Extending the Reach of Exendin-4: New Pathways in the Control of Body Weight and Glucose Homeostasis

Deborah J. Good

Department of Human Nutrition, Foods and Exercise, Virginia Tech, Blacksburg, Virginia 24061

According to the Centers for Disease Control and Prevention, approximately 1.9 million adults aged 20 yr and older were newly diagnosed with diabetes in 2010 (1). Up to 72% of diabetics in the United States take a prescription medication to treat the disease, either alone or in combination with insulin (1). Glucagon-like 1 peptide, a 39-amino acid peptide derived from the proglucagon gene, has been shown to have antidiabetic properties using both humans and rodent models (2, 3). Most of these studies have shown a direct effect on pancreatic cells, but GLP-1 has previously been shown to increase serotonin synthesis and may be a promising treatment for Alzheimer's disease (4, 5). Five different drugs that act through the glucagon-like 1 peptide receptor (GLP-1R) are either in development or use for the treatment of diabetes (2). Of these, Exenatide (marketed as Byetta) has been approved for use in type 2 diabetes since 2005 (6). A longer-lasting form of Exenatide (marketed as Bydureon) received Food and Drug Administration approval in January 2012 (7). Exenatide is the synthetic form of Exendin-4, a 39-amino acid peptide originally identified in Gila monster saliva (6). Although Exendin-4 shares 50% homology with the amino acids in the glucagon-like 1 peptide (GLP-1) peptide, it is considered an agonist and not an analog of the peptide. GLP-1 is normally synthesized in the gut and brain and is dependent on prohormone convertase 1/3 for cleavage from proglucagon (3). The hypothalamic neuronal populations and signaling pathways involved in GLP-1 or Exendin-4's anorexic and antiobesity/diabetes functions have not been understood, despite numerous rodent and human clinical studies showing reduced food intake and body weight after treatment with Exendin-4 or GLP-1 peptide agonists [most recently reviewed by Gallwitz (7)].

In this issue of *Endocrinology*, Dalvi *et al.* (8) identified hypothalamic proopiomelanocortin (POMC), neuropeptide Y, neurotensin, and ghrelin neurons as targets of Exendin-4 therapy. They show that intracerebroventricular injections of Exendin-4, at a dose that could induce both anorexia and weight loss in rodents, activated these specific neurons within several hypothalamic regions. Furthermore, using a hypothalamic neuronal cell line developed by the same group, the authors were able to demonstrate that the cAMP/protein kinase A signaling pathway (previously identified as the signaling mechanism for GLP-1R in pancreatic β-cells) also acted in hypothalamic neurons (8, 9). Signaling through GLP-1R results in phosphorylation and activation of the cAMP-response element binding protein (CREB) transcription factor and transcriptional regulation of target genes. Obvious next steps in these studies would be to show binding by CREB/activating transcription factor and c-fos transcription factors to the promoters of genes regulated by Exendin-4, as identified by Dalvi *et al.* (8). Previous studies by others have identified active CREB response elements in the POMC (10), neurotensin (11), and neuropeptide Y promoters (12) but not yet in the gherlin promoter.

Based on the findings presented by Dalvi *et al.* (8) in this issue of *Endocrinology*, I propose a possible mechanism by which GLP-1 analogs and agonists act to induce hypothalamic neurogenesis and serve to promote long-term glucose and body weight homeostasis (Fig. 1). Indeed, GLP-1 and Exendin-4 have been previously shown to potentiate proliferation in pancreatic β-cells (13). The new data from Dalvi *et al.* (8) suggest that cell proliferation in response to activation of the GLP-1 pathway in target cells could occur in the central nervous system, as it does in peripheral target cells. This is based largely on the finding

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Abbreviations: CNTF, Ciliary neurotrophic factor; CREB, cAMP-response element binding protein; GLP-1, glucagon-like 1 peptide; GLP-1R, GLP-1 receptor; POMC, proopiomelanocortin.

