Mechanisms of Action and Therapeutic Application of Glucagon-like Peptide-1

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Glucagon-like peptide-1 (GLP-1) released from gut enteroendocrine cells controls meal-related glycemic excursions through augmentation of insulin and inhibition of glucagon secretion. GLP-1 also inhibits gastric emptying and food intake, actions maximizing nutrient absorption while limiting weight gain. Here I review the circuits engaged by endogenous versus pharmacological GLP-1 action, highlighting key GLP-1 receptor (GLP-1R)-positive cell types and pathways transducing metabolic and non-glycemic GLP-1 signals. The role(s) of GLP-1 in the benefits and side effects associated with bariatric surgery are discussed and actions of GLP-1 controlling islet function, appetite, inflammation, and cardiovascular pathophysiology are highlighted. Refinement of the risk-versus-benefit profile of GLP-1-based therapies for the treatment of diabetes and obesity has stimulated development of orally bioavailable agonists, allosteric modulators, and unimolecular multi-agonists, all targeting the GLP-1R. This review highlights established and emerging concepts, unanswered questions, and future challenges for development and optimization of GLP-1R agonists in the treatment of metabolic disease.

The molecular cloning of the cDNAs and genes encoding mammalian proglucagon in the early 1980s revealed the sequences of two tandemly linked glucagon-like peptides, GLP-1 and GLP-2 (Figure 1). Both GLP-1 and GLP-2 are continuously secreted from enteroendocrine cells (EECs) at low basal levels in the fasting or interprandial state, and circulating levels of these peptides rise briskly within minutes of food ingestion. GLP-2 was shown to exhibit intestinotrophic and proabsorptive activity, enabling development of a GLP-2 receptor (GLP-2R) agonist, teduglutide, for the treatment of short bowel syndrome (Drucker et al., 2017). GLP-1R was found to exhibit incretin-like activity, potentiating glucose-dependent insulin secretion in normal and diabetic animals and humans (Drucker et al., 2017). These findings, followed rapidly by demonstration that GLP-1 inhibited glucagon secretion, food intake, and gastric emptying, supported development of GLP-1 receptor (GLP-1R) agonists for the treatment of type 2 diabetes (T2D) and, subsequently, obesity (Drucker et al., 2017).

Genetic Linkage of GLP-1R Variation with Cardiometabolic Traits

The actions of GLP-1 are transduced by a single GLP-1R, exhibiting structure and signaling properties similar to those of related class B receptor family members encoding receptors for glucagon (GCG), GLP-2, and glucose-dependent insulinotrophic polypeptide (GIP) (Mayo et al., 2003). GLP-1Rs were first identified in islet β cells and the central nervous system (CNS). Subsequently, expression of the canonical GLP-1R was identified in islet and pancreatic exocrine cells, the autonomic and enteric nervous systems, blood vessels, Brunner’s glands, and sinocarotid node (Figure 1). Genetic evidence supporting a role for the human proglucagon (GCG) gene encoding GLP-1, or the GLP-1 receptor (GLP1R), in the susceptibility to diabetes or obesity is limited. Inactivation of the mouse Glp1r produces mild fasting hyperglycemia, glucose intolerance, and defective glucose-stimulated insulin secretion, delineating an essential role for Glp1r in control of β cell function (Scrochi et al., 1996). Initial studies examining the genetic susceptibility of T2D did not associate variation within the GLP1R locus with glycemic traits or control of body mass index; more recent analyses have linked variation within the GLP1R to the control of glucose and β cell function. A low-frequency missense variant Ala316Thr; rs10305492 in the GLP1R gene was associated with fasting glucose and a lower risk of T2D (Scott et al., 2016), findings independently confirmed in separate human cohorts (Wessel et al., 2015). Moreover, 12 additional single nucleotide variants within GLP1R (from among 150 studied) associated with fasting glucose (Wessel et al., 2015). Intriguingly, the fasting glucose-lowering allele of GLP1R was also associated with protection against coronary heart disease (Scott et al., 2016), whereas these same genetic glucose-lowering variants were not associated with an increased risk of common neoplasms (Scott et al., 2016). Complete loss of function at the GLP1R locus is not invariably associated with glycemic phenotypes in humans, although limited numbers of individuals have been identified (Scott et al., 2016). Less information is available surrounding the genetic determinants of response to GLP-1 pharmacotherapy. The acute insulin response to GLP-1 infusion over 3 hr in 88 healthy individuals subjected to a hyperglycemic clamp was associated with non-synonymous variation (rs6923761 and rs3765467) within the GLP1R (Smushkin et al., 2012). However, whether genetic variation within GLP1R predicts the magnitude of the glucose-lowering or weight loss response in subsets of individuals remains to be determined.

Post hoc genetic analysis of patient subgroups in the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial identified two markers (rs57922 and rs9299870) that were significantly
associated with cardiovascular mortality in subjects assigned to the intensive glycemic control arm. Intriguingly, analysis of plasma biomarkers demonstrated a significant correlation between carriers of the C/C genotype at rs57922, lower mortality in the intensive-treated subgroup, and increased circulating levels of GLP-1 (Shah et al., 2018). In contrast, subjects with the T/T genotype had lower plasma levels of GLP-1 and increased cardiovascular mortality. These associations provide intriguing hypotheses; however, it is not immediately apparent how variation at rs57922, within an intergenic non-coding region, influences GLP-1 secretion or cardiovascular outcomes. It seems unlikely that the modest changes in circulating GLP-1 associated with genetic variation at this locus directly contribute to cardioprotection in ACCORD.

Human subjects with loss-of-function mutations in hERG (human ether-a-go-go-related gene, KCNH2, encoding the Kv11.1 voltage-gated potassium channel) develop long-QT syndrome type 2 (LQT2) and exhibit symptomatic hyperinsulinemic hypoglycemia after oral glucose challenge, associated with defective glucagon responses and increased plasma levels of GIP and GLP-1 (Hyldén-Cavallius et al., 2017). Moreover, small interfering RNA-mediated knockdown of Kcnh2 in the GLUTag enteroendocrine cell line increased the magnitude of glucose-stimulated GLP-1 release, implicating a direct role for hERG in control of GLP-1 secretion. It seems likely that ongoing genetic studies coupled with more detailed phenotyping will refine our understanding of the genetic determinants of GLP-1 secretion and metabolic action.

**GLP-1 and Control of Islet Function**

The actions of GLP-1 to potentiate glucose-dependent insulin secretion and inhibit glucagon secretion in islet cells while minimizing hypoglycemia supported the development of multiple structurally distinct GLP-1R agonists for the treatment of T2D (Drucker et al., 2017; Drucker and Nauck, 2006; Ussher and Drucker, 2014). The physiological importance of endogenous GLP-1 is illustrated by studies employing the GLP-1R antagonist exendin(9–39), which increased glycemic excursion following oral glucose ingestion in human subjects (Edwards et al., 1999). Complementary studies demonstrated impairment of glucose tolerance and defective glucose-stimulated insulin secretion in Glp1r−/− mice (Scrochi et al., 1996). Remarkably, the insulinotropic properties of GLP-1 are preserved in human subjects with T2D who fail to respond to sulfonylurea therapy (Nauck et al., 1998).

The mechanisms through which GLP-1R signaling rapidly restores glucose sensitivity to failing diabetic human β cells are incompletely understood and likely involve crosstalk between membrane ion channels, cyclic AMP (cAMP)-dependent signaling, and intracellular glucose metabolism.

