



● PERSPECTIVE

Efficacy of glucagon-like peptide-1 mimetics for neural regeneration

Glucagon-like peptide 1 (GLP-1) is secreted from enteroendocrine L cells in response to nutrient ingestion and exhibits insulinotropic properties by stimulating specific G protein-linked receptors (GLP-1Rs) on pancreatic β cells. Several GLP-1 mimetics, such as exenatide (exendin-4 (Ex-4)), liraglutide, and lixisenatide, have been developed and approved as treatments for patients with type 2 diabetes. These peptides show bioactivities almost identical to those of GLP-1 and have a substantially longer plasma half-life than GLP-1 because of their resistance to dipeptidyl peptidase-4, a GLP-1 degrading enzyme. GLP-1Rs are found in not only the pancreas but also the extrapancreatic tissues, including the nervous tissues (Harkavyi and Whitton, 2010). It is important to note that GLP-1 mimetics can cross the blood brain barrier and directly act on neurons in the central nervous system. In addition to the inhibition of appetite, the neuroprotective properties of GLP-1 have been receiving increasing attention. Recent studies have suggested that GLP-1 mimetics confer beneficial effects in neurodegenerative disorders, such as Parkinson's disease (PD), Alzheimer's disease, amyotrophic lateral sclerosis, ischemia and stroke, and multiple sclerosis (Holscher, 2014). In particular, the neuroprotective properties of Ex-4 have been demonstrated in animal and cell culture models of PD. A single-blinded clinical trial with 45 PD patients revealed that the treatment with Ex-4 significantly improved the cognition and memory of patients (Aviles-Olmos et al., 2013). The beneficial effects of GLP-1 mimetics on the peripheral nervous system (PNS) have also been reported. Both GLP-1 and Ex-4 delivered *via* osmotic minipumps prevented axonal degeneration in a rat model of pyridoxine-induced neuropathy (Perry et al., 2007). Treatment of streptozotocin (STZ)-induced diabetic mice with Ex-4 for 4 weeks restored motor and sensory nerve conduction velocities and hypoalgesia without normalizing blood glucose levels (Himeno et al., 2011). In addition, repeated intraperitoneal injections of Ex-4 significantly promoted axonal regeneration and functional recovery following sciatic nerve crush injury in normal adult rats (Yamamoto et al., 2013). These findings are in agreement with *in vitro* studies that revealed that GLP-1 and Ex-4 promoted neurite outgrowth of rat pheochromocytoma-derived PC12 cells (Perry et al., 2002) and adult mouse dorsal root ganglion (DRG) neurons (Himeno et al., 2011). Together these results provide further evidence of the direct actions of Ex-4 on the PNS; however, the underlying mechanisms remain unclear. Our recent study (Tsukamoto et al., 2015) aimed to elucidate the precise localization of GLP-1R in adult rat DRG *in vivo* and *in vitro* as well as to determine the neurotrophic and neuroprotective properties of Ex-4 in adult rat DRG neurons.

Double immunofluorescence histochemistry was performed using anti-GLP-1R antibody and specific neuron markers (anti-200 kDa neurofilament (NF200) and anti-calcitonin gene-related peptide (CGRP) antibodies and *Griffonia simplicifolia* isolectin B4 (IB4)). Staining results revealed that GLP-1R was predominantly found in NF200-immunoreactive large neurons and CGRP-immunoreactive small peptidergic neurons rather than IB4-binding small non-peptidergic neurons. It is generally accepted that large sensory neurons transmit proprioception and vibration and that small peptidergic and non-peptidergic neurons transmit nociception and thermoreception; whether these two groups of small neurons have distinct functions has been the subject of controversy (Takaku et al., 2013). This distribution pattern of GLP-1R agrees with previous findings that

GLP-1 and Ex-4 restores pyridoxine-induced large fiber neuropathy (Perry et al., 2007) and diabetes-induced large and small fiber dysfunction (Himeno et al., 2011; Jolivald et al., 2011).

We maintained adult rat DRG neurons in serum-free culture conditions with different concentrations of Ex-4 (0, 1, 10, and 100 nM) in the presence or absence of insulin, and evaluated neurite outgrowth at 2 days and neuronal survival at 7 days in culture, respectively. For the neurite outgrowth assay, DRG neurons were immunostained with the anti- β III tubulin antibody and the number of neurite-bearing cells was expressed as a relative value wherein the total number of neurons per well was assumed to be 100. The length of the neurites (in μ m) was measured from digital images of the stained neurites, and expressed as the average value calculated from the measurements of about 60 neurites in each experimental group. For the survival assay, dead neurons were detected by positive trypan blue staining. The number of viable neurons at 7 days was expressed as a relative value when the original number measured 16 hours after seeding was assumed to be 100. Ex-4 promoted neurite outgrowth and survival of DRG neurons in a dose-dependent manner (1 nM < 10 nM < 100 nM), and effects of Ex-4 were more noticeable in the absence of insulin than in its presence. Treatment with 100 nM Ex-4 almost completely restored the reduced neurite outgrowth and viability of DRG neurons caused by insulin removal from the medium. Insulin is recognized as a neuroprotective molecule, and the predominant distribution of insulin receptors in small peptidergic and non-peptidergic DRG neurons has been documented (Baiou et al., 2007). Insulin and GLP-1 have been suggested to activate common signaling pathways, such as the phosphatidylinositol 3'-phosphate kinase (PI3K) and Ras/Raf/mitogen-activated protein kinase (MAPK) pathways (Holscher, 2014). Thus, the findings of our study suggest that GLP-1 mimetics confer a neuroprotective function, at least partly, by compensating for the absence of insulin receptor activation. Considering the differences in the distribution patterns between GLP-1R and insulin receptor described above, it seems plausible that Ex-4 and insulin exhibit synergistic effects on the small peptidergic neurons but complementary effects on the other subtypes of neurons. To further confirm this theory,