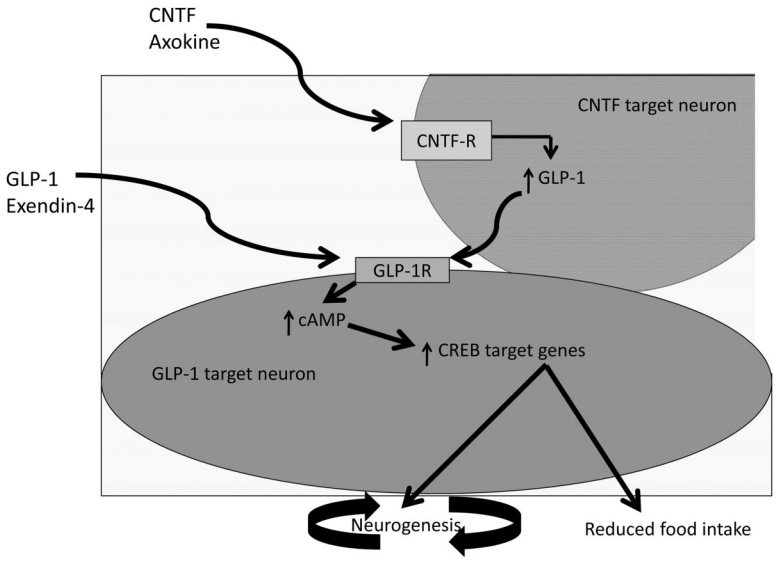

FIG. 1. Proposed mechanism of action for GLP-1 and the GLP-1R agonist, Exendin-4. GLP-1 or Exendin-4 act on hypothalamic target neurons to induce reduced food intake through neurotensin, gherlin, and POMC gene regulation by CREB (8) and induce hypothalamic neurogenesis either in the same neurons and/or adjacent neurons (9). CNTF (9) or Axokine could also act through this pathway by inducing hypothalamic GLP-1 expression.

by Dalvi *et al.* that phosphorylation and activation of the CREB transcription factor, one of the key steps in the cAMP signaling pathway controlling adult neurogenesis (14), occurs in response to Exendin-4 treatment.

It is now widely accepted that adult neurogenesis occurs in discrete areas of the nervous system, including the dentate gyrus of the hippocampus, the lateral ventricles (15), and recently around regions of the third ventricle of the hypothalamus, as well as within specific hypothalamic nuclei (16, 17). Much of this work, especially that related to body weight control, was done using ciliary neurotrophic factor (CNTF). A CNTF-related peptide was once used in the treatment of human obese patients who also had diabetes (trade name Axokine), and both mice and humans treated with Axokine were able to lose body weight and maintain weight loss (18, 19). CNTF can stimulate neurogenesis in discrete hypothalamic regions, including the ependymal layer and surrounding neuronal parenchyma (17). In the diabetic *db/db* mouse model, treatment with Axokine improved glucose, insulin, and triglyceride levels better than reduced caloric intake alone (20). As previously shown by Belsham *et al.* (9), CNTF treatment of mice or hypothalamic neuron cultures lead to increased proglucagon production (the precursor of GLP-1), and treatment with either Exendin-4 or CNTF resulted in increased neurogenesis (9). It is intriguing to speculate that the efficacy of GLP-1 mimics and agonists in the treatment of diabetes is actually due to the production of new hypothalamic neurons with the potential to form new neuronal connections or simply increase the number of neurons responsive to peripheral signals of energy availability. These new neurons, in combination with the gene regulatory effects that Extendin-4 has on anorexic neuropeptide synthesis, could account for the efficacy in patients taking Extendin-4-related drugs. Indeed, new work from the group who originally characterized CNTF's role in adult hypothalamic neurogenesis shows that animals with diet-induced obesity fail to have the same levels of neurogenesis as normal weight animals; however, a calorie restriction, perhaps similar to that which could be brought about by the anorexic neuropeptides induced by treatment with Exendin-4 or similar drugs, can partially restore the number of proliferating cells in the hypothalamus to levels in the normal-weight animals (21).

The new work by Dalvi *et al.* (8) bridges the fields of diabetes and hypothalamic neurogenesis and suggests that GLP-1R agonists work by inducing specific neuropeptides that act to reduce food intake as well as by inducing hypothalamic neurogenesis, which could ultimately increase the number of healthy, GLP-1-responsive neurons in these body weight control centers. Use of adult hypothalamic neurons [also developed by Belsham *et al.* (9)] along with additional diabetic and gene-specific knockout or transgenic rodent models will help to solidify the proposed mechanism. Although the POMC and neurotensin neuropetides induced by GLP-1 or Exendin-4 are known anorexigenic peptides (22, 23), the CREB-responsive genes that lead to neurogenesis in this system need to be identified and further characterized. Axokine is no longer in use, due to the development of antibodies against the drug in patients taking it, but this new research suggests that GLP-1R agonists could prove to work through a similar mechanism of hypothalamic neurogenesis and may be useful not only in treatment type 2 diabetes but, like Axokine, in prediabetic, obese individuals needing to lose weight and improve glucose regulation. Only time will tell whether Extendin-4-based treatments prove to be the drug that can treat and prevent complications from diabetes.

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Address all correspondence and requests for reprints to: Dr. Deborah J. Good, Human Nutrition, Foods, and Exercise, 1981

Kraft Drive (0913), ILSB Room 1020, Blacksburg, Virginia 24061. E-mail: goodd@vt.edu.

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