PERSPECTIVE

# Glucagon peptide-like 1 receptor (GLP-1R) expression *per se*: a new insight into neurodegenerative disease?

**Glucagon peptide-like 1 (GLP-1) and GLP-1 receptor (GLP-1R):** GLP-1 is an incretin hormone secreted from gut L cells. GLP-1 exerts its action through binding to its specific receptor, GLP-1R, which is a member of the G protein-coupled receptor superfamily. GLP-1R is reportedly expressed in various organs, such as the liver, kidney, and peripheral tissues. GLP-1 not only stimulates insulin secretion in pancreatic β-cells, but also ameliorates insulin resistance in insulin-dependent organs. However, endogenous GLP-1 is rapidly degraded within a few minutes by the enzyme dipeptidyl peptidase-4 (DPP-4). Therefore, GLP-1 analogs that are resistant to DPP-4, such as liraglutide and exenatide (a synthetic version of exendin-4), are used clinically to treat type 2 diabetes (T2DM). These drugs promote insulin secretion in a glucose-dependent manner, decrease appetite, inhibit body weight gain, and lower blood triglyceride levels.

In rodents and primates, GLP-1R is expressed in the regions of the central nervous system (CNS) responsible for controlling energy homeostasis. Moreover, endogenous GLP-1 and exendin-4 can cross the blood-brain barrier (BBB). In addition, the progression of T2DM and neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD) share several pathways in common. These findings led us to hypothesize that GLP-1 analogs represent potential drugs for treating diseases of the brain.

**Efficacy of GLP-1 analogs in AD and PD:** AD and PD are two major human neurodegenerative diseases. The etiology of AD is various, but the majority of AD cases are characterized by cognitive dysfunction as well as amyloid β (Aβ)- and tau-mediated neuronal cell death in the hippocampus and cerebral cortex. Insulin resistance may play a role in AD, as the development of AD increased in elderly patients with T2DM. Indeed, epidemiological studies have reported that people with T2DM have a 1.5- to 2.5-fold greater risk of developing cognitive impairment and dementia (Ninomiya, 2014). Further, insulin signaling in the brain is impaired in most AD patients. Many preclinical studies have attempted to discover treatments for AD, and recent findings suggest that GLP-1 analogs may be a potential solution. Yoshino et al. (2015) reported that treatment with GLP-1(7-36), a metabolically active form of GLP-1, protected hippocampal neurons against various insults, such as Aβ, L-glutamate, oxidative stress, and endoplasmic reticulum (ER) stress. Bomfim et al. (2012) reported that exendin-4 blocked Aβ oligomer-induced insulin signaling impairment in hippocampal neurons. Furthermore, liraglutide prevented memory impairment and reduced Aβ plaques in an AD mouse model. Intracerebroventricular injection of GLP-1 enhanced associative and spatial learning abilities, which are impaired in GLP-1R-deficient mice and enhanced in hippocampal GLP-1R-overexpressing mice (During et al., 2003). Based on these reports, GLP-1 analogs seem to be potent drugs for the treatment of AD. In fact, the National Institute on Aging (NIA, USA) is currently conducting an ongoing phase 2 clinical trial of exendin-4 in AD (www.ClinicalTrials.gov: NCT01255163).

The precise etiology of PD is unknown. However, it is characterized by accumulation of alpha-synuclein (αSyn) and apoptosis of dopaminergic neurons in the substantia nigra of the midbrain. Patients with T2DM had shown an increasing risk (~40%) to develop PD (Xu et al., 2011). Moreover, ob/ob and db/db mice, typical rodent models of T2DM, are more susceptible to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced dopaminergic cell death (Wang et al., 2014). These results suggest that PD and T2DM are closely related and that these two conditions may have similar etiologies. GLP-1R is reportedly expressed in the substantia nigra. Further, exendin-4 administration protected dopaminergic neurons in MPTP-treated mice (Li et al., 2009). In phase 2 clinical trials, the safety and efficacy of long-term exendin-4 treatment were evaluated (Aviles-Olmos et al., 2013). Forty-four patients were randomly divided two groups (exendin-4 group 20 patients *vs.* control group 24 patients) and exendin-4 was administrated for 12 months. Exendin-4 treatment ameliorated PD symptoms determined by Movement Disorders Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) for a difference of 4.9 points (mean improvement of exendin-4 group: 2.7 points *vs.* mean worsening of control group: 2.2 points). Exendin-4 treatment, however, induced weight loss (mean exendin-4 group weight loss: 3.2 kg/year *vs.* mean control group weight loss: 0.8 kg/year).