The physiological importance of the β cell GLP-1R has been demonstrated by restoration or deletion of GLP-1R within mouse β cells. Selective transgenic targeting of GLP-1R expression to GLP-1R-deficient mouse β cells under the control of the Pdx1 promoter normalized glucose tolerance and glucose-stimulated insulin secretion following oral and intraperitoneal glucose challenge in Glp1r−/− mice (Lamont et al., 2012). Conversely, mice with conditional selective genetic knockdown of Glp1r in β cells exhibited impairment of intraperitoneal glucose tolerance and defective insulin secretion following parenteral, but not oral, glucose administration (Smith et al., 2014). Loss of GLP-1R selectively in β cells also impaired the acute glucoregulatory action of GLP-1R agonists such as liraglutide. Hence, GLP-1R signaling within β cells is essential for glucose homeostasis and the pharmacological actions of GLP-1R agonists. Whether how GLP-1Rs expressed beyond the β cell, possibly within the enteric and peripheral nervous system, contribute to physiological enteral glucose-mediated augmentation of β cell function requires further study. Although genetic loss or acute pharmacological antagonism of CNS GLP-1Rs transiently impairs glucose tolerance (Burmeister et al., 2017; Sandoval et al., 2008), the available evidence suggests that the CNS GLP-1R system is not required for physiological regulation of glycemia by endogenous GLP-1 or the pharmacological GLP-1R-dependent control of glucose homeostasis (Lamont et al., 2012; Sisley et al., 2014).

The importance of neural transmission for GLP-1-initiated signals controlling glycemia, body weight, or gastrointestinal motility in human subjects is less clear. Attenuation of efferent vagal cholinergic transmission using atropine to block muscarinic receptor activity did not impair the acute insulinotropic response to exogenous GLP-1 in healthy human subjects (Plamboeck et al., 2015). In contrast, GLP-1 reduced postprandial glycemic excursions but no longer inhibited food intake or the rate of gastric emptying in non-diabetic normal-weight men after truncal vagotomy and pyloroplasty (Plamboeck et al., 2013).
Hence, the importance of vagal circuits for endogenous GLP-1 action and the pharmacological responses to GLP-1R agonists in humans requires more investigation.

GLP-1 also regulates glycemia through suppression of glucagon secretion from α cells (Figure 2). Multiple products secreted from β cells inhibit glucagon secretion, including insulin, zinc, and γ-aminobutyric acid, and might theoretically contribute to GLP-1R-dependent inhibition of α cell secretory activity. Some studies have reported GLP-1R expression within a subset of α cells, supporting the possibility of direct GLP-1R-mediated suppression of glucagon secretion (Richards et al., 2014). Nevertheless, the GLP1R is expressed at very low levels or is undetectable in the majority of human α cells using single-cell RNA sequencing (RNA-seq) or immunohistochemistry for detection of the GLP1-1R protein (Muraro et al., 2016; Segerstolpe et al., 2016; Waser et al., 2015). Furthermore, GLP-1 stimulates islet somatostatin secretion directly via the canonical GLP1-R, localized by immunohistochemistry to human islet δ cells (Waser et al., 2015); somatostatin in turn inhibits α cell glucagon secretion through somatostatin receptor-2 (Figure 2) (de Heer et al., 2008). Whether GLP-1R agonists predominantly inhibit glucagon secretion in humans through the islet α cell SSTR2 requires clinical studies employing selective SSTR2 antagonists.

GLP-1 inhibits β cell death, induces β cell proliferation, and promotes expansion of β cell mass (Figure 2) in experimental models of diabetes (Campbell and Drucker, 2013). Notably, activation of GLP-1R signaling upregulates insulin biosynthesis and secretion while simultaneously attenuating the development of ER stress in β cells via cAMP-dependent potentiation of ATF4 translation (Yusta et al., 2006). These findings supported the hypothesis that sustained GLP-1R agonism might result in disease-modifying activity in human subjects with T2D, through preservation or enhancement of functional β cell mass.

Improved β cell function is generally maintained during therapy with GLP-1R agonists in T2D; however, most clinical trials are 6–24 months in duration and only limited evidence supports a disease-modifying effect following discontinuation of these agents. Treatment of human subjects with T2D for 48 weeks using liraglutide did not result in sustained improvement in β cell function, assessed 2 weeks following discontinuation of liraglutide (Retnakaran et al., 2014). Although modest improvements in β cell function and disposition index were detected 4 weeks following drug discontinuation in subjects with T2D treated with multiple daily injections of exenatide for 3 years, interpretation of these findings is complicated by substantial reductions in body weight (5.7 kg) in exenatide-treated subjects (Bunck et al., 2011). Notwithstanding current limitations in imaging of β cell mass in human subjects, the available data do not prove the hypothesis that treatment with GLP-1R agonists will produce sustained improvement in human β cell function through durable changes in β cell mass.

How does one reconcile the extensive preclinical evidence linking augmentation of GLP-1R signaling to preservation and expansion of functional β cell mass with the available clinical data? Although β cells from young animals proliferate in response to GLP-1, older rodent β cells exhibit a generalized loss of proliferative capacity (Rankin and Kushner, 2009; Tschen et al., 2011), including attenuated proliferation in response to GLP-1R agonism (Rankin and Kushner, 2009). The loss of basal and GLP-1-stimulated proliferative capacity is also a feature of older human β cells (Dai et al., 2017) and appears independent of relative levels of GLP1R mRNA transcripts that were comparable in juvenile versus adult human islet β cells (Dai et al., 2017). The mitogenic action of GLP-1R agonists in juvenile human islets was linked to functional integrity of calcineurin/nuclear factor of activated T cells (NFAT) signaling, as the NFAT inhibitor, FK506, abrogated the exendin-4-dependent induction of β cell proliferation in juvenile human islets (Dai et al., 2017). Moreover, the NFAT signaling pathway was impaired in islets from adult (20 years of age or older) human donors (Dai et al., 2017). These findings have implications for efforts directed at using GLP-1R agonists for therapeutic augmentation of human β cell mass.

**GLP-1, β Cell Function, and Type 1 Diabetes**

GLP-1R agonists also reduce islet inflammation and delay or prevent development of experimental β cell failure in the NOD (non-obese diabetic) mouse model of autoimmune diabetes, prompting exploration of the therapeutic potential of GLP-1R agonists in the treatment of T1D. Intensive treatment of individuals with long-standing (mean duration of disease 21 years) T1D with exenatide (up to 40 μg daily) with or without daclizumab for...
6 months resulted in weight loss, reduction in insulin dose, and improved insulin sensitivity (Rother et al., 2009). Nevertheless, despite inclusion criteria requiring detectable C-peptide levels at trial entry, no improvement in residual β cell function was detected in exenatide-treated subjects.

GLP-1R agonists improve the survival of human islets ex vivo, or following transplantation into animals (Campbell and Drucker, 2013). However, their effectiveness as adjuvant therapy for preservation of β cell function in human subjects with T1D undergoing islet transplantation remains unproven. Despite preclinical studies linking GLP-1R agonism to reduced inflammation, and enhanced β cell function and survival of transplanted islets, compelling clinical evidence demonstrating a benefit for GLP-1R agonists in the islet transplant setting has not been forthcoming. Equally disappointing are results of clinical trials examining the efficacy of liraglutide as adjuvant therapy to insulin in human subjects with T1D. Addition of liraglutide, either in a treat-to-target trial design or on top of capped insulin regimens, resulted in weight loss and reduction in insulin dose, but only modest reduction in A1c, and increased rates of gastrointestinal adverse events (AEs), ketoacidosis, and symptomatic hypoglycemia (Ahrén et al., 2016; Mathieu et al., 2016). Hence, the potential impact of GLP-1 therapy in human subjects with T1D, ranging from preservation of endogenous β cell function, to sustained improvement in function of transplanted islets, to an adjunctive therapy to insulin, has not yet been realized.

GLP-1 and Insulin Sensitivity in Peripheral Tissues

Weight loss associated with sustained GLP-1R agonism improves insulin sensitivity in animals and humans. However, the available evidence suggests that GLP-1R agonism, independent of stimulation of insulin and inhibition of glucagon secretion, does not directly affect insulin signaling or glucose uptake in liver, muscle, or adipose tissue. Studies using RNA-seq, in situ hybridization, or validated GLP-1R antisera have not detected expression of the canonical GLP-1R in hepatocytes, skeletal myocytes, or adipocytes (Campbell and Drucker, 2013; Panjwani et al., 2013). CNS GLP-1Rs control insulin sensitivity in animals, and activation of GLP1Rs within peripheral nerves or blood vessels may augment tissue blood flow, indirectly regulating insulin action. For example, GLP-1 infusion increases microvascular recruitment in human skeletal muscle independent of insulin, which may potentiate local insulin action (Sjoberg et al., 2014). On the other hand, chronic administration of liraglutide, 1.8 mg daily for 12 weeks, in subjects with T2D did not change capillary perfusion or vasomotor activity assessed in the fasted and postprandial states, despite a 1.7% reduction in hemoglobin A1c (HbA1c) (Smits et al., 2016b). These contrasting results may reflect differences in vascular beds, differences in acute versus chronic GLP-1R activation, structural and functional differences in GLP-1R agonists, or differences in study populations.