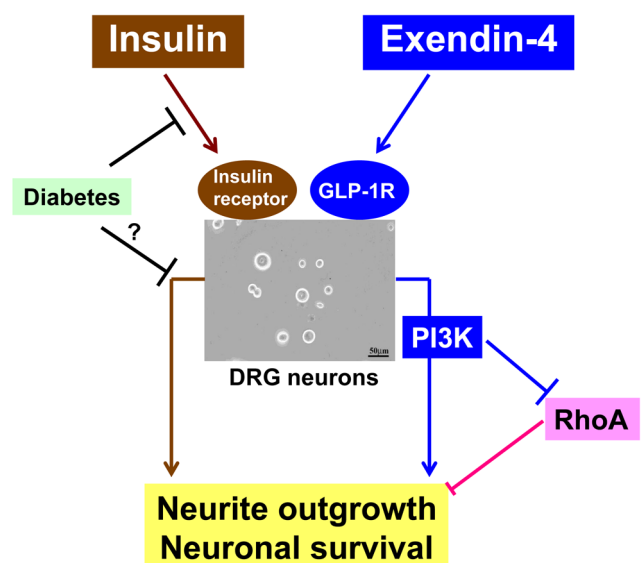


Figure 1 Possible signaling pathways mediating the effects of exendin-4 (Ex-4) on cultured dorsal root ganglion (DRG) neurons. Ex-4 may compensate for reduced insulin actions under diabetic conditions. GLP-1R: Glucagon-like peptide 1 receptor; PI3K: phosphatidylinositol 3'-phosphate kinase.



we are currently investigating the colocalization of GLP-1R with insulin receptor in DRG and the possible association between insulin and GLP-1 signaling pathways in DRG and other neurons (**Figure 1**).

GLP-1 mimetics have been shown to activate several signaling pathways in the nervous system, including cyclic AMP (cAMP)/protein kinase A/nuclear transcription activator CREB, PI3K/Akt, and MAPK/ERK (Harkavyi and Whitton, 2010; Holscher, 2014). The upregulation of cAMP (Liu et al., 2011) and activation of ERK signaling (Jolivald et al., 2011) were suggested to play a role in the restoring effects of GLP-1 mimetics against peripheral nerve dysfunction in STZ-diabetic rats. In our study, the promoting effects of Ex-4 on the neurite outgrowth and survival of DRG neurons were abolished by PI3K inhibitor LY294002. Furthermore, pretreatment with LY294002 in PC12 cells canceled Ex-4-induced inactivation of RhoA, an inhibitory regulator for peripheral nerve regeneration. These findings suggest that Ex-4 exhibits neurotrophic and neuroprotective activities through the activation of PI3K signaling pathway, which negatively regulates RhoA activity (**Figure 1**). The implication of RhoA was confirmed in PC12 cells but not in DRG neurons because insufficient amount of protein was obtained from the latter to measure RhoA activity. Therefore, we cannot definitely state that this hypothesis holds true in DRG neurons. However, on the basis of the evidence of the inverse relation between the RhoA activity/expression and the neurite outgrowth activity and viability of DRG neurons, the inhibition of RhoA activity appears to be one of the mechanisms accounting for the neuroprotective effects of GLP-1 mimetics. In agreement with our study, Wang et al. (2013) reported the involvement of RhoA inhibition in the cytoprotective actions of GLP-1 in cultured cardiac microvascular endothelial cells under diabetic conditions. The possible association between PI3K and the other signaling pathways described above and the target genes upregulated through PI3K-RhoA signaling remain to be determined.

In summary, the neurotrophic and neuroprotective properties of Ex-4 illustrated in our study (Tsukamoto et al., 2015) imply their efficacy for the acceleration of axonal regeneration and functional repair following peripheral nerve injury. Impaired axonal regeneration and remyelination are the characteristic features in the pathobiology of diabetic neuropathy; therefore, therapeutic approaches of Ex-4 and other GLP-1 mimetics against diabetic neuropathy can be expected. Classical neurotrophic factors, such as nerve growth factor (NGF) and glial cell line-derived neurotrophic factor (GDNF), exhibited more potent bioactivities on the neurite outgrowth of DRG neurons as compared with Ex-4 (Tsukamoto et al., personal data), but clinical trials with NGF resulted in painful side effects and failed to confirm its efficacy for diabetic neuropathy. Although Ex-4 has milder neurotrophic effects than NGF, Ex-4 and other GLP-1 mimetics are now available to patients with type 2 diabetes and may be beneficial for both stable glycemic control and the restoration of peripheral nerve function in patients with diabetic neuropathy. Moreover, the direct actions of GLP-1 mimetics on PNS suggest their therapeutic utility against the neuropathy caused by type 1 diabetes. In addition to its efficacy for neurite outgrowth and neuronal survival, we are currently investigating whether Ex-4 promotes myelin formation in cocultured DRG neurons and immortalized adult rat Schwann cells. The growing evidence that GLP-1 mimetics accelerate peripheral nerve regeneration and remyelination in preclinical studies will encourage us to consider the clinical trials with the drugs for diabetic and other peripheral neuropathies as well as traumatic nerve injury. Because the safety of GLP-1 mimetics in long-term use for patients with type 2 diabetes has been proved and their clinical trials for PD and Alzheimer's disease are in progress, we believe that their clinical applications for the PNS disorders described above will launch in the near future.

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