**GLP-1R expression in diabetic retinopathy (DR):** In a previous study, we reported on the role of GLP-1R in DR. The retina is comprised of 10 layers, among which the retinal pigment epithelium (RPE), the outermost layer of the retina, is a crucial component of the blood-retinal barrier (BRB). RPE cell apoptosis is closely related to BRB breakdown, which leads to visual impairment. Therefore, RPE cells are believed to play an important role in the pathogenesis of DR. It was recently reported that GLP-1R was expressed in RPE cells; we found that GLP-1R expression was downregulated in high glucose (HG)-treated RPE cells (Kim et al., 2015), and that this downregulation *per se* induced RPE cell apoptosis *via* the reactive oxygen species (ROS)/ER stress/p53/Bax pathway. In the initial stages of the study, we focused on the efficacy of exendin-4 against HG-induced RPE cell apoptosis. GLP-1R was investigated simply to confirm its expression. Interestingly, however, we observed that GLP-1R expression

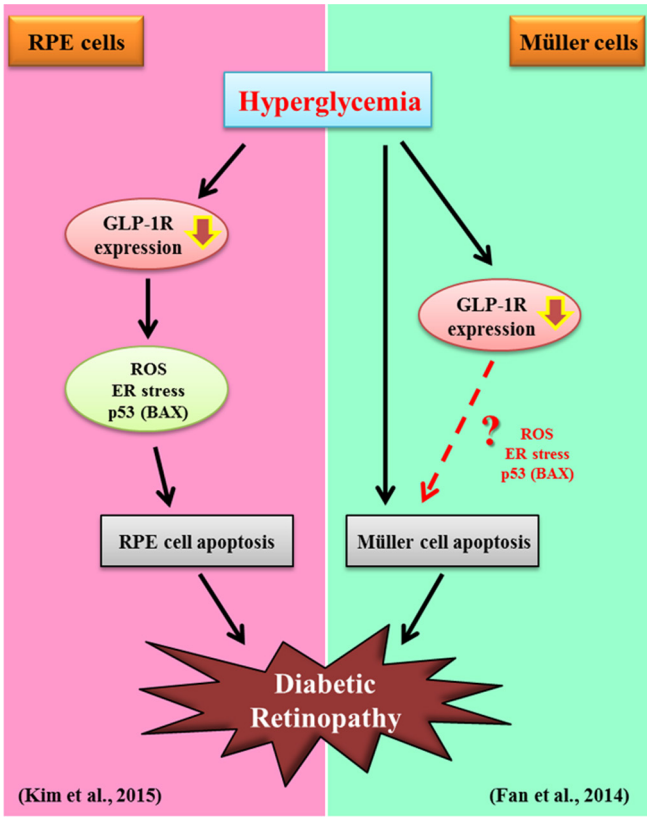


**Figure 1 Hyperglycemia-induced glucagon peptide-like 1 receptor (GLP-1R) downregulation causes apoptosis in retinal pigment epithelium (RPE) cells and Müller cells: potential role in the development of diabetic retinopathy (DR).**
ROS: Reactive oxygen species; ER: endoplasmic reticulum.

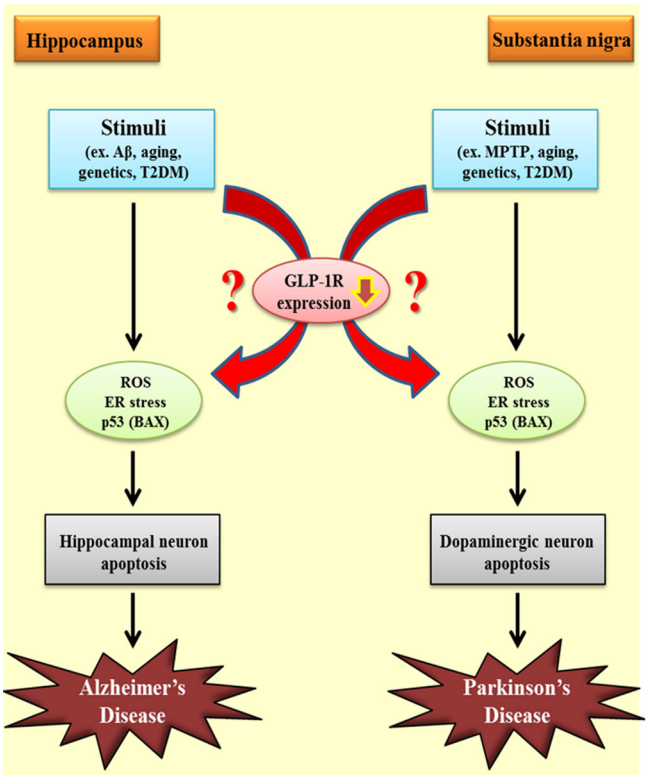


**Figure 2 Hypothesized signaling cascades of glucagon peptide-like 1 pathophysiology in the hippocampus and substantia nigra in the development of Alzheimer's disease (AD) and Parkinson's disease (PD).**
Aβ: Amyloid β; T2DM: type 2 diabetes mellitus; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; ROS: reactive oxygen species; ER: endoplasmic reticulum.