GLP-1R Agonism and Control of Body Weight

Extensive evidence demonstrates that GLP-1R agonism reduces food intake and promotes weight loss (Campbell and Drucker, 2013; Drucker and Nauck, 2006), and a single GLP-1R agonist, liraglutide, has been approved for the treatment of obesity (Astrup et al., 2009). GLP-1R agonists activate brown fat and increase energy expenditure in rodents independently of locomotor activity through sympathetic nervous system (SNS) pathways. Central GLP-1 infusion also decreases peripheral lipid storage in white adipocytes from lean mice through mechanisms dependent on SNS activation, actions blunted in high-fat diet (HFD)-fed mice (Nogueiras et al., 2009). However, sustained treatment of obese human subjects with liraglutide (1.8 or 3 mg daily) resulted in reduced energy intake and weight loss, a modest relative shift toward fat oxidation, and reductions in energy expenditure assessed by calorimetry (van Can et al., 2014). The available data strongly suggest that weight loss ensuing from GLP-1R agonism in humans largely reflects reductions in food intake.

Identification of Endogenous Gcg and Glp1r Pathways Controlling Food Intake

The importance of brainstem nucleus tractus solitarius (NTS) neurons that express the Gcg and the proglucagon-derived peptides (PGDPs) for control of food intake and glycemia has been interrogated through use of chemogenetics and optogenetics. Activation of murine hindbrain Gcg+ neurons transiently reduced food intake, metabolic rate, and glucose production as assessed by a pyruvate tolerance test, but had no effect on oral glucose tolerance or insulin sensitivity in lean animals (Gaykema et al., 2017). Interestingly, sustained activation of Gcg+ neurons failed to reduce food intake or body weight in lean mice; however, mice with diet-induced obesity exhibited reduced food intake and weight loss over a 48-hr period, but failed to exhibit suppression of glucose production after Gcg neuronal activation (Gaykema et al., 2017). Similarly, Wang et al. (2015) demonstrated that chemogenetic activation of brainstem NTS Gcg+ neurons attenuated food intake in HFD-fed mice, actions sensitive to peripheral administration of exendin(9–39). As these chemogenetic approaches target entire populations of potentially heterogeneous neurons, it is not always possible to ascertain whether the anorectic effects observed following Gcg neuronal activation reflect integrated or dominant contributions from various PGDPs such as glicentin, oxyntomodulin, glucagon, GLP-1, or GLP-2 (Figure 1), or perhaps non-PGDP-related signals.

Complementary studies identified an anatomical brainstem Gcg-hypothalamic paraventricular nucleus (PVN)-corticotropin-releasing hormone (CRH)+ excitatory neuronal circuit transducing an anorectic response following Gcg activation (Liu et al., 2017). Selective inhibition of Gcg-originated signaling within the PVN increased feeding, whereas stimulation of GLP-1 release reduced food intake in a presynaptic glutamate-independent manner. Blockade of glutamate receptor 1 (GluA1) membrane trafficking in hypothalamic CRH neurons using dominant-negative GluA1 receptors, or administration of the PKA inhibitor H-89, blunted the inhibitory effects of exendin-4 on food intake. These findings support a model wherein GLP-1 stimulates PKA-dependent phosphorylation of GluA1 at S845, thereby promoting GluA1 membrane trafficking and anorectic activity of CRH neurons, without activation of the hypothalamic-pituitary axis (Liu et al., 2017). As some chemogenetic studies activating Gcg neurons do not include simultaneous application of receptor antagonists or studies with receptor knockout mice, interpretation of the downstream peptidergic...
signaling pathways mediating actions such as reduced feeding ensuing from activation of \textit{Gcg}⁺ neurons may be challenging.

**GLP-1R-Dependent Vagal Circuits, Food Intake, and Glucose Homeostasis**

Optogenetic and chemogenetic manipulation of GLP-1R⁺ circuits has enabled delineation of neural pathways transducing signals emanating from \textit{Gcg}⁺ or GLP-1R⁺ neurons. Williams et al. (2016) used \textit{Glp1r}-ires-Cre mice to study the functional signals propagated by GLP-1R⁺ afferent sensory vagal neurons in the proximal gastrointestinal tract. Within the duodenum, vagal GLP-1R⁺ neurons were predominantly localized to the muscle layer and not the villi; GLP-1R⁺ fibers also corresponded to the majority of intraganglionic laminar endings within stomach muscle. Consistent with these anatomical findings, optogenetic activation of GLP-1R⁺ vagal neurons increased gastric pressure and produced small reductions in heart rate and increases in respiratory rate (Williams et al., 2016). Conversely, only a small number (9.0%) of vagal neurons sensed intraluminal nutrient within the duodenum, whereas the majority responded to gastric or intestinal distension. Intriguingly, vagal GLP-1R sensory neurons did not exhibit acute responses to exogenous administration of GLP-1R agonists. Hence, the proximal gut (stomach and duodenum) vagal GLP-1R system predominantly transduces signals initiated by changes in gut distension, rather than by local nutrient influx (Williams et al., 2016). However, the relative functional importance of GLP-1R⁺ neurons distal to the duodenum was not interrogated, and administration of sugars such as D-allulose triggers GLP-1R-dependent circuits within the nodose ganglia to control food intake and glycemia in HFD-fed mice (Iwasaki et al., 2018).

Experiments using genetics or viral RNAi knockdown to reduce \textit{Glp1r} expression within the nodose ganglion revealed that pharmacological GLP-1R agonism to promote weight loss does not require engagement of nodose ganglion GLP-1R receptors. Lentiviral-mediated bilateral knockdown of nodose ganglion GLP-1R-1Rs (50% reduction of \textit{Glp1r} expression) in Sprague-Dawley rats enhanced the rate of gastric emptying and increased post-meal (but not oral glucose-induced) glycemic excursions in association with reduced meal-stimulated insulin levels (Krieger et al., 2016). Although the actions of peripherally administered low-dose GLP-1 or exendin-4 to acutely reduce gastric emptying or food intake were attenuated following nodose ganglion \textit{Glp1r} knockdown, loss of the nodose ganglion GLP-1R did not affect basal control of food intake or body weight (Krieger et al., 2016).

Similar conclusions were obtained in studies examining the metabolic consequences ensuing from genetic reduction of mouse nodose ganglion \textit{Glp1r} expression. Mice with \textit{Phox2b}-directed Cre expression to inactivate the nodose ganglia \textit{Glp1r} exhibited no perturbation in basal food intake, body weight gain, glucose tolerance, or the pharmacological response (decreased food intake, reduced glucose, and weight loss) to GLP-1R agonists (Sisley et al., 2014). Consistent with the dispensable role of vagal GLP-1R circuits for the pharmacological responses to GLP-1R agonism, peripheral administration of twice-daily liraglutide reduced food intake and body weight gain over 14 days in rats following subdiaphragmatic vagal afferent deafferentation (Secher et al., 2014).

**Multiple GLP-1R⁺ Regions in the CNS Regulate Food Intake**

Studies employing GLP-1R antagonists or genetic reduction of \textit{Glp1r} expression support a role for both hypothalamic and hind-brain GLP-1Rs in the physiological control of food intake. Central intracerebroventricular (i.c.v.) administration of exendin(9–39) alone increased food intake and body weight over 3 days in regular chow-fed rats, and i.c.v. exendin(9–39) potentiated the stimulatory actions of co-administered NPY on food intake and body weight over 7 days (Meeran et al., 1999). Moreover, acute administration of exendin(9–39) into the fourth ventricle of rats increased food intake over 24 hr and blocked the gastric distension-associated suppression of food intake (Hayes et al., 2009).

