The discovery, characterization, and clinical development of glucagon-like-peptide-1 (GLP-1) spans more than 30 years and includes contributions from multiple investigators, science recognized by the 2017 Harrington Award Prize for Innovation in Medicine. Herein, we provide perspectives on the historical events and key experimental findings establishing the biology of GLP-1 as an insulin-stimulating glucoregulatory hormone. Important attributes of GLP-1 action and enteroendocrine science are reviewed, with emphasis on mechanistic advances and clinical proof-of-concept studies. The discovery that GLP-2 promotes mucosal growth in the intestine is described, and key findings from both preclinical studies and the GLP-2 clinical development program for short bowel syndrome (SBS) are reviewed. Finally, we summarize recent progress in GLP biology, highlighting emerging concepts and scientific insights with translational relevance.
The discovery, characterization, and clinical development of glucagon-like peptides

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Discovery of GLP-1
Although GIP was isolated through classical peptide purification and protein sequencing methodology, the discovery of the GLP-1 sequence stemmed from the application of recombinant DNA approaches developed in the laboratories of Stanley Cohen, Paul Berg, and Herb Boyer in the early 1970s. This remarkable new technology allowed for a rapid and accurate prediction of the amino acid sequences of proteins by the decoding of the nucleotide sequences of cloned recombinant cDNA copies of messenger RNAs. The Habener lab utilized this technology to elucidate proglucagon amino acid sequences from cDNAs and genes isolated from anglerfish in the early 1980s (3–5) and the rat proglucagon cDNA and gene sequences followed shortly thereafter (refs. 6, 7, Figure 1, and Figure 2). Corresponding proglucagon sequences from hamster, bovine, and human were identified by Graeme Bell and others in the early 1980s (8–10). These sequences revealed that glucagon and related GLP sequences were encoded by larger protein precursors, termed preprohormones (Figure 2). The anglerfish proproglucagons (Figure 2A), isolated and characterized by Lund and Goodman (3–5), were interesting as there were two different cDNAs encoded by separate (nonallelic) genes and they each contained a glucagon-related sequence, in addition to glucagon. The two anglerfish glucagon-related peptides resembled GIP, a glucoincretin hormone released from the gut into the circulation during meals, subsequently shown by Dupre and Brown in 1973 to augment glucose-dependent insulin secretion (11). Unlike the two anglerfish proproglucagons, each of which harbored glucagon and a single glucagon-related peptide, the mammalian proproglucagons all contained glucagon and two additional glucagon-related peptides, designated GLP-1 and GLP-2 (Figure 2B). Notably, the corresponding amino acid sequences of the GLP-1s in the four mammalian species were identical (12), with conservation of sequence implying as yet unknown but potentially important biological actions of GLP-1.

Collectively, these findings further supported the evolving notion at the time that small peptide hormones are synthesized in the form of larger prohormones and that the final bioactive peptides are formed posttranslationally by selective enzymatic cleavages from the prohormones (Figure 2B). Earlier studies

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Structure-activity properties of GLP-1

Examination of the amino acid sequence of proglucagon initially presented a conundrum regarding the processes by which potentially bioactive GLP-1 peptides might be liberated from the prohormone (Figure 2B). In keeping with the rule that bioactive peptides are cleaved from prohormones at sites consisting of two basic amino acids (13), several investigators initially predicted that bioactive GLP-1 would be a peptide of 37 amino acids beginning with histidine and ending in glycine, GLP-1(1-37). However, further inspection of the prohormone sequences revealed a second single basic amino acid followed by histidine residing 6 amino acids carboxyl-proximal to the first histidine, predicting a GLP-1 peptide of 31 amino acids, GLP-1(7-37). Further, at the carboxy-terminal region of the putative GLP-1 peptide resides a sequence RGRR predicting a prohormone convertase–directed cleavage site followed by an amidation of the penultimate arginine by a peptidylglycine α-amidating monoxygenase (14) resulting in peptides of 36 and 30 amino acids, GLP-1(1-36)_amide and GLP-1(7-36)_amide.