was decreased following HG treatment at both the mRNA and protein levels. We further confirmed that GLP-1R knockdown by glp-1r-specific small interfering (si) RNA resulted in increased ROS generation, p53-mediated Bax promoter activity, and apoptotic cell number, in addition to activation of signaling molecules involved in ER stress. Moreover, GLP-1R expression in the retina of rats treated with streptozotocin (STZ), which induced hyperglycemia due to its selective pancreatic β-cell toxicity, was decreased compared with vehicle-treated rats. These results suggested that GLP-1R expression is modifiable and decreased by HG treatment. Coincident with our results, around the same time, Fan et al. (2014a, b) reported that intravitreal injection of exendin-4 alleviated DR by protecting the BRB and reducing vascular permeability in diabetic Goto-Kakizaki (GK) rats, a well characterized model of non-insulin-dependent diabetes mellitus. In those studies, the authors found that GLP-1R was expressed throughout the entire retina, including the ganglion cell layer, inner plexiform layer, inner nuclear layer, outer plexiform layer, and inner segment. GLP-1R expression in these regions was decreased in GK rats compared with age-matched Wistar rats. Furthermore, in primary rat Müller cells, a type of retinal glial cell, HG treatment resulted in decreased GLP-1R expression. It is well known that Müller cells undergo apoptotic cell death under HG conditions, similarly to RPE cells. These two types of cells share two HG-induced outcomes: GLP-1R downregulation and apoptosis. In RPE cells, however, we revealed that GLP-1R downregulation was directly involved in RPE cell apoptosis (**Figure 1**).

Interestingly, we and Fan et al. observed that HG-induced GLP-1R downregulation rebounded following exendin-4 administration in the retina of GK rats as well as in RPE and primary rat Müller cells (Fan et al., 2014a; Kim et al., 2015). Furthermore, exendin-4 addition recovered HG-induced apoptosis of RPE and Müller cells. Therefore, we hypothesize that a positive feedback loop exists: GLP-1R activation by exendin-4 upregulates GLP-1R expression. This feedback loop subsequently protects these cells from apoptotic cell death.

**Alterations in GLP-1R expression and diabetes:** Until now, the majority of studies have focused on the efficacy of GLP-1 and its analogs, while very few have focused on altered GLP-1R expression. Pan et al. (2009) reported that additional glucose treatment decreased GLP-1R expression in INS-1 β-cells. Xu et al. (2007) reported that GLP-1R expression was reduced in the islets of pancreatectomized hyperglycemic rats and by overexpression of

protein kinase C alpha (PKCα), which is activated by HG treatment and under diabetic conditions. Furthermore, Mima et al. (2012) reported that glomerular GLP-1R expression was also decreased in diabetic mice *via* ubiquitination-dependent degradation. Growing evidence suggests that GLP-1R expression is decreased in diabetic conditions, which may be important, as decreased GLP-1R expression means decreased GLP-1 sensitivity. We recently revealed that GLP-1R expression *per se* was critically involved in apoptosis, even in the absence of GLP-1 (Kim et al., 2015). This suggests that even when ligand-mediated GLP-1R activation is absent, GLP-1R expression *per se* is important for maintenance of normal cell physiology.

**GLP-1R expression and new insights into neurodegenerative diseases:** It is noteworthy that AD and PD are closely associated with T2DM. ROS, ER stress, and p53 signaling, which are activated by GLP-1R knockdown in RPE cells, are involved in the initiation and progression of both AD and PD. In addition, GLP-1R is expressed in the hippocampus and substantia nigra. However, no study has been conducted to determine potential alterations in brain GLP-1R expression under either AD- or PD-like conditions.

As mentioned above, exendin-4 administration effectively improved PD symptoms in patients during a phase 2 clinical trial. However, unexpectedly, it also induced weight loss. It is believed that exendin-4 treatment is a good therapy. Nevertheless, better drugs with fewer side effects are always needed. In an effort to discover new drugs and improve existing ones, one viable approach may be to examine altered GLP-1R expression in AD and PD (**Figure 2**). New strategies to preserve GLP-1R expression could be used to treat diabetes as well as neurodegenerative disease.