Selective postnatal disruption of \textit{Glp1r} expression in the hypothalamic PVN of 6- to 8-week-old mice using targeted injection of adeno-associated virus Cre resulted in increased food intake, decreased locomotor activity, and increased body weight over a 6-week period (Liu et al., 2017). Similarly, adenoviral short hairpin RNA-mediated knockdown of \textit{Glp1r} expression in the lateral hypothalamus of rats resulted in increased food intake and weight gain, evident over several weeks (López-Ferreras et al., 2017). Consistent with findings from antagonism or disruption of CNS GLP-1R circuits, once-daily subcutaneous administration of an acylated long-acting GLP-1R antagonist, Jant-4(9–40)a Lys40-C16, increased food intake and body weight over 7 days in mice with diet-induced obesity (Patterson et al., 2011).

Hence, multiple clusters of GLP-1R⁺ nuclei within the CNS control food intake in mice and rats; however, little information is available about the physiological importance of endogenous GLP-1 for control of body weight in humans, and genetic variants of GLP1R that associate with lower glucose are not linked to reduction in body mass index (Scott et al., 2016). Acute infusion of exendin(9–39) for 4 hr in overweight or obese subjects with T2D prevented meal-induced reductions in bilateral insula activation in response to food pictures, assessed using functional magnetic resonance imaging (fMRI) (ten Kuile et al., 2015). However, chronic studies employing GLP-1 receptor antagonists to assess food intake and body weight in normal humans have not been reported.

**GLP-1 Action in the Hypothalamus**

The widespread distribution of GLP-1R expression within the CNS as well as the autonomic and enteric nervous systems has spurred investigation into how physiological gut-derived GLP-1, as well as pharmacological activation of the GLP-1R, controls appetite and body weight. Germline disruption of \textit{Glp1r} expression within the CNS, including knockdown of hypothalamic and brainstem \textit{Glp1r} expression achieved through Nestin Cre-mediated recombination of a floxed \textit{Glp1r} allele, attenuated the effect of peripherally administered liraglutide to reduce food intake and body weight in mice (Sisley et al., 2014). Twice-daily subcutaneous injection of liraglutide for 28 days in rats with diet-induced obesity increased \textit{Cart} and reduced the relative expression of \textit{Npy}, \textit{Agrp}, \textit{Ghsr}, and \textit{Lepr} mRNA transcripts in the arcuate nucleus (ARC) (Secher et al., 2014). Moreover, a fluorescent liraglutide analog was detected within the ARC, PVN, suprachiasmatic nuclei, and suprachiasmatic decussation in both rats and mice after peripheral administration, with brain uptake dependent on a functional GLP-1R (Secher et al., 2014).
Consistent with localization of GLP-1R to the ARC, infusion of exendin(9–39) into the ARC, but not the PVN, partially attenuated the extent of liraglutide-induced weight loss (Secher et al., 2014). Consistent with localization of GLP-1R to the ARC, infusion of exendin(9–39) into the ARC, but not the PVN, partially attenuated the extent of liraglutide-induced weight loss (Secher et al., 2014). 

Burmeister et al. (2017) used Nkx2.1-Cre, Sim1-Cre, or Pomc-Cre mice to knock down hypothalamic Glp1r expression. Selective or broad disruption of hypothalamic Glp1r expression did not impair the ability of GLP-1R agonists to reduce food intake and body weight. Moreover, interpretation of the putative role(s) of hypothalamic GLP-1Rrs in the control of body weight was complicated by concomitant increases in energy expenditure, consistent with resistance to diet-induced obesity described in whole-body HFD-fed Glp1r$^{−/−}$ mice (Hansotia et al., 2007). The magnitude of liraglutide-induced weight loss was partially attenuated in HFD-fed mice with ablation of the Gip1r in Nkk2.1$^{+}$ neurons (Burmeister et al., 2017). Hence, neurons within the hypothalamus mediate a component of, but not the full spectrum of, anorexigenic responses to peripheral GLP-1R agonism.

**GLP-1 Action in the Hindbrain**

Hindbrain GLP-1Rs also mediate physiological control of food intake and the response to GLP-1R agonism (Hayes et al., 2009). Direct administration of exendin-4 into the fourth ventricle of rats activated protein kinase A and enhanced phosphorylation of extracellular signal-regulated kinase (ERK)-1/2, which in turn suppressed AMP-activated protein kinase. These actions were associated with acute reductions of food intake and blocked by co-administration of inhibitors of cAMP-dependent signaling (Hayes et al., 2011). A role for hindbrain astrocytes in the transduction of anorectic GLP-1R-dependent signals has also been proposed. Fluorescently labeled exendin-4 administered peripherally, or via direct injection into the fourth ventricle, was detected on the surface of and internalized within a subset of neurons and glial fibrillary acidic protein (GFAP)$^{+}$ rat hindbrain astrocytes; its binding was attenuated by co-administration of exendin(9–39) (Reiner et al., 2016). Moreover, immunohistochemistry identified GLP-1-immunopositive axons in close proximity to GFAP$^{+}$ astrocytes and hindbrain injection of the astrocyte Krebs cycle inhibitor fluorocitrate attenuated the actions of exogenous exendin-4 to inhibit food intake and reduce body weight over 24 hr (Reiner et al., 2016). Hence, the available evidence from studies of pharmacological GLP-1R blockade or selective genetic disruption of GLP-1R signaling in rodents supports the importance of endogenous CNS GLP-1R signaling within both the hypothalamus and brainstem for the physiological control of food intake and body weight.

**The Role of GLP-1 in Bariatric Surgery**

Circulating GLP-1 levels rise rapidly and are often substantially elevated in human subjects after Roux-en-Y gastric bypass (RYGB) and to a lesser extent following vertical sleeve gastrectomy (VSG) (Madsbad et al., 2014). In some individuals, exaggerated brisk elevations in plasma GLP-1 levels contribute to the development of hyperinsulinemic hypoglycemia, which can be difficult to treat and may require partial pancreatectomy for alleviation of symptoms. The hypoglycemia is mitigated by short-term GLP-1R blockade using exendin(9–39) (Salehi et al., 2014), prompting clinical development of GLP-1R antagonists for the treatment of refractory hypoglycemia after bariatric surgery.

The contribution of GLP-1 to the weight loss and improved glucose control ensuing after bariatric surgery is uncertain (Figure 3). Acute infusion of exendin(9–39) in obese women prior to and after RYGB demonstrated the presence of GLP-1R-sensitive food-activated circuits in response to food pictures or chocolate milk ingestion, as assessed by fMRI (Ten Kulve et al., 2017). Nevertheless, the importance of GLP-1 for the sustained reduction in appetite and weight loss after RYGB or VSG has not been conclusively demonstrated. Analysis of the acute metabolic effects of GLP-1R blockade using exendin(9–39) was carried out in obese human subjects with T2D, achieving similar weight loss (~12 kg) after 24 weeks of intensive lifestyle management or RYGB surgery (Vetter et al., 2015). Postprandial glycemic excursions increased in both groups after infusion of exendin(9–39), although postprandial glucose tolerance was similar in both groups despite much higher plasma levels of GLP-1, attenuation of meal-stimulated C peptide, and increased plasma glucagon responses following exendin(9–39) in the RYGB group. Notably, hepatic insulin sensitivity and suppression of endogenous glucose production were greater in the RYGB group, and insensitive to infusion of exendin(9–39) (Vetter et al., 2015). Qualitatively similar results were obtained in studies using exendin(9–39) to block GLP-1 action in non-diabetic subjects with elevated GLP-1 levels studied ~5 years after RYGB, with results compared with data obtained in weight-matched controls. Although exendin(9–39) produced a modest deterioration in insulin secretion after meal ingestion, there was no effect of GLP-1R blockade on insulin action, endogenous glucose production, or glucose disappearance (Shah et al., 2014). Hence, increased GLP-1R signaling contributes to improvement of β cell function but does not fully explain the marked improvement in glycemic control, insulin sensitivity, or weight loss achieved after bariatric surgery (Figure 3).