The availability of the amino acid sequences of GLP-1 and GLP-2 obtained from the decoding of the nucleotide sequences based on the genetic code allowed for the chemical synthesis of the predicted peptides, examination of their biological activities, and preparation of peptide-specific antisera. Daniel Drucker joined the Habener laboratory in the summer of 1984, with the original intent of studying the molecular control of thyroid hormone biosynthesis. However, the thyroid group, led by Bill Chin, was decamping for the Brigham and Women’s Hospital, and Daniel was assigned to work on the proglucagon gene. To understand whether proglucagon might be processed to yield multiple proglucagon-derived peptides (PGDPs), including GLP-1 and GLP-2, Drucker transfected a proglucagon cDNA expression vector into fibroblasts, pituitary cells, and islet cells (15). Although minimal processing was observed in fibroblasts, immunoreactive GLP-1 and GLP-2 was detected by chromatography and radioimmunoassays in medium and extracts from transfected pituitary and islet cells (15). Application of similar chromatography and radioimmunoassay techniques in studies of rat (16), pig (17), and human (18) tissues revealed distinct profiles of PGDPs in pancreas and gut (Figure 3). Of note, in addition to glucagon, the pancreas contained a large peptide with immunoreactive determinants for both GLP-1 and GLP-2 (but not glucagon), consistent with incompletely cleaved proglucagon (major proglucagon fragment [MPGF]) (Figure 3, A and C, and ref. 19). In contrast to findings in pancreas, immunoreactive GLP-1 peptides detected in gut extracts consisted entirely of smaller peptides (refs. 15 and 16, and Figure 3, B and C). These findings indicated that the cleavage of proglucagon into small GLP-1-immunoreactive peptides was more efficient in the gut compared with pancreas. These observations were also consistent with the incretin concept in which, in response to oral nutrients, glucoincretin hormones such as GIP (and subsequently GLP-1) originate from the gut and not the pancreas.

Svetlana Mojsov in the Habener laboratory detected glycine-extended and arginine-amidated isoforms of GLP-1 as well as both the amino-terminally extended peptides GLP-1(1-37) and GLP-1(1-36)_amide and the amino-truncated peptides GLP-1(7-37) and GLP-1(7-36)_amide — in extracts of pancreas (Figure 3A). Nevertheless, the abundance of these peptides was much greater in the gut (20).

Bioactivities of the GLP-1 peptides

The earliest studies of the bioactivities of GLP-1(1-37) were indecisive. One study found that the 37 amino acid peptide activated adenyl cyclase in membranes prepared from rat pituitary and hypothalamus (21), whereas another study failed to detect any effects of the peptide on glucose and insulin in cortisone-treated rabbits.
The Journal of Clinical Investigation

Figure 2. Structure and processing of anglerfish and human proglucagon. Representation of the structures of proglucagon cDNAs from Anglerfish (A) and Human (B), with tissue-specific liberation of individual proglucagon-derived peptides in pancreas or intestine. PC1, prohormone convertase 1; PC2, prohormone convertase 2; MPGF, major proglucagon fragment. Arrows in A represent sites of cleave by prohormone convertase enzymes.

The demonstration that GLP-1 directly increased cAMP levels provided conditional evidence for the existence of a Gs protein-coupled receptor in β cells. In studies of insulin secretion using the isolated perfused pancreas, Mojsov and Weir demonstrated that GLP-1(7-37) and not GLP-1(1-37) stimulated insulin secretion at concentrations as low as 50 pM (ref. 24 and Figure 5, A–C). Likewise, as outlined below, Jens Holst and colleagues showed that luminal glucose stimulated GLP-1 secretion from the perfused intestine (Figure 5D), and doses from 500 pM to 5 nM GLP-1(7-36)amide stimulated insulin secretion in the perfused pig pancreas (Figure 5E and ref. 25). Thus, it turned out that a cleavage in proglucagon at the single basic amino acid, arginine, and not the double basic amino acids, generated the active GLP-1 peptides, GLP-1(7-37) and GLP-1(7-36)amide (Figure 5C). First in man studies reported in December 1987 by Kreymann and Bloom (26) rapidly established the insulinotrophic actions of GLP-1(7-36)amide in human subjects. Although numerous studies have demonstrated that the amino-terminally truncated forms of GLP-1, GLP-1(7-37), and GLP-1(7-36)amide are active glucoregulatory hormones, no compelling bioactivities for the extended forms, GLP-1(1-37) and GLP-1(1-36)amide, have yet been determined. Furthermore, no distinctive physiological activities have been attributable specifically to the amidated forms of GLP-1.

