Regulation of Glucose Homeostasis by GLP-1

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Abstract

Glucagon-like peptide-1(7–36)amide (GLP-1) is a secreted peptide that acts as a key determinant of blood glucose homeostasis by virtue of its abilities to slow gastric emptying, to enhance pancreatic insulin secretion, and to suppress pancreatic glucagon secretion. GLP-1 is secreted from L cells of the gastrointestinal mucosa in response to a meal, and the blood glucose-lowering action of GLP-1 is terminated due to its enzymatic degradation by dipeptidyl-peptidase-IV (DPP-IV). Released GLP-1 activates enteric and autonomic reflexes while also circulating as an incretin hormone to control endocrine pancreas function. The GLP-1 receptor (GLP-1R) is a G protein-coupled receptor that is activated directly or indirectly by blood glucose-lowering agents currently in use for the treatment of type 2 diabetes mellitus (T2DM). These therapeutic

agents include GLP-1R agonists (exenatide, liraglutide, lixisenatide, albiglutide, dulaglutide, and langlenatide) and DPP-IV inhibitors (sitagliptin, vildagliptin, saxagliptin, linagliptin, and alogliptin). Investigational agents for use in the treatment of T2DM include GPR119 and GPR40 receptor agonists that stimulate the release of GLP-1 from L cells. Summarized here is the role of GLP-1 to control blood glucose homeostasis, with special emphasis on the advantages and limitations of GLP-1-based therapeutics.

LIST OF ABBREVIATIONS

ACh acetylcholine
ANF atrial natriuretic factor
CICR Ca^{2+}-induced Ca^{2+} release
CNS central nervous system
CBP CREB-binding protein
CRE cAMP response element
CREB cAMP response element-binding protein
CRTC CREB-regulated transcriptional coregulator
DPP-IV dipeptidyl-peptidase-IV
Epac cAMP-regulated guanine nucleotide exchange factor
ER extended release
GLP-1R GLP-1 receptor
GPCR G protein–coupled receptor
GSIS glucose-stimulated insulin secretion
HFD high-fat diet
IRS-2 insulin receptor substrate 2
K_{ATP} ATP-sensitive K^+ channel
KO knockout
MEN2 multiple endocrine neoplasia syndrome type 2
NDM neonatal diabetes mellitus
NPY neuropeptide tyrosine
PACAP pituitary adenylyl cyclase-activating peptide
PG proglucagon
PIP_2 phosphatidylinositol 4,5-bisphosphate
PKA protein kinase A
PTH parathyroid hormone
PYY peptide tyrosine tyrosine
RCT randomized control trial
RYGB Roux-en-Y gastric bypass
SNARE soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor
SUR1 sulfonylurea receptor type 1
T2DM type 2 diabetes mellitus
TMAC transmembrane adenylyl cyclase
TxNIP thioredoxin-interacting protein
VDCC voltage-dependent Ca^{2+} channel
WT wild type

1. INTRODUCTION

Systemic blood glucose homeostasis in humans is under the control of glucagon–like peptide-1(7–36)amide (GLP-1), a peptide secreted from intestinal enteroendocrine L cells in response to a meal.[1] L cells are located within the gastrointestinal mucosa and they act as nutrient sensors to release GLP-1 in response to luminal sugars, amino acids, and fatty acids.[2] Released GLP-1 acts locally within the intestinal wall to activate enteroenteric reflexes important to the control of gastric motility, thereby slowing gastric emptying.[3] Simultaneously, released GLP-1 activates vagal sensory nerve terminals that innervate the intestinal wall, and in this manner, GLP-1 initiates vagal–vagal autonomic reflexes that control endocrine pancreas function.[4] Circulating GLP-1 also acts as a hormone at the islets of Langerhans in the endocrine pancreas to stimulate the release of insulin, while suppressing the release of glucagon.[5] During the postprandial phase of blood glucose control, these immediate and multiple actions of GLP-1 act in concert to lower levels of blood glucose.