Consistent with studies in humans, a functional GLP-1R is not required for weight loss or improved glucose tolerance after experimental RYGB or VSG in Glp1r$^{−/−}$ mice or in wild-type mice chronically treated with i.c.v. infusion of exendin(9–39) (Mokadem et al., 2014; Wilson-Pérez et al., 2013; Ye et al., 2014).
Nevertheless, chronic peripheral, but not i.c.v., infusion of exendin(9−39) blunted improvements in oral glucose tolerance following RYGB in obese mice (Carmody et al., 2016) and selective β cell disruption of the GLP-1R in mice attenuated the improvement in insulin secretion and glucose tolerance 3 weeks after VSG (Garibay et al., 2016). Given the rapid improvement in insulin sensitivity after RYGB and VSG (not a consistent feature of GLP-1 action), and the pleiotropic changes in bile acids, gut microbial populations, and numerous gut peptides and metabolites, it seems likely that elevated levels of GLP-1 contribute to improved β cell function but do not mediate the majority of the multiple metabolic benefits evident after bariatric surgery (Figure 3).

GLP-1, Inflammation, and the Gut Microbiome

Administration of GLP-1R agonists to animals and humans with T2D or obesity is frequently associated with reduction of local or systemic inflammation (Drucker, 2016a). GLP-1 in turn may mediate a subset of its metabolic actions through immune cells, increasing the number and activity of invariant natural killer T (iNKT) cells, thereby triggering production of fibroblast growth factor 21 and induction of weight loss in mice (Lynch et al., 2016). Interpretation of the anti-inflammatory actions of GLP-1 may be complicated by concomitant changes in glycemic state, reduction of caloric intake and weight loss, and the absence of functional GLP-1Rs in most immune cell subtypes, including iNKT cells (Drucker, 2016a). The putative physiological importance of endogenous GLP-1 for reduction of inflammation, independent of glucose control and weight loss, is highlighted by demonstration that GLP-1-secreting EECs not only respond to nutrients, but also act as inflammation sensors (Drucker, 2016a). GLP-1 secretion increases rapidly in response to cytokines, most notably interleukin-6 (Ellingsgaard et al., 2011; Lebrun et al., 2017), bacterial metabolites, lipid amides and proteins secreted by commensal and pathobiont bacteria.

Conversely, complete loss of the normal gut microbiome, as seen in germ-free mice or dysbiosis arising from antibiotic administration in mice, increases gut GLP-1 synthesis and secretion (Hwang et al., 2015; Wichmann et al., 2013). Moreover, germ-free or antibiotic-treated mice, or mice with diet-associated dysbiosis, exhibit reduced GLP-1R expression in the ileum (Glashoff et al., 2016) and correlated with the severity of illness in critically ill human subjects with sepsis, though it remains unclear whether altered GLP-1 response to administration of LPS or acute transient intestinal ischemia is preserved in humans (Lebrun et al., 2017), consistent with a role for L cell secretory products, including GLP-2, in the response to and defense against gut mucosal injury and barrier dysfunction (Drucker and Yusta, 2014). Moreover, circulating GLP-1 levels are elevated in critically ill human subjects with sepsis, and correlated with the severity of illness and clinical outcomes (Lebherz et al., 2017). Given the heterogeneity in GLP-1-secreting EEC populations along the gut, identification of L cell subtypes that differentially express bacterial metabolite and pattern recognition receptors requires further scrutiny.

GLP-1 acts locally to modulate intestinal inflammatory responses through the canonical GLP-1R, expressed on intestinal intraepithelial lymphocytes (IELs) in the small and large bowel (Yusta et al., 2015). Although diet-induced gut microbial dysbiosis in mice is associated with reduced Glp1r expression in the enteric nervous system (ENS) and impaired metabolic responses to GLP-1 (Grasset et al., 2017), whether the ENS GLP-1R also transduces anti-inflammatory signals is uncertain. The distal gut of Glp1r−/− mice displays microbial dysbiosis, is more sensitive to inflammation-related injury, and exhibits a signature of dysregulated inflammation-related gene expression in the basal state that is substantially corrected following transplantation of Glp1r+/+ bone marrow (Yusta et al., 2015). Whether the gut IEL GLP-1Rs indirectly propagate anti-inflammatory signals that attenuate inflammation in the liver, or in more distant organs, has not yet been determined (Drucker, 2016a).

Figure 4. The Role of Microbial Products and Metabolites in the Control of GLP-1 Secretion

TLR, Toll-like receptor; IEL, intraepithelial lymphocyte; EEC, enteroendocrine cell.
pattern receptor signaling (Grasset et al., 2017). These findings support a role for microbial populations and their downstream effectors as modifiers of GLP-1 secretion and ENS sensitivity to GLP-1 action in mice.

On the other hand, treatment of lean non-diabetic human subjects with a 4-day course of a broad-spectrum antibiotic cocktail produced substantial alterations in the number and abundance of gut microbial populations, yet no change in meal-related glucose tolerance or plasma levels of GLP-1 in the fasting or postprandial state (Mikkelsen et al., 2015). Reijnders et al. (2016). These analyses were extended to overweight and obese human subjects with prediabetes (impaired fasting glucose and/or impaired glucose tolerance). A 7-day course of vancomycin administration reduced gut microbial diversity but did not affect insulin sensitivity, intestinal energy harvest, gut permeability, circulating levels of LPS and plasma cytokines, glucose homeostasis, or plasma levels of gut hormones, including GLP-1 as assessed 1 week or 8 weeks after the intervention. Hence, there is insufficient information from human studies of subjects with T2D or obesity to link specific patterns of gut microbial dysbiosis with clinically relevant alterations in GLP-1 secretion or responsivity.

Studies of metformin action in human subjects with T2D reveal alteration of the gut microbiome and generation of microbial metabolites, including butyrate and fatty acids, which in turn might contribute to increased GLP-1 secretion and the glucoregulatory properties of metformin (Wu et al., 2017), even at very low “gut-restricted” doses. Indeed, delayed release metformin improved glucose control and increased plasma levels of GLP-1 at systemic exposures much lower than those achieved with conventional metformin administration (DeFronzo et al., 2016). Nevertheless, metformin acts within minutes to increase plasma GLP-1 levels and lowers glucose rapidly in mice and humans (Maida et al., 2011; Migoya et al., 2010), a time frame inconsistent with an important role for alterations in the microbiome as a target for acute metformin actions on GLP-1 secretion. Furthermore, metformin does not require GLP-1 signaling to robustly lower glucose in mice (Maida et al., 2011) and exerts pleiotropic actions to lower glucose independent of GLP-1 action in humans. Nevertheless, a pilot study of probiotic administration (twice-daily controlled-release capsules of Lactobacillus reuteri) in non-diabetic lean and obese human subjects increased plasma levels of GLP-1 and GLP-2, and improved glucose-stimulated insulin levels, without altering glucose tolerance, systemic insulin sensitivity, circulating cytokine levels, or the composition of the fecal microbiota (Simon et al., 2015). Hence, the importance and therapeutic potential of targeting gut microbial dysbiosis for regulation of GLP-1 secretion and glucose tolerance across a range of human patient populations require more study.

GLP-1 Actions in the Gastrointestinal Tract and Liver

GLP-1R agonists inhibit gastric and small bowel motility, although the precise neuronal circuits responsible for these actions remain unclear, and the inhibition is subject to rapid tachyphylaxis and wanes with sustained GLP-1R activation (Nauck et al., 2011). GLP-1 also inhibits postprandial chylomicron secretion and lowers circulating triglyceride levels in rodents and humans (Heish et al., 2010; Xiao et al., 2012); however, neither enterocytes nor hepatocytes express the canonical GLP-1R (Panjwani et al., 2013; Pyke et al., 2014; Richards et al., 2014), suggesting that these actions are indirectly mediated, possibly through neural circuits. The actions of GLP-1 to attenuate gut motility have prompted exploration of GLP-1R agonism for the treatment of irritable bowel syndrome, although the available results of human studies are preliminary and inconclusive.