The view from Denmark

In Copenhagen, Jens Holst and colleagues were interested in the incretin effect and were studying the condition of postprandial reactive hypoglycemia after gastric surgery (27). This type of hypoglycemia was clearly hyperinsulinemic, yet the signal for insulin secretion was unknown. Looking for possible candidates, they were inspired by Lise Heding’s work on glucagon and her identification of the immunological differences between gut and pancreatic glucagon (28). Knowing that glucagon would stimulate insulin secretion, they were interested in the numerous cells in the gut that produce immunoreactive glucagon (29). Eventually, this work led to the identification of glicentin and oxyntomodulin (Figure 2B), which both contain the full glucagon sequence, explaining the immunoreactivity in the gut (30–32).

Having identified all of the molecular components of glicentin also in the pancreas (33), they proposed that glicentin represents at least part of a common gut and pancreatic glucagon precursor, which undergoes differential processing in the two tissues, a hypothesis subsequently confirmed through identification of the human proglucagon gene by Graeme Bell and colleagues (7). However, it was also clear that proglucagon was larger than glicentin, and the interest focused on peptides contained within the MPGF representing the remainder of proglucagon (minus glicentin) (34). The early work decoding the anglerfish proglucagon cDNA by Lund and Habener (3) followed by elucidation of the hamster proglucagon cDNA by Graeme Bell (9) supported a hypothesis that cleavage of MPGF might result in liberation of the GLPs. The Holst group quickly developed radioimmunoassays for GLP-1 and GLP-2 to test this hypothesis. To their excitement, they found that MPGF was indeed differentially processed in the gut, but not in the pancreas, to yield two GLPs (ref. 17 and Figure 3C). However, using the perfused pancreas preparation, they soon realized that neither of the two GLPs used in these
Importantly, this hormone amidated, corresponding to proglucagon (Figure 5C) and subsequently found to be representing proglucagon (aa 78-108) ring peptide was a truncated form of GLP-1 and they found that the naturally occurring hormone from porcine and human and gut extracts, to isolate the naturally occurring hormone secretion. They therefore decided studies had any effect on pancreatic hormone secretion. They therefore decided to isolate the naturally occurring hormone from porcine and human and gut extracts, and they found that the naturally occurring peptide was a truncated form of GLP-1 representing proglucagon (aa 78-108) (Figure 5C) and subsequently found to be amidated, corresponding to proglucagon 78-107 amide (35). Importantly, this hormone was potently insulinotropic (Figure 5E and ref. 25), so they had also described a new wonder whether GLP-1 was more interesting than the already known incretin GIP, which exhibited a diminished effect on insulin secretion in patients with T2D (36). They soon found, using the perfused pancreas, that GLP-1—in contrast to GIP—also powerfully inhibits glucagon secretion (37). Eventually, they demonstrated that during infusions of physiological amounts of GLP-1 into humans, insulin secretion would be stimulated and glucagon secretion inhibited, resulting in a decrease in hepatic glucose production (38). However, the effect was self-limiting, with the insulin-stimulating activity attenuated as plasma glucose levels started to fall, limiting the fall to 0.5–1 mmol/l.