Clinical studies demonstrate that the blood glucose-lowering action of GLP-1 is itself glucose-dependent.[6–8] More specifically, GLP-1 reduces levels of blood glucose only when concentrations of blood glucose are elevated above fasting levels, as is the case after a meal. As the postprandial blood glucose levels fall in response to GLP-1, the blood glucose-lowering action of GLP-1 is self-terminating. This remarkable glucose-dependent property of GLP-1 action results in a situation in which intravenously administered GLP-1 fails to reduce levels of blood glucose below fasting levels.[6–8] Since administered GLP-1 does not produce hypoglycemia, these clinical findings have led to the use of GLP-1 receptor (GLP-1R) agonists as a new class of blood glucose-lowering agents for use in the treatment of type 2 diabetes mellitus (T2DM).[9,10]
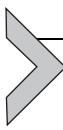
2. GLP-1 BIOSYNTHESIS, SECRETION, AND DEGRADATION

Proglucagon gene expression in the intestinal L cells generates proglucagon (PG) that is processed by prohormone convertases (PC1/3) to liberate the GLP-1(1–37) peptide precursor.[11,12] Endopeptidase-catalyzed cleavage of GLP-1(1–37) generates two peptides with insulin secretagogue properties. These are GLP-1(7–37) that is processed by

amidating enzyme to generate GLP-1(7–36)amide.[13–15] Although glucagon gene expression also generates PG in islet α-cells, it was thought that α-cells fail to synthesize GLP-1 due to the fact that these endocrine cells contain a prohormone convertase (PC2) that preferentially processes PG to glucagon.[16] However, it is now apparent that endocrine cell "plasticity" exists within the islets such that α-cells synthesize GLP-1 under stressful or pathophysiological conditions including T2DM.[17] Thus, it seems likely that GLP-1 can also act as an intraislet paracrine hormone but in a context-dependent manner.

GLP-1 is packaged in secretory granules and it is released from intestinal L cells by exocytosis in response to an elevation of cytosolic Ca^{2+} and cAMP.[18] In this regard, it is important to note that L cells are electrically excitable and that glucose transporter-mediated uptake of glucose by L cells is Na^+-dependent and electrogenic. Thus, L cells respond to orally administered glucose by generating action potentials that trigger depolarization-induced Ca^{2+} influx through voltage-dependent Ca^{2+} channels (VDCCs).[19] Ca^{2+} mobilized from intracellular Ca^{2+} stores is also a stimulus for GLP-1 secretion, and this Ca^{2+} mobilization is initiated by the binding of fatty acids to a receptor designated as GPR40 located on L cells.[20] GLP-1 secretion is also stimulated by fatty acid amides (oleoylethanolamide) and monoacylglycerols (2-oleoyl glycerol) that activate GPR119, a receptor that is positively coupled to cAMP production in L cells.[20]

GLP-1 released from L cells acts locally within the hepato–portal circulation to activate the GLP-1R located on vagal sensory neurons. These neurons constitute the hepato–portal glucose sensor that communicates with brainstem neurons in order to regulate whole-body metabolism.[4] Highest concentrations of released GLP-1 are found immediately within the hepato–portal circulation since GLP-1 is quickly metabolized to GLP-1 (9–36)amide by dipeptidyl-peptidase-IV (DPP-IV).[21] DPP-IV exists in two forms—a 766-amino-acid transmembrane protein and a smaller soluble form found in the plasma.[22] Both forms of DPP-IV have enzymatic activity, and the preferred substrates are peptides such as GLP-1 that contain penultimate N-terminus alanine or proline residues. The half-life for intravenously administered GLP-1 is less than 5 min owing to its rapid degradation by DPP-IV.[23] However, picomolar concentrations of GLP-1 activate the GLP-1R,[24] and concentrations of circulating GLP-1 are sufficiently high to allow it to activate the GLP-1R on islet β-cells.