The actions of GLP-1R agonists to reduce hepatic steatosis, decrease liver inflammation, and attenuate hepatocyte injury in preclinical models of non-alcoholic steatohepatitis (NASH) may be secondary to weight loss or reflect other indirect mechanisms governing hepatocyte lipid synthesis and oxidation, inflammation, or fibrosis (Campbell and Drucker, 2013; Drucker, 2016b). Remarkably, liraglutide (1.8 mg once daily for 48 weeks) improved liver histology, increased resolution of NASH, and decreased the rate of progression to fibrosis in overweight and obese human subjects with biopsy-proven NASH (Armstrong et al., 2016). One-third of the subjects had concomitant T2D, predominantly treated with metformin. Body weight was reduced by ~5% and HbA1c decreased by 0.5%, in liraglutide-treated (n = 23) versus placebo-treated (n = 22) subjects. Although changes in body weight and glycemic control were not different in liraglutide responders versus non-responders, the mechanisms linking liraglutide action to attenuation of disease progression remain uncertain. The canonical GLP-1R is not expressed in hepatocytes (Flock et al., 2007; Landgraf et al., 2015; Panjwani et al., 2013), and expression of the GLP-1R within non-hepatocyte liver cell types has not been conclusively established. Given the importance of weight loss in the therapeutic approach to NASH, together with potential confounding indirect contributions arising from GLP-1R-dependent reductions in postprandial lipemia, glycemia, and inflammation, a mechanistic understanding of how GLP-1R agonists attenuate NASH remains unclear.

Cardiovascular Safety, Outcomes, and Mechanisms

The demonstration that some GLP-1R agonists reduce the rates of major adverse cardiovascular events (MACEs) in cardiovascular outcome trials (CVOTs) (Marso et al., 2016a, 2016b), whereas other agents exhibited cardiovascular safety (Holman et al., 2017; Pfeffer et al., 2015) (Table 1), has enhanced interest in understanding GLP-1R-dependent mechanisms of action in the cardiovascular system (Drucker, 2016a).

The safety of lixisenatide or placebo was studied over a mean period of 25 months in 6,068 human subjects with T2D who had experienced a myocardial infarction or hospitalization for unstable angina within the previous 180 days (Pfeffer et al., 2015). Administration of once-daily lixisenatide did not modify the numbers of reported events reflecting the primary composite of cardiovascular death, myocardial infarction, stroke, or hospitalization for unstable angina, nor did it change rates of hospitalization for heart failure. Reassuringly, no unexpected safety concerns were detected in lixisenatide-treated subjects.

Cardiovascular safety of once-daily liraglutide was evaluated in the Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results (LEADER) trial, which randomized 9,340 subjects with T2D, 81.3% with established cardiovascular disease (CVD), who were followed for a mean period of 3.8 years (Marso et al., 2016b). Occurrence of MACEs, total mortality, and cardiovascular death was reduced in liraglutide-treated subjects, who also experienced a numerical reduction.
in myocardial infarction. Rates of microvascular disease, primarily reflecting reduced microalbuminuria events, were also lower in the liraglutide arm (Marso et al., 2016b).

The cardiovascular safety of semaglutide, a GLP-1R agonist structurally related to liraglutide but suitable for once-weekly administration (Table 1), was evaluated at two different doses over 104 weeks in subjects with T2D. Fewer MACEs occurred in semaglutide-treated subjects, primarily reflecting reduced numbers of non-fatal strokes. Non-fatal myocardial infarction was numerically reduced; however, reports of retinopathy-associated complications were higher in semaglutide-treated individuals (Marso et al., 2016a).

Once-weekly exenatide, a long-acting GLP-1R agonist, was evaluated compared with placebo in 14,752 subjects with T2D, 73.1% with established CVD, followed for a mean period of 3.2 years in the Exenatide Study of Cardiovascular Event Lowering (EXSCEL) (Holman et al., 2017). Numerically fewer MACEs were reported in exenatide-treated subjects with \( p = 0.06 \) for superiority.

In the absence of head-to-head trials, it is not possible to directly compare results obtained with structurally different GLP-1R agonists, as each trial enrolled different proportions of subjects with established CVD, and patients were followed for different lengths of time using different study protocols. Nevertheless, the cumulative data, buttressed by a meta-analysis, demonstrated that all drugs examined to date in this class exhibit reduced macrovascular complications as higher in semaglutide-treated individuals (Marso et al., 2016a).

Table 1. Clinically Approved and Investigational Glucagon-like Peptide-1 Receptor Agonists

<table>
<thead>
<tr>
<th>Drug</th>
<th>Frequency</th>
<th>Properties and Dosing</th>
<th>CVOT, Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liraglutide</td>
<td>daily</td>
<td>acylated peptide, 0.6–1.8 mg, T2D</td>
<td>LEADER, reduced MACEs</td>
</tr>
<tr>
<td>Liraglutide</td>
<td>daily</td>
<td>acylated peptide, 3 mg, obesity</td>
<td>ND</td>
</tr>
<tr>
<td>Lixisenatide</td>
<td>daily</td>
<td>peptide 44 aa, 10–20 ( \mu g )</td>
<td>ELIXA, neutral</td>
</tr>
<tr>
<td>Semaglutide</td>
<td>weekly</td>
<td>acylated peptide, 0.5–1 mg</td>
<td>SUSTAIN-6, reduced MACEs</td>
</tr>
<tr>
<td>Epeglenatide</td>
<td>weekly</td>
<td>exenatide-4-non-glycosylated Fc</td>
<td>investigational, ND</td>
</tr>
<tr>
<td>ITCA650</td>
<td>3–6 months</td>
<td>exenatide osmotic minipump</td>
<td>investigational, FREEDOM-CVO, NR</td>
</tr>
</tbody>
</table>

NR, not reported; ND, not done; aa, amino acid; T2D, type 2 diabetes; MACEs, major adverse cardiovascular events.

Although therapy with GLP-1R agonists favorably modifies risk factors such as body weight, blood pressure, and lipids, the modest changes in these parameters over time in the LEADER trial do not seem to fully explain the reduction in MACE reported with liraglutide therapy (Marso et al., 2016b).

Similarly, the reduction in non-fatal myocardial infarction detected with liraglutide is unlikely to be completely explained by actions mediated through the cardiac GLP-1R, which has been detected predominantly in the atrium in rodents (Kim et al., 2013; Richards et al., 2014), and within the sinoatrial node in monkey and human heart (Baggio et al., 2018; Pyke et al., 2014). GLP1R mRNA transcripts have been detected in RNA isolated from the human left ventricle, including cardiomyocytes (Baggio et al., 2018; Wallner et al., 2015), although the precise cellular localization and biological importance of a translated ventricular GLP-1R remains uncertain.

Therapy with GLP-1R agonists increases heart rate in non-diabetic human subjects and in individuals with T2D and obesity, without any apparent adverse rhythm-related outcomes in multiple large CVOTs studying the safety of GLP-1R agonists in T2D (Holman et al., 2017; Marso et al., 2016a, 2016b; Pfeffer et al., 2015). Nevertheless, increased reports of arrhythmias were described with liraglutide administration in human subjects with moderate to advanced heart failure and impaired ventricular function, suggesting caution in this subset of individuals (Jorsal et al., 2017; Margules et al., 2016).

GLP-1R agonism also reduces cardiovascular inflammation through incompletely understood mechanisms (Drucker, 2016a). The localization of GLP-1R expression to intestinal IELS within the immune system (Yusta et al., 2015) does not link direct activation of the immune GLP-1R to reduction of vascular or cardiac inflammation. Nevertheless, it remains possible that GLP-1R expression may be induced in some immune cells during the inflammatory or atherosclerotic process. Although GLP-1R agonism reduces the development of atherosclerosis in genetically sensitized animal models, the weight loss-independent actions of GLP-1 that account for these benefits have not been conclusively identified (Drucker, 2016a).

Hence, the mechanisms enabling GLP-1R activation to reduce rates of myocardial infarction, stroke, and cardiovascular death require further elucidation.

