular adhesion molecule-1 (ICAM-1) and inhibits BBB leakage (73). Expression of endothelial NOS (eNOS), which plays a role in the maintenance of vascular homeostasis, is increased by recombinant GLP-1, while exenatide reduces the vascular extravasation of immunoglobulin G (IgG), reducing endothelial leakage in the late inflammatory response to ischemia (71,74). Production of brain-derived neurotrophic factor (BDNF) was increased with pre-ischemic treatment with alogliptin, which reduced the release of neurotoxic glutamate and stabilized the neuroprotective GABAergic synapses with continuous exposure (75).

Oxidative stress: Both liraglutide and exenatide decreased the production of reactive oxygen species (ROS) after cerebral artery occlusion in diabetic mice (76). Exendin-4 decreased superoxide anion (O_2^-) (73). In sitagliptin-treated animals, the enzyme NADPH oxidase (NOx), which participates in the generation of ROS, was normalized (72).

Apoptosis and neuronal damage: To identify DNA damage, the "terminal deoxynucleotidyl transferase dUTP nick-end labeling" (TUNEL) assay is used. In ischemic lesions, the count of TUNEL-positive cells was significantly reduced by exenatide, liraglutide, and

modified GLP-1R agonists (77). Exenatide also reduced the activity of Caspase-3, which takes part in apoptosis (78).

Infarct volume: When administered pre-ischemia or post-ischemia, GLP-1R agonists are observed to reduce infarct volume (79). High doses of exenatide were observed to be neuroprotective when administered 1.5 h and 3 h post-ischemia, and the activity diminished upon late administration. Exenatide administered early resulted in the greatest reduction in the infarct volume compared to later delivery (80). In contrast, the effects of DPP-4 inhibitors are more variable.

Functional outcome and cerebral blood flow: Studies on GLP-1R agonists generally reported improved functional outcomes, whereas only one out of two studies using DPP-4 inhibitors demonstrated such an effect. In the first study, Ma et al. reported that linagliptin ameliorated cognitive impairment in diabetic mice, while the other study on alogliptin reported no beneficial effects of the compound on neurologic deficits when administered daily for three weeks before the stroke, to seven days post-ischemia (75,81). Conflicting results for the cerebrovascular effects of GLP-1RAs have been reported. In one study, treatment with exendin-4 was observed to increase blood flow, suggesting that GLP-1RAs impaired vascular function in ischemia, while another study reported that liraglutide did not affect the cerebral blood flow (73,82). The effects of DPP-4I administration on cerebral blood flow were also variable.

In conclusion, GLP-1-based therapies have been mainly used in diabetes and obesity, but recent studies have demonstrated their protective roles in the central nervous system. These agents, which have experimentally proven benefits both in neurodegenerative diseases and ischemic neuronal injuries, should be examined in this field by clinical studies. Randomized, placebo-controlled, prospective, double-blind open-ended studies and systematic meta-analyses will indicate whether GLP-based treatments have neuroprotective effects in clinical practice, regardless of the antidiabetic effect. Moreover, the new GLP-1 combinations, developed together with GIP and glucagon agonists, also need well-planned

clinical trials, even though their benefits have been demonstrated experimentally (83). It does not appear far-fetched to use these agents as neuroprotective agents in diabetic or non-diabetic patients soon.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Okan Sefa Bakiner; Design: Okan Sefa Bakiner; Control/Supervision: Akadlı Kürşad Özşahin; Data Collection and/or Processing: Okan Sefa Bakiner; Analysis and/or Interpretation: Okan Sefa Bakiner; Literature Review: Okan Sefa Bakiner; Writing the Article: Okan Sefa Bakiner; Critical Review: Okan Sefa Bakiner; Other (Language edition): Akadlı Kürşad Özşahin.

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