At that time, the Copenhagen group realized that GLP-1 was extremely interesting and, in further studies, demonstrated that it strongly inhibited gastric motility and gastric and pancreatic exocrine secretion (39), consistent with an important role for this hormone as a regulator of upper gastrointestinal function. They also demonstrated that infusions of GLP-1 in humans inhibited appetite and food intake, actions subsequently exploited in the clinic to treat obesity (40). In studies published in 1993 by Michael Nauck and colleagues in Göttingen, i.v. infusion of GLP-1 completely normalized severely elevated fasting glucose concentrations in patients with long-standing T2D as a consequence of the actions of GLP-1 to stimulate insulin and inhibit glucagon secretion (41). Although GLP-1 clearly had therapeutic potential, s.c. injections of GLP-1 were disappointingly ineffective (42). The explanation was an extremely rapid metabolism and inactivation of GLP-1. With inspiration from Rolf Mentlein in Kiel, Holst and Deacon showed that the GLP-1 molecule was cleaved by the enzyme dipeptidyl-peptidase-4 (DPP-4) in vivo and that inhibitors of this enzyme could completely protect the molecule (43). In fact, the circulating half-life of GLP-1 was only 1.5–2 minutes in human subjects with diabetes, and they proposed that inhibitors of DPP-4 could maintain higher levels of intact active endogenous GLP-1 for therapeutic purposes (44). Subsequent studies soon demonstrated that DPP-4-resistant GLP-1 analogues were longer-acting than native GLP-1 (45). Furthermore, inhibitors of DPP-4 completely prevented the breakdown of GLP-1 in the circulation and amplified the insulinotropic actions of GLP-1 (46). This exciting development, presented in a Perspectives article in Diabetes in 1998 (47), was soon followed by the development of clinically useful inhibitors, first vildagliptin and subsequently sitagliptin.

It remained to be understood whether GLP-1 receptor agonists would actually be useful for clinical diabetes therapy or whether tachyphylaxis would develop upon chronic administration. The group in Copenhagen administered synthetic GLP-1 by constant s.c. infusion for 6 weeks to a group of individuals with long-standing T2D (48). Fortunately, no tachyphylaxis was observed; GLP-1 therapy reduced fasting and mean plasma glucose by 4.3 and 5.5 mmol/l; glycated hemoglobin by 1.3%; and body weight by 2 kg. Moreover, insulin sensitivity and β cell function, assessed by clamp studies, greatly improved. Importantly, no limiting side effects were recorded (48), providing proof of concept in 2002 for GLP-1 therapy in subjects with T2D. It was now clear that GLP-1–based therapies had tremendous potential.

The Toronto perspective: the physiology of GLPs and discovery of GLP-2

Building on the availability of cloned proglucagon gene sequences in the Habener lab (6), Daniel Drucker and Jacques Philippe pursued the analysis of the molecular control of islet α cell proglucagon gene expression in the mid 1980s (49, 50). Upon returning to Toronto in 1987, Drucker extended these studies to examine proglucagon gene expression in the intestine

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**Figure 3. Processing of proglucagon and glucagon-like peptides in the pancreas and intestine.**

Detection of immunoreactive forms of GLP-1 in extracts from rat pancreas (A) and intestine (B) as adapted from ref. 16. Characterization of proglucagon-derived peptide immunoreactivity secreted from perfused pig pancreas and intestine (C) using peptide-specific antisera reveal tissue-specific posttranslational processing of the PGDPs, as outlined in ref. 17.
and CNS. He isolated human neonatal brainstem cDNAs encoding proglucagon, which exhibited an identical sequence to that described for islet proglucagon (51). Molecular cloning of the rat intestinal proglucagon cDNA similarly revealed an open reading frame identical to that elucidated for the rat pancreatic islet proglucagon cDNA (52). Moreover, in studies carried out in collaboration with Patricia Brubaker, forskolin, cholera toxin, and dibutyryl cyclic AMP increased the synthesis and secretion of intestinal PGDPs from primary cultures of rat intestinal cells (52). At the time, there were no differentiated GLP-1-secreting enteroendocrine cell lines suitable for studies of intestinal proglucagon gene expression. Accordingly, Ying Lee, a fellow in the Drucker laboratory, generated a transgenic mouse expressing the SV40 T antigen cDNA under the control of the proglucagon gene promoter. This transgenic mouse reproducibly developed GLP-1-secreting enteroendocrine tumors of the colon (53), enabling isolation of the first differentiated GLP-1-producing enteroendocrine L cell line in 1992, designated GLUTag cells. GLUTag cells were easily propagated ex vivo; secreted immunoreactive GLP-1, glicentin, oxyntomodulin, and GLP-2 in response to cyclic AMP analogues (54, 55); and resembled primary cultures of nonimmortalized gut endocrine cells in regard to their response to a battery of secretagogues (56).