Adverse Events Associated with GLP-1R Agonism

The predominant AEs associated with therapeutic administration of GLP-1R agonists are gastrointestinal, specifically nausea, diarrhea, and vomiting (Drucker and Nauck, 2006). Most human subjects never report nausea or vomiting during treatment with a GLP-1R agonist; however, up to 50% of treated individuals experience at least one episode of nausea and some patients discontinue use of GLP-1R agonists due to problems with tolerability (Drucker and Nauck, 2006). New titration regimens with slower escalation to maximum effective doses of GLP-1R agonists have markedly reduced rates of nausea and vomiting. Distinct regions within the rat brain mediate the aversive versus...
the anorectic responses to i.c.v. administration of GLP-1R agonists. GLP-1R activation in the hypothalamus and brainstem reduces food intake in rats and mice, whereas the central nucleus of the amygdala transduces the aversive response to GLP-1R agonists (Kinzig et al., 2002). GLP-1R agonists inhibit gastrointestinal motility, likely contributing to the pathophysiology of diarrhea reported with these agents. GLP-1R agonism also enhances intestinal growth in rats and mice (Koehler et al., 2015b; Simonsen et al., 2007) and intestinal polyp formation via stimulation of crypt fission in genetically susceptible mice (Koehler et al., 2015b). However, there is no evidence from human clinical trials that GLP-1R agonists increase rates of benign or malignant colorectal neoplasms (Holman et al., 2017; Marso et al., 2016a, 2016b; Pfeffer et al., 2015).

Initial concerns about the pancreatic safety of GLP-1R agonists stemmed from preclinical reports of acute and chronic pancreatitis in animals treated with the DPP-4 inhibitor sitagliptin, concurrent with clinical reports of pancreatitis in exenatide-treated subjects, and subsequent demonstrations of preneoplastic pancreatic ductal lesions in animals treated with GLP-1R agonists (Drucker, 2013). More extensive studies demonstrated widespread spontaneous occurrence of focal pancreatitis, atrophy, necrosis, and ductal proliferation in normal and diabetic rats in the absence of exposure to GLP-1R agonists (Chadwick et al., 2014). Moreover, characterization of reagents used to detect the GLP-1R revealed generalized problems with impaired sensitivity and specificity of multiple commercially available GLP-1R antisera, questioning the accuracy of reports of GLP-1R localization using these antisera (Drucker, 2013; Panjwani et al., 2013; Pyke et al., 2014). A comprehensive independent review of the preclinical toxicology and carcinogenicity studies associated with GLP-1R agonists was carried out by regulatory authorities in Europe and the United States. Scrutiny of the available data, including independent studies by the Food and Drug Administration examining the pancreatic actions of exenatide in normal and diabetic mice and rats, yielded no evidence for pancreatic toxicity of incretin-based therapies (Egan et al., 2014). Subsequently, several large cardiovascular outcome studies examining the safety of GLP-1R agonists in thousands of subjects with T2D (described above) have demonstrated no clear imbalance in rates of pancreatitis or pancreatic cancer (Holman et al., 2017; Marso et al., 2016a, 2016b; Pfeffer et al., 2015). Hence, the conclusion of regulatory authorities that assertions of pancreatic toxicity “are inconsistent with the available scientific data” (Egan et al., 2014) has been validated by rigorous basic and clinical science.

Plasma levels of pancreatic enzymes, predominantly lipase and to a lesser extent amylase, rapidly increase in many human (diabetic or obese) subjects treated with GLP-1R agonists, in the absence of symptoms or signs associated with pancreatitis. Preclinical studies demonstrate that GLP-1R agonists increase expression of genes and proteins important for pancreatic protein synthesis in the mouse pancreas, such as increased S6 kinase phosphorylation, actions sensitive to rapamycin (Koehler et al., 2015a). Low-level GLP-1R expression in acinar cells was revealed through detection of Glp1r mRNA transcripts in isolated mouse pancreatic acini ex vivo (Hou et al., 2016) and through immunohistochemical detection of the GLP-1R in acinar cells (Pyke et al., 2014), including analysis of histology sections from the pancreas of non-diabetic subjects and individuals with T2D (Kirk et al., 2017). GLP-1 directly increased cAMP accumulation, protein kinase A-dependent protein phosphorylation, and amylase secretion from wild-type, but not Glp1r−/−, mouse acini ex vivo. Hence, these findings are consistent with GLP-1R-dependent augmentation of enzyme synthesis and secretion from pancreatic acinar cells. Moreover, the increase in plasma enzyme levels in human subjects treated with GLP-1R agonists is reversible, with levels of amylase and lipase returning to normal following discontinuation of liraglutide therapy (Steinberg et al., 2017).

Regulatory authorities also expressed concerns about the theoretical risk of medullary thyroid cancer (MTC) in human subjects treated with GLP-1R agonists, stemming from preclinical observations linking sustained GLP-1R agonism to development of C cell hyperplasia and MTC in rats and mice (Parks and Rosebraugh, 2010). GLP-1Rs are expressed and functional in rodent thyroid C cells and contribute to regulation of bone mass through GLP-1R-dependent calcitonin secretion. Indeed, Glp1r−/− mice exhibit cortical osteopenia, increased bone resorption, and reduced calcitonin expression in the thyroid, revealing a functional nutrient-sensitive enteroendocrine-thyroid C cell axis for regulation of bone resorption (Yamada et al., 2008). In contrast, the density of GLP-1Rs on monkey and human thyroid cells is extremely low, and sustained GLP-1R agonism does not cause calcitonin secretion, C cell hyperplasia, or MTC in non-human primates (Bjerre Knudsen et al., 2010; Vahle et al., 2015). Calcitonin levels have been measured in tens of thousands of human subjects with T2D or obesity treated with GLP-1R agonists for months to years, and no evidence for changes in calcitonin levels or increased rates of MTC has been forthcoming (Hegedüs et al., 2011; Holman et al., 2017; Marso et al., 2016a, 2016b; Pfeffer et al., 2015). Nevertheless, the GLP-1R may be expressed in some human MTCs (Waser et al., 2015), and it remains prudent to eschew use of GLP-1R agonists in subjects with a family or personal history of MTC, or multiple endocrine neoplasia type 2.

The use of GLP-1R agonists to treat T2D and obesity has also been associated with increased reports of gallbladder disease, including gallstones, acute cholecystitis, and rates of cholecystectomy in some (Faille et al., 2016; Holman et al., 2017; Marso et al., 2016b), but not all (Marso et al., 2016a; Pfeffer et al., 2015), clinical studies. A single dose of short-acting exenatide had no effect on gallbladder volume or emptying in humans (Keller et al., 2012); however, gallbladder volume was greater and cholecystokinin-stimulated ejection fraction was lower in healthy human subjects treated with a single 50-mg dose of albiglutide (Shaddinger et al., 2017). In contrast, 12 weeks of 1.8-mg daily administration of liraglutide in human subjects with T2D was not associated with changes in gallbladder volume or ejection fraction (Smits et al., 2016a). Moreover, exendin-4 has no acute effect on gallbladder volume in mice, and levels of Glp1r expression in the murine gallbladder were extremely low, ~50-fold lower than corresponding levels of Glp2r mRNA transcripts (Yusta et al., 2017). Hence, whether sustained GLP-1R agonism directly affects the biliary system in some subjects through direct actions on gallbladder or duct motility, or indirectly through weight loss and alteration of lithogenic bile composition, requires further clarification.
New Medicinal Approaches to the GLP-1 Receptor as a Therapeutic Target

Recent advances have enabled elucidation of the structure of the GLP-1 receptor, its membrane topology, and more detailed understanding of how structurally distinct ligands interact with multiple extracellular regions of the receptor (Jazayeri et al., 2017; Song et al., 2017; Wootten et al., 2016; Zhang et al., 2017). These insights, coupled with delineation of receptor-G-protein interactions critical for signal transduction, have re-energized efforts directed at development of new peptide and small-molecule GLP-1R agonists, including full and biased agonists and allosteric modulators (Figure 5) (Jazayeri et al., 2017; Song et al., 2017; Wootten et al., 2016; Zhang et al., 2017). Indeed, a “holy grail” of GLP-1 therapeutics, the development of small-molecule GLP-1R agonists, is likely more achievable with advanced understanding of GLP-1R structures. Sustained GLP-1R agonism does not lead to broad receptor desensitization or diminution of glucoregulatory or anorectic responses, although tachyphylaxis for gastric emptying, nausea, and vomiting develops rapidly (Campbell and Drucker, 2013; Graaf et al., 2016). Nevertheless, it has been proposed that identification of peptides with biased agonist activity at the GLP-1R (less or absent β-arrestin-linked signaling) may enable greater therapeutic activity, with reduced potential for receptor desensitization upon sustained administration (Zhang et al., 2015). Whether GLP-1R ligands promoting biased signaling will confer greater tolerability or potency while preserving safety, and established clinical benefits, remains to be determined. It seems likely that the resolution of GLP-1 receptor structures and enhanced understanding of the complexity of peptide receptor binding and activation, together with extensive knowledge gleaned from studies of receptor mutagenesis, trafficking, and signaling, may enable development of more efficacious and selective small-molecule and peptide GLP-1R agonists and allosteric modulators (Graaf et al., 2016). The latter class of agents depends on the presence of endogenous GLP-1 and might be particularly well suited to work in subjects after bariatric surgery, or in combination with metformin, which increases plasma levels of GLP-1 in both non-diabetic individuals (Migoya et al., 2010; Preiss et al., 2017) and in subjects with T2D (Napolitano et al., 2014; Preiss et al., 2017).