Exenatide is a DPP-IV-resistant peptide that shares structural homology with GLP-1.[25] It is the synthetic form of exendin-4, a peptide isolated from

the Gila monster lizard *Heloderma*.[26] Exenatide is a GLP-1R agonist in humans, and it has a half-life of ca. 20 min when it is administered by the intravenous route.[9] Patients with T2DM receive exenatide by subcutaneous injection, and circulating exenatide produces a blood glucose-lowering effect since it directly activates the GLP-1R.[9,10] Orally administrable DPP-IV inhibitors such as sitagliptin and vildagliptin are also useful for the treatment of T2DM.[27,28] By slowing degradation of GLP-1, these DPP-IV inhibitors enhance the action of endogenous GLP-1 to activate vagal sensory neurons that compose the hepato–portal glucose sensor.[29] This intestinal action of DPP-IV inhibitors is of significance since it is the predominant means by which low concentrations of DPP-IV inhibitors exert a blood glucose-lowering effect.[29]

3. INSULINOTROPIC AND GROWTH FACTOR PROPERTIES OF GLP-1

Soon after the cloning of the anglerfish *PG* gene by the Habener laboratory in 1982,[11] the sequence of a hamster *PG* cDNA was reported by Bell and coworkers.[12] Bioinformatics analysis of the hamster *PG* cDNA revealed that it encoded GLP-1(1–37) derived from exon 4 of the *PG* gene.[12] Subsequently, it was demonstrated by the laboratories of Creutzfeldt,[30] Holst,[31] Habener,[13] Weir,[32] and Bloom[15] that pancreatic insulin secretion could be stimulated in a glucose–dependent manner by derivates of GLP-1(1–37) that included GLP-1(1–36), GLP-1(7–36)amide, and GLP-1(7–37). Cloning of the 463-amino-acid rat pancreatic islet GLP-1R by Thorens in 1992 revealed that nanomolar high-affinity agonist binding to the recombinant GLP-1R was measurable using a truncated metabolite of GLP-1(1–37) that corresponded to GLP-1(7–36)amide.[33]

It is now recognized that GLP-1(7–36)amide is the predominant bioactive GLP-1 present in human serum. It has an MW of 3298 Da and is composed of 30-amino-acid residues with the sequence HAEGTFTSDVSSYLEGQAAKEFIAWLVKGR–NH$_2$.[34] This truncated and fully bioactive GLP-1 binds to the human GLP-1R[35,36] expressed on islet β-cells in order to potentiate glucose-stimulated insulin secretion *in vivo* (GSIS).[6–8] Importantly, the insulin secretagogue action of GLP-1 is accompanied by its ability to stimulate insulin gene transcription, insulin mRNA translation, and proinsulin biosynthesis in β-cells.[37–40] Potentially just as important, GLP-1 also acts as a β-cell growth factor so that in mice, it stimulates β-cell proliferation while also exerting a prosurvival

(antiapoptosis) action to protect against β–cell death.[41–45] Thus, there is great interest to determine whether such preclinical findings concerning GLP-1 are applicable to T2DM patients treated with GLP-1R agonists.

In addition to improving β–cell insulin secretion and insulin biosynthesis, a modern treatment for T2DM might also be capable of increasing β–cell "mass," either by stimulating β–cell replication or by slowing β–cell death. Furthermore, for certain forms of T2DM, it might be useful to identify GLP-1R agonists that selectively "bias" GLP-1R signal transduction in order to achieve a desired therapeutic outcome. In this regard, attention has recently focused on whether it might be possible to synthesize GLP-1R agonists that allosterically induce a GLP-1R conformation that enhances receptor coupling to select downstream effectors such as GTP-binding proteins, mitogen-activated protein kinases, c-src kinase, and β-arrestin.[46] In this context, it is valuable to summarize what is currently known concerning signal transduction pathways activated by the GLP-1R.