Two unexpected observations were made during isolation of GLUTag cells. First, mice harboring s.c. GLUTag cell tumors exhibited a marked reduction of pancreatic islet α cell mass (57). Second, mice with s.c. GLUTag, InR1-G9, or RIN1056A glucagon-producing tumors all exhibited marked enlargement of the small bowel. These findings led Drucker to reinvestigate the link between glucagon-producing tumors and gut growth, first reported in a human subject studied at the Hammersmith hospital in 1970 by Dowling and colleagues in London (58). A series of simple experiments from the Drucker lab published in 1996 identified GLP-2 as the PGDP with the most potent intestino-trophic activity in mice (58). Remarkably, although immunoreactive GLP-2 had been detected in intestinal extracts of various species (refs. 16, 59, and Figure 3), no previous biological activity had yet been identified for GLP-2 in vivo.

The actions of GLP-2 to stimulate small bowel growth were rapid, detectable within days, and associated with increased crypt cell proliferation (58). Surprisingly, when similar doses of GLP-2 were administered to rats, intestinal growth was not significantly increased, although an increase in crypt plus villus height was observed (60). With hindsight, these findings reflected the importance of DPP-4 for the degradation of GLP-2, more evident in rats than in mice. Subsequent studies in the Drucker lab demonstrated that native GLP-2 robustly increased small bowel growth in Fischer 344 rats with an inactivating mutation in the Dpp4 gene (60). Furthermore, a GLP-2 analogue with a single amino acid substitution [Gly2]-GLP-2 exhibited substantial resistance to DPP-4 cleavage and robust intestinotrophic activity in normal rats in vivo (60). Hence, the importance of DPP4 for cleavage of both GLP-1 and GLP-2 became evident very early in the study of the GLPs.

The identification of GLP-2 as an intestinal growth factor spurred a series of experiments examining the actions of GLP-2 in the context of experimental gut injury. GLP-2 administration was generally associated with preservation of gut mucosal structure and function in the setting of chemical, radiation, or surgically induced intestinal injury in preclinical studies (61–63). Notably, GLP-2 also rapidly increased nutrient absorption in normal rodents (64) and in animals with surgical gut resection mimicking SBS (65). Excitingly, the findings in animals were soon extended to humans with SBS. In a pilot study carried out around 2000–2001, Jeppesen and colleagues demonstrated that native GLP-2 administered for 35 days increased nutrient absorption, energy absorption, and weight gain in human subjects with SBS (66). These findings, namely expansion of intestinal mucosal surface area coupled with...
enhanced nutrient absorption (67), together with substantial preclinical data, supported the initiation of a drug development program to test the efficacy of GLP-2 in human subjects with parenteral nutrition-dependent (PN-dependent) SBS. Clinical studies were initiated with h[Gly2]-GLP-2, a degradation-resistant GLP-2 analogue discovered in the Drucker lab (60) and subsequently designated teduglutide. Once daily teduglutide administration in human subjects with PN-dependent SBS resulted in increased fluid and energy absorption, while reducing the requirements for PN, in two separate placebo-controlled Phase 3 studies (67, 68). Teduglutide was approved for chronic therapy of subjects with PN-dependent SBS in the US in December 2012 (67).

The physiology of GLP-1 action
Complementary studies in the Drucker lab were focused on defining the physiological importance of endogenous GLP-1 through generation of Glp1r<sup>−/−</sup> mice. These animals generated by Louise Scroccchi exhibited impaired oral glucose tolerance and reduced insulin levels after glucose stimulation, demonstrating the critical role of endogenous GLP-1 as an incretin hormone in 1996 (69). Unexpectedly, Glp1r<sup>−/−</sup> mice also exhibited fasting hyperglycemia, and impaired i.p. glucose tolerance, establishing the importance of the GLP-1 for β cell function beyond its original description as an incretin. Moreover, GLP-1R-deficient β cells exhibit enhanced sensitivity to apoptotic injury, whereas pharmacological activation of GLP-1R signaling attenuated β cell death in mice in vivo, as well as in cultures of purified rat β cells studied ex vivo (70). The importance of GLP-1R signaling for the response to cellular stress was highlighted by findings that activation of GLP-1R signaling attenuated the development of ER stress in β cells by enhancing ATF-4 induction and accelerating recovery from translational repression via augmentation of ER stress–stimulated ATF-4 translation (71). Collectively, these findings explain how GLP-1, despite acting to simultaneously enhance insulin biosynthesis and secretion, maintains functional β cell mass through attenuation of ER stress.

The actions of GLP-1 to stimulate insulin and inhibit glucagon secretion, together with its inhibitory effects on food intake and gastric emptying, spurred considerable effort in development of GLP-1-based therapeutics. Remarkably, the first GLP-1R agonist approved for clinical use was exenatide (synthetic exendin-4), a peptide originally isolated from <i>Heloderma suspectum</i> lizard venom by John Eng in 1992 (72). Subsequent molecular cloning studies published by the Drucker laboratory in 1997 demonstrated that the lizard genome contains two distinct proglucagon gene sequences encoding GLP-1 and a separate proexendin gene, largely restricted in its expression to the salivary gland (73). The original clinical development program for exendin-4 utilized twice-daily injections of the unmodified peptide. Pivotal studies in subjects with T2D ultimately resulted in the approval of twice-daily exenatide, the first GLP-1R agonist in 2005 (Figure 1). These efforts spurred the development of a once-weekly form of exenatide in a microsphere suspension, ultimately the first once-weekly therapy approved for diabetes in 2012 (Figure
The Journal of Clinical Investigation

1. Exenatide once weekly was more effective than the twice-daily preparation, with greater reduction of glycemia and comparable control of body weight (74). Today, multiple GLP-1R agonists (small peptides and high molecular weight proteins) are approved for the treatment of T2D, and a single drug, liraglutide, was approved for the treatment of obesity in 2014 (Figure 1).

The widespread distribution of GLP-1 receptors in extrapancreatic tissues stimulated considerable research into the nonglycemic actions of GLP-1. Indeed, we now understand that GLP-1 controls inflammation (75), reduces experimental kidney injury (76), and like GLP-2, acts as a potent intestinal growth factor through mechanisms including stimulation of crypt fission (ref. 77 and Figure 6). Among the actions of GLP-1 that have engendered the most interest are its effects in the cardiovascular system. Native GLP-1 increases flow-mediated vasodilation, enhances heart rate (HR) and cardiac output, and is cardioprotective in preclinical studies, most notably in animals with ischemic cardiac injury (78). Although degradation-resistant GLP-1R agonists have minimal effects on blood vessels (79), they increase HR and reduce cardiac inflammation and infarct size in animals with experimental myocardial infarction (75, 80). Understanding the mechanisms through which GLP-1 exerts cardioprotection is challenging, since the GLP-1R is largely expressed in atrial and not ventricular cardiomyocytes (81). Furthermore, the GLP-1R-dependent control of inflammation in heart and blood vessels is complex, as the predominant site of GLP-1R expression in the (murine) immune system is within the intestinal intraepithelial lymphocyte (82).

Several cardiovascular outcome studies have documented the safety of GLP-1R agonists in human subjects with T2D and preexisting cardiovascular disease (83, 84). Excitingly, reduction of major adverse cardiovascular events was reported in human subjects treated with long-acting liraglutide and semaglutide (85, 86). These findings, revealed more than a decade after the first approval of twice-daily exenatide in 2005, have rekindled enthusiasm for the use of GLP-1R agonists, alone or in combination, in the treatment of T2D and obesity, most compellingly in subjects at high risk for cardiovascular disease. Today, only two drug classes used for treatment of T2D, the GLP-1R agonists and SGLT-2 inhibitors, produce weight loss, effective reduction of A1c, a low incidence of hypoglycemia, and reductions in rates of major adverse cardiovascular events and cardiovascular death (75, 87).

**Challenges in studies of GLP-1 and in development of GLP-1R agonists**

In the era of rapid daily progress in biomedical science, it is surprising that it took almost 20 years from identification of GLP-1 to successful approval of the world’s first clinically utilized GLP-1R agonist. The rapid renal clearance of many small peptides like GLP-1, together with its inactivation by DPP4, required development of longer-acting peptides with greater stability and enhanced resistance to DPP-4. The development of nausea and vomiting, encountered in every GLP-1 development program, often limited dose escalation and likely delayed introduction of GLP-1R agonists, utilized at higher doses, with greater efficacy. Indeed, it has now become apparent that rates of nausea and vomiting can be greatly reduced with much slower titration regimens, as has been the case in development of titratable GLP-1–insulin combination therapies. Indeed, the development of liraglutide for obesity using a 3-mg daily dosing regimen further illustrates that much greater tolerability to GLP-1 can be achieved using progressive stepwise titration regimens.

Studies of GLP-1 synthesis, secretion, and action have also been hampered by lack of suitable reagents and experimental challenges. Circulating GLP-1 levels are very low, and measurement of GLP-1 in the circulation is technically challenging; indeed, not all commercial assays produce consistent or accurate results, and many exhibit problematic sensitivity and specificity (88). Similar challenges surround the quantitative detection of GLP-2 (89), which is challenging to measure with available commercial assays. Supplementary problems have been identified with the antisera used to detect the GLP-1 and GLP-2 receptors, with problematic antisera utilized in dozens of publications. The majority of commercially available antisera used to detect GLP-1R and GLP-2R exhibit impaired sensitivity and specificity (67, 90), challenging published conclusions surrounding receptor expression and localization.

Elucidation of the physiological roles of endogenous GLP-1 and GLP-2 in humans has been limited in part due to limitations associated with the available antagonists. Exendin(9-39) has been widely used as a GLP-1 receptor antagonist, yet only in short-term human studies. It seems likely that more selective antagonists with optimized pharmacokinetic profiles would advance our understanding of the importance of endogenous GLP-1 action. Similarly, GLP-2(3-33), often employed as a GLP-2 receptor antagonist, is both a weak antagonist and a partial agonist, and little data is available to date that illuminates the physiological importance of endogenous GLP-2 in human subjects.

Analysis of proglucagon synthesis and secretion in rare enteroendocrine L cell populations in the small and large bowel is also technically challenging. Transgenic technologies have enabled isolation of primary L cell populations suitable for secretion and electrophysiological analyses (91), and stem cell–derived gut organoids, including those of human origin, herald considerable potential for generation of diverse enteroendocrine cell populations from different sites along the gastrointestinal tract (92). Remarkably, despite the theoretical potential of enhancing L cell secretion for the treatment of metabolic disorders (1), there has been scant progress in clinical development of GLP-1 secretagogues exhibiting sustained efficacy in both preclinical and clinical studies.

**GLPs: current use and future considerations**

What does the second decade of GLP-1–based therapies portend? Currently, multiple GLP-1R agonists are indicated for the treatment of T2D, and liraglutide is also approved for obesity; however, indications such as nonalcoholic steatohepatitis, Parkinson’s disease, and Alzheimer’s disease are being explored. Indeed, several randomized controlled clinical trials have demonstrated significant clinical improvement in human subjects with Parkinson’s disease (93). Despite the appeal of current GLP-1R agonists for the treatment of T2D, market penetration for many GLP-1R agonists remains disappointing, raising questions about the potential clinical
appeal, expense, and commercial success of newer formulations. Ongoing attempts to improve the therapeutic efficacy of GLP-1R agonists, exemplified by once-weekly semaglutide for diabetes or once-daily semaglutide for obesity, are under active investigation. ITCA-650 is a long-acting version of exendin enabling sustained peptide delivery with implantation of a small s.c. osmotic minipump every 3–6 months. Semaglutide is also being studied as a once-daily oral formulation (94), potentially extending the appeal of GLP-1R agonists to a subset of patients that may traditionally eschew injectable therapies.

Considerable effort is being expended to combine the established efficacy of GLP-1R agonism with additional therapies, enabling greater therapeutic efficacy delivered through a single injection. Two insulin–GLP-1 combination therapies have been approved, lixisenatide-insulin glargine and liraglutide-insulin degludec, enabling enhanced control of glucose without weight gain. These fixed-ratio combination therapies represent attractive options for some patients; however, the current expense of and unfamiliarity with these new drugs may limit their appeal in some markets. Whether longer-acting versions, perhaps weekly insulin–GLP-1 combinations, are feasible and efficacious requires additional investigation. Moreover, GLP-1 may yet emerge as an ideal partner for peptide combinations that enable greater weight loss. Unimolecular coagonists and triagonists exhibit substantial preclinical efficacy (95) and are under active clinical investigation. Nevertheless, it may be challenging to predict the ideal ratio(s) of 2–3 peptide epitopes combined in a single therapy that produces enhanced control of metabolic disease, while maintaining an acceptable safety profile (96).

The observations that GLP-1R signaling in β cells is essential for control of insulin secretion, together with data suggesting that exendin(9-39) may attenuate insulin secretion through reduction of β cell cyclic AMP (97), has supported investigational use of exendin(9-39) for the treatment of severe hyperinsulinemic hypoglycemia. Preliminary short-term data supports the feasibility of using exendin(9-39) in the therapy of bariatric surgery-associated hypoglycemia (98). Whether exendin(9-39) will exhibit sustained efficacy and safety and be similarly useful for treatment of genetic forms of hyperinsulinemic hypoglycemia requires further study. The elevated levels of GLP-1 detected in most subjects after bariatric surgery are widely viewed as contributing to the enhanced β cell function and reduced appetite evident in these subjects (99). Nevertheless, insulin sensitivity is also rapidly improved after bariatric surgery, and some studies suggest that GLP-1 (9-36) and its degradation products, which circulate at elevated levels after bariatric surgery, may enhance insulin sensitivity in peripheral tissues. Further research into the potential contribution of these PGDPs in the context of bariatric surgery may illuminate the role(s) of these peptides in this unique clinical context.

The cardiovascular safety and benefits (Figure 6) associated with the use of GLP-1R agonists (75) raises further clinically relevant questions. Might nondiabetic subjects with obesity expect comparable cardioprotection with sustained GLP-1R agonism? Alternatively, can we identify the cardioprotective mechanisms of GLP-1 action and extend the therapeutic possibilities of GLP-1 therapy to nondiabetic subjects at high risk for cardiovascular disease? Similarly, will human subjects with prediabetes, or those at low risk for cardiovascular disease, be suitable candidates for GLP-1R agonism without long-term data establishing durability and reduction of complications? Additional clinical trials will be required to answer these questions.

The translational success of GLP-1 from bench to bedside reflects important contributions from dozens of colleagues studying GLP-1 over 3 decades, to whom we extend our thanks and appreciation. The history of GLP-1 science provides instructive lessons relevant to modern-day experimentation and drug development. First, the field was built slowly with a series of nonflashy yet solid studies examining the physiology, pharmacology, and pleiotropic actions of native GLP-1 and multiple GLP-1R agonists. From the onset, the availability of the native peptide enabled rapid proof of concept in human subjects, quickly validating discoveries made in preclinical studies. Most of the early, and the majority of subsequent, GLP-1 studies were published in tradition-
al physiology or endocrinology subspecialty journals and might be described as “descriptive” or “incremental” by many colleagues. The elusive distribution of GLP-1 receptors, often expressed in small numbers of endocrine cells, CNS or enteric neurons, or rare immune cells, makes it challenging to decisively identify cells, pathways, and reductionist mechanisms linking receptor engagement to physiological actions and therapeutic efficacy. Nevertheless, the multiple actions of GLP-1, described in dozens of laboratories, have largely been reproducible, and occasional questionable findings and controversies have been extensively studied and subsequently discarded by more careful investigation (100). Taken together, the story of the discovery and characterization of the GLPs highlights how old-fashioned biochemistry, physiology, molecular biology, and traditional analyses of hormone action provides a firm scientific foundation for the development of multiple novel therapeutics for the treatment of obesity, diabetes, and intestinal disorders.

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