Progress in the development of peptide formulations suitable for oral administration enabled initiation of an investigational drug development program for oral semaglutide, a small acylated peptide agonist (Figure 5) now approved for the treatment of T2D as a once-weekly injectable formulation. Semaglutide for oral use is co-formulated with sodium N-[8 (2-hydroxybenzoyl)amino]caprylate (SNAC), enabling a pH-dependent enhancement of solubility, resistance to proteolytic degradation, and enhanced transcellular permeability, predominantly in the stomach (Davies et al., 2017). Dose-ranging phase 2 studies examining the actions of oral semaglutide over 26 weeks in subjects with T2D demonstrated a profile of efficacy and tolerability comparable with that achieved using once-weekly semaglutide, including gastrointestinal AEs as the dose-limiting side effect (Davies et al., 2017).

A complementary approach to improving the efficacy of GLP-1R agonists, stimulated in part by the putative contributions of multiple gut peptides acting coordinately in the setting of bariatric surgery, entails the development of unimolecular multi-agonists, containing two or three peptide epitopes within a single peptide.
molecule. The prototype co-agonist oxyntomodulin (Figure 1) is a naturally occurring 37-amino-acid PGDP co-secreted with GLP-1 and GLP-2 from gut EECs, which transduces its metabolic actions through activation of the GLP-1R and glucagon receptor (GCGR). Native oxyntomodulin exhibits biased signaling at the GLP-1R, away from cAMP accumulation and calcium influx, with a preference for ERK1/2 activation (Wootten et al., 2016). Administration of oxyntomodulin three times daily for 4 weeks to overweight or obese human subjects reduced food intake and induced weight loss (Wynne et al., 2005); oxyntomodulin also increased activity-related energy expenditure over a 4-day treatment period (Wynne et al., 2006). Several long-acting glucagon/GLP-1 co-agonists produce greater weight loss than GLP-1 alone in preclinical studies while improving glucose control via GLP-1R signaling (Sadry and Drucker, 2013). Whether novel unimolecular multi-agonists will induce robust weight loss and comparable or superior glycemic control relative to GLP-1R agonists in clinical studies is under examination.

An acylated co-agonist that recognizes both the GLP-1 and GIP receptors produced greater metabolic benefits (glucose control and weight loss) in preclinical studies (including acute studies in non-human primates) relative to GLP-1R agonism alone (Finan et al., 2013). Moreover, administration of a pegylated GLP-1/GIP co-agonist produced dose-dependent reductions in HbA1c over 6 weeks, associated with a low rate of gastrointestinal AEs, in human subjects with T2D (Finan et al., 2013). An open-label study of a balanced acylated GLP-1/GIP co-agonist, NNC0090-2746, was carried out over 12 weeks in human subjects with T2D inadequately controlled on metformin. Treatment with 1.8 mg daily of NNC0090-2746 reduced HbA1c (0.68 and 0.96 decrements assessed at 8 and 12 weeks, respectively), with reductions in body weight (1.8% at 8 weeks) and fasting lipid levels, and an acceptable gastrointestinal AE profile (Frias et al., 2017). The metabolic benefits achieved with NNC0090-2746 were comparable, but not superior, to reductions in HbA1c and body weight observed in an open-label comparator group treated with liraglutide (Frias et al., 2017).

Unimolecular tri-agonists containing peptide epitopes that simultaneously recognize the glucagon, GLP-1, and GIP receptors (Figure 5) exhibit robust glucose-lowering activity and substantial weight loss in preclinical studies (Finan et al., 2015). These molecules exploit the premise that simultaneous activation of complementary mechanisms for inhibition of food intake (GLP-1 and glucagon), increased energy expenditure (GLP-1 and glucagon), and enhancement of β cell function (GLP-1 and GIP) will produce substantially greater metabolic benefits than those demonstrated for GLP-1 or emerging co-agonists (Finan et al., 2015).

Several observations and assertions surrounding the development of these new molecular entities require careful consideration and validation in human clinical trials. First, the rapidity and magnitude of weight loss detected with co-agonists and tri-agonists in preclinical studies may reflect not only enhanced potency, but also fewer limitations on dose escalation in mice and rats relative to humans. Furthermore, enhanced energy expenditure detected with GLP-1R and GCGR agonism contributes to weight loss in animals (Finan et al., 2015), yet is less likely to be evident in humans (van Can et al., 2014). Moreover, fixed ratios of each peptide epitope optimized for agonism at the respective rodent receptors may not necessarily translate into optimized activation of the cognate human receptors. Finally, whether the clinical safety profile, including cardiovascular benefit (Drucker, 2016a), now established for GLP-1R mono-agonism can be recapitulated through simultaneous activation of multiple widely distributed GPCRs remains to be established. These challenges suggest that the impressive metabolic benefits achieved with unimolecular multi-agonists in preclinical studies may be difficult to fully recapitulate in subjects with T2D or obesity.

**Emerging Investigational Studies for GLP-1R Agonists**

The use and safety profile of GLP-1R agonists in the treatment of T2D and obesity, together with the pleiotropic mechanisms of GLP-1 action, have stimulated considerable interest in potential non-cardiometabolic indications for GLP-1 therapies. As noted, GLP-1R agonism appears promising for the treatment of NASH, and phase 3 trials with semaglutide are planned. A large body of preclinical science demonstrates that GLP-1R agonists exert neuroprotective and neurotrophic actions, and two clinical trials have demonstrated therapeutic efficacy of twice-daily or once-weekly exendin in the treatment of Parkinson’s disease (Athauda et al., 2017; Aviles-Olmos et al., 2013). GLP-1R agonists also improve cognition as modeled in some preclinical studies and are under investigation in clinical studies for the treatment of Alzheimer’s disease. Activation of GLP-1R signaling also modifies the behavior of animals with alcohol, nicotine, or cocaine dependence, supporting pilot studies in human subjects. Hence, it remains possible that GLP-1R agonists may be effective in therapeutic areas beyond diabetes, heart disease, and obesity, pending more rigorous analyses in larger and longer clinical trials.

**Conclusions**

Building on the first 10 years of GLP-1 therapeutics, the use of GLP-1R agonists for the treatment of T2D and obesity is growing. The compelling demonstration that these agents, unlike insulin, reduce myocardial infarction, stroke, and cardiovascular death, with a favorable benefit/risk profile, will broaden their clinical use and further enhance interest in mechanisms of GLP-1 action. The delineation of long-term safety in GLP-1-treated subjects with obesity at high risk for cardiovascular disease remains an important challenge for expansion of GLP-1 therapeutics in non-diabetic populations. Development of next-generation improved GLP-1 therapies requires a more precise understanding of the cellular sites and mechanisms of action, and greater insight into control of GLP-1 receptor signaling in the CNS and peripheral tissues. Advances in understanding how nutrients, bacterial metabolites, and microbial populations control EEC GLP-1 secretion may enable the development of GLP-1 secretagogues that are more potent and better tolerated than metformin. Moreover, multiple new GLP-1-based therapeutic agents, be they small molecules, peptides, and larger hybrid proteins, continue to be developed. Hence, ongoing delineation of the mechanisms of GLP-1 action should continue to have immediate translational relevance for scientists and healthcare providers focused on the treatment of diabetes, obesity, and related complications.
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DECLARATION OF INTERESTS

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