*This work was supported financially by a research grant from National Research Foundation of Korea (NRF-2010-0023627).*

**Dong-il Kim, Soo-hyun Park***

**College of Veterinary Medicine, Chonnam National University, Gwangju, Republic of Korea**

***Correspondence to:** Soo Hyun Park, Ph.D., parksh@chonnam.ac.kr.*
***Accepted:** 2015-04-28*
***doi:**10.4103/1673-5374.160078* ***http**://www.nrronline.org/*
*Kim DI, Park SH (2015) Glucagon peptide-like 1 receptor (GLP-1R) expression per se: a new insight into neurodegenerative disease? Neural Regen Res 10(7):1055-1057.*

## References

Aviles-Olmos I, Dickson J, Kefalopoulou Z, Djamshidian A, Ell P, Soderlund T, Whitton P, Wyse R, Isaacs T, Lees A, Limousin P, Foltynie T (2013) Exenatide and the treatment of patients with Parkinson's disease. J Clin Invest 123:2730-2736.

Bomfim TR, Forny-Germano L, Sathler LB, Brito-Moreira J, Houzel JC, Decker H, Silverman MA, Kazi H, Melo HM, McClean PL, Holscher C, Arnold SE, Talbot K, Klein WL, Munoz DP, Ferreira ST, De Felice FG (2012) An anti-diabetes agent protects the mouse brain from defective insulin signaling caused by Alzheimer's disease- associated Abeta oligomers. J Clin Invest 122:1339-1353.

During MJ, Cao L, Zuzga DS, Francis JS, Fitzsimons HL, Jiao X, Bland RJ, Klugmann M, Banks WA, Drucker DJ, Haile CN (2003) Glucagon-like peptide-1 receptor is involved in learning and neuroprotection. Nat Med 9:1173-1179.

Fan Y, Liu K, Wang Q, Ruan Y, Ye W, Zhang Y (2014a) Exendin-4 alleviates retinal vascular leakage by protecting the blood-retinal barrier and reducing retinal vascular permeability in diabetic Goto-Kakizaki rats. Exp Eye Res 127:104-116.

Fan Y, Liu K, Wang Q, Ruan Y, Zhang Y, Ye W (2014b) Exendin-4 protects retinal cells from early diabetes in Goto-Kakizaki rats by increasing the Bcl-2/Bax and Bcl-xL/Bax ratios and reducing reactive gliosis. Mol Vis 20:1557-1568.

Kim DI, Park MJ, Choi JH, Lim SK, Choi HJ, Park SH (2015) Hyperglycemia-induced GLP-1R downregulation causes RPE cell apoptosis. Int J Biochem Cell Biol 59:41-51.

Li Y, Perry T, Kindy MS, Harvey BK, Tweedie D, Holloway HW, Powers K, Shen H, Egan JM, Sambamurti K, Brossi A, Lahiri DK, Mattson MP, Hoffer BJ, Wang Y, Greig NH (2009) GLP-1 receptor stimulation preserves primary cortical and dopaminergic neurons in cellular and rodent models of stroke and Parkinsonism. Proc Natl Acad Sci U S A 106:1285-1290.

Mima A, Hiraoka-Yamomoto J, Li Q, Kitada M, Li C, Geraldes P, Matsumoto M, Mizutani K, Park K, Cahill C, Nishikawa S, Rask-Madsen C, King GL (2012) Protective effects of GLP-1 on glomerular endothelium and its inhibition by PKCbeta activation in diabetes. Diabetes 61:2967-2979.

Ninomiya T (2014) Diabetes mellitus and dementia. Curr Diab Rep 14:487.

Pan QR, Li WH, Wang H, Sun Q, Xiao XH, Brock B, Schmitz O (2009) Glucose, metformin, and AICAR regulate the expression of G protein-coupled receptor members in INS-1 beta cell. Horm Metab Res 41:799-804.

Wang L, Zhai YQ, Xu LL, Qiao C, Sun XL, Ding JH, Lu M, Hu G (2014) Metabolic inflammation exacerbates dopaminergic neuronal degeneration in response to acute MPTP challenge in type 2 diabetes mice. Exp Neurol 251:22-29.

Xu G, Kaneto H, Laybutt DR, Duvivier-Kali VF, Trivedi N, Suzuma K, King GL, Weir GC, Bonner-Weir S (2007) Downregulation of GLP-1 and GIP receptor expression by hyperglycemia: possible contribution to impaired incretin effects in diabetes. Diabetes 56:1551-1558.

Xu Q, Park Y, Huang X, Hollenbeck A, Blair A, Schatzkin A, Chen H (2011) Diabetes and risk of Parkinson's disease. Diab Care 34:910-915.

Yoshino Y, Ishisaka M, Tsujii S, Shimazawa M, Hara H (2015) Glucagon-like peptide-1 protects the murine hippocampus against stressors via Akt and ERK1/2 signaling. Biochem Biophys Res Commun 458:274-279.