The GLP-1R is a G protein–coupled receptor (GPCR) that is a member of the secretin receptor–like family of seven transmembrane-spanning domain proteins. These group B receptors include GPCRs that selectively bind secretin, glucagon, glucose-dependent insulinotropic peptide (GIP), vasoactive intestinal peptide (VIP), and pituitary adenylyl cyclase-activating peptide (PACAP).[47] Heterotrimeric G_S GTP-binding proteins are activated in response to agonist binding to the GLP-1R, and they couple GLP-1R agonist occupancy to the stimulation of transmembrane adenylyl cyclases (TMACs). In β–cells, TMACs catalyze conversion of ATP to cytosolic cAMP, a second messenger that activates either protein kinase A (PKA) or the cAMP-regulated guanine nucleotide exchange factor designated as Epac2.[48] PKA is a serine/threonine protein kinase that phosphorylates key substrate proteins of the β–cell stimulus-secretion coupling and gene regulatory networks. In contrast, Epac2 acts via Rap1 GTPase to activate a novel phospholipase C-epsilon (PLCε) that specifically hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2).[49]

In β–cells, there exists a PKA–mediated action of GLP-1R agonists to phosphorylate Snapin.[50] Snapin is a protein that associates with SNAP-25, a component of the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex that couples an increase of cytosolic Ca^{2+} concentration to insulin secretory granule exocytosis. By phosphorylating Snapin, GLP-1R agonists potentiate GSIS, thus lowing levels of blood glucose.[50] An additional PKA-mediated action of GLP-1R agonists controls β–cell gene expression by phosphorylating CREB, a *cAMP response*

*e*lement-*b*inding protein.[51] Activated CREB binds cAMP response elements (CREs) located in 5′ gene promoters, and it couples PKA activation to the stimulation of gene transcription. This CREB-mediated action of PKA is facilitated by CREB coactivators such as p300, CREB-binding protein (CBP), and CRTC (CREB-regulated transcriptional coregulator).[52] Numerous CREB-regulated genes are under the control of GLP-1R agonists in β-cells, as demonstrated for CREB-dependent stimulation of insulin gene expression and insulin receptor substrate-2 (IRS-2) gene expression.[51–53]

Particularly interesting is the role of Epac2 in the control of pancreatic insulin secretion. Studies of mice with a knockout (KO) of Epac2 gene expression demonstrate that Epac2 mediates the action of GLP-1R agonist exendin-4 to potentiate the first phase kinetic component of GSIS.[54] Since first phase GSIS is defective in patients with T2DM,[55] and since exendin-4 restores first phase GSIS under conditions of T2DM,[56] it appears that Epac2 activation might play an especially important role when considering how GLP-1-based therapeutic agents restore normoglycemia in patients with T2DM. Interestingly, Epac proteins may also play a role in the central nervous system (CNS) control of glucose homeostasis and energy expenditure since their activation appears to reduce leptin sensitivity in neural networks controlling feeding behavior.[57]

When considering how GLP-1R agonists act as β-cell trophic factors to increase β-cell mass, studies of β-cell lines or neonatal mouse β-cells indicate that it is PKA that mediates transcriptional induction of cyclin D1 expression by GLP-1 in order to stimulate β-cell proliferation.[58] Interestingly, a proliferative action of GLP-1 also results from PKA-mediated phosphorylation of β-catenin, thereby indicating that the β-cell cAMP–PKA signaling branch exhibits signal transduction cross talk with a noncanonical Wnt signaling pathway that uses the transcription factor TCF7L2 to control gene expression.[59] An additional surprising finding is that a truncated GLP-1 designated as GLP-1(28–36)amide stimulates cAMP production in β-cells, thereby activating the β-catenin/TCF7L2 signaling pathway.[60] Furthermore, GLP-1(28–36)amide protects against β-cell glucotoxicity by improving mitochondrial function.[61] GLP-1(28–36)amide is a cell-penetrating peptide that does not exert its effects by binding to the GLP-1R, but instead acts intracellularly.[61] Thus, it is not clear how GLP-1(28–36)amide stimulates cAMP production.

PKA-mediated induction of IRS-2 expression also promotes β-cell growth in response to GLP-1,[53,62] and PKA mediates the action of

GLP-1 to promote translocation of transcription factor PDX-1 to the nucleus, thereby enhancing the differentiated state of β-cells.[63] In contrast, Epac2 participates in the protection of β-cells from cytotoxicity induced by reactive oxygen species (ROS).[64,65] Redox control in β-cells is under the control of thioredoxin (TxN), and TxNIPs are thioredoxin-interacting proteins that downregulate the ROS buffering capacity of thioredoxin.[66] Thus, it is significant that GLP-1 acts via Epac2 to suppress TxNIP expression and to enhance ROS buffering in β-cells.[64] To what extent Epac2 also mediates actions of GLP-1R agonists to control β-cell mass and/or survival remains an active field of investigation.
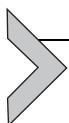
4. MOLECULAR BASIS OF GLP-1 RECEPTOR ACTIVATION

Like other group B GPCRs, the GLP-1R possesses a long extracellularly oriented N-terminus of ca. 150 amino acids in which three pairs of disulfide bonds create a secondary structure important to ligand binding. This N-terminal extracellular domain is connected to a core domain of the receptor consisting of seven transmembrane α-helices interconnected by three extracellular loops (ECL 1–3) and three intracellular loops (ICL 1–3). Based on findings originally obtained in studies of the group B GPCR for parathyroid hormone (PTH),[67] a "two-domain model" for GLP-1R activation exists in which the α-helical C-terminus of GLP-1 (7–36)amide interacts with the receptor's N-terminal domain, while the N-terminus of GLP-1(7–36)amide interacts with ECL-1 and ECL-2 of the GLP-1R.[68–72] The affinity and selectivity of ligand binding is determined by interactions of the GLP-1(7–36)amide C-terminus with the receptor's N-terminal domain, whereas coupling of the GLP-1R to intracellular signaling pathways is strongly influenced by the receptor core domain and its intracellular loops. In this regard, ICL-3 is of major importance to GLP-1R–stimulated adenylyl cyclase activity.[73]

There exists an alternative model of GLP-1R activation in which it is proposed that binding of GLP-1(7–36)amide to the receptor results in a structural rearrangement of the receptor's N-terminal domain so that a pentapeptide NRTFD signature sequence within the N-terminus of the receptor acts as an "endogenous agonist" at ECL-2 or ECL-3 of the receptor.[74] In this model, the signature sequence acts as a "tethered ligand" to promote GLP-1R activation.[74] Adding to this complexity, the signaling properties of the GLP-1R are also dictated by its ability to form homodimers in which receptor dimerization occurs at the interface of transmembrane helix four of each receptor protomer.[75]

Since small molecule GLP-1R agonists are highly desired for the treatment of T2DM, considerable effort has been exerted in an attempt to identify the precise mechanisms of ligand binding to the GLP-1R. Nuclear magnetic resonance (NMR) analysis using isolated N-terminal extracellular domains of group B GPCRs reveals that these ligand-binding domains contain a core structure composed of two antiparallel β-sheets stabilized by three disulfide bonds. X-ray crystallographic analysis also reveals that these β-sheets are linked to an N-terminal α-helix in order to form a "fold" that is highly conserved among the group B GPCRs.[69] Binding of GLP-1(7–36) amide within this fold leads to a structural rearrangement of GLP-1(7–36) amide so that it transitions from its disordered solution structure to an induced α-helical conformation with hydrophobic residues buried within the fold.[69]

Since binding of GLP-1(7–36)amide to the GLP-1R is accompanied by a structural rearrangement of the peptide in order for it to stimulate receptor signaling, it is understandable that the rationale design of small molecule GLP-1R agonists is complex and is not guided simply by the disordered solution structure of GLP-1(7–36)amide. However, the flexibility of GLP-1(7–36)amide to adopt multiple conformations might be of significance in view of current efforts to design small molecule allosteric modulators of the GLP-1R.[76–78] These modulators bind regions of the receptor distinct from GLP-1(7–36)amide, and they might cause GLP-1(7–36)amide to adopt a conformation that "biases" its signal transduction properties so that it activates select downstream pathways. Given that potentially dangerous islet cell hyperplasia is reported to occur in some T2DM patients treated with GLP-1R agonists,[79] it might be possible to design allosteric modulators of the GLP-1R that preferentially stimulate insulin secretion rather than islet growth.

5. CONTROL OF PANCREATIC β-CELL INSULIN SECRETION BY GLP-1

In studies of isolated islets, a stepwise increase of the glucose concentration from 2.8 to 16.7 mM leads to an initial first phase kinetic component of insulin secretion, followed by a delayed second phase, and the amplitudes of both phases of GSIS are potentiated by GLP-1.[80–83] These insulin secretagogue actions of GLP-1 are also measurable *in vivo* under conditions of a glucose clamp in which a GLP-1R agonist is infused intravenously while raising the blood glucose concentration in a stepwise manner.[56] In patients with prediabetes, there is a characteristic

loss of first phase GSIS that can be restored quickly under conditions of administered GLP-1. As T2DM progresses, there is an additional loss of second phase GSIS, and it too can be restored under conditions of acute GLP-1 administration. Such findings indicate that in T2DM, GLP-1 has the capacity to quickly restore GSIS independently of any long-term action to increase islet insulin content. Presumably, such acute actions of GLP-1 reflect, at least in part, its physiological role as an incretin hormone in which it activates the GLP-1R located on islet β-cells.

When considering the acute insulin secretagogue action of GLP-1 *in vivo*, it is also thought that oral administration of glucose leads to activation of vagal–vagal reflexes that allow GLP-1 to control insulin exocytosis indirectly. Thus, GLP-1 released from L cells activates vagal sensory neurons that project to the brainstem in order to initiate efferent vagal reflexes via the parasympathetic branch of the autonomic nervous system. Parasympathetic neurons release acetylcholine (ACh) in the islets in order to activate muscarinic cholinergic receptors that stimulate Ca^{2+} mobilization in β-cells, and these neurons also release PACAP to stimulate cAMP production in β-cells. The net effect is an indirect and neurally mediated action of GLP-1 to potentiate GSIS.[84]

As illustrated in Fig. 2.1, the direct action of GLP-1 to activate the GLP-1R on β-cells leads to dual activation of the PKA and Epac2 branches of the cAMP signaling mechanism. In this manner, GLP-1 facilitates glucose-dependent closure of ATP-sensitive K^+ channels (K_{ATP}).[85] The net effect is β-cell depolarization with consequent activation of VDDCs in order to allow Ca^{2+} influx that stimulates Ca^{2+}-dependent insulin secretion. Simultaneously, GLP-1 enhances a mechanism of Ca^{2+}-induced Ca^{2+} release (CICR) in which Ca^{2+} influx triggers the release of Ca^{2+} from intracellular Ca^{2+} stores.[86–90] This mobilized Ca^{2+} then acts as an additional stimulus for Ca^{2+}-dependent insulin secretion. In patients with T2DM, β-cell glucose metabolism is dysfunctional so that glucose is not fully capable of closing K_{ATP} channels in order to stimulate Ca^{2+} influx.[84] Under these pathophysiological conditions, glucose alone fails to generate the critically important cytosolic Ca^{2+} signal that initiates insulin exocytosis. By facilitating glucose-dependent K_{ATP} channel closure and by enhancing CICR, GLP-1 restores the Ca^{2+} signal, thereby allowing GSIS to occur.

When considering how GLP-1 potentiates GSIS from β-cells of healthy individuals, a different scenario exists. Under these nonpathological conditions, coupling of glucose metabolism to K_{ATP} channel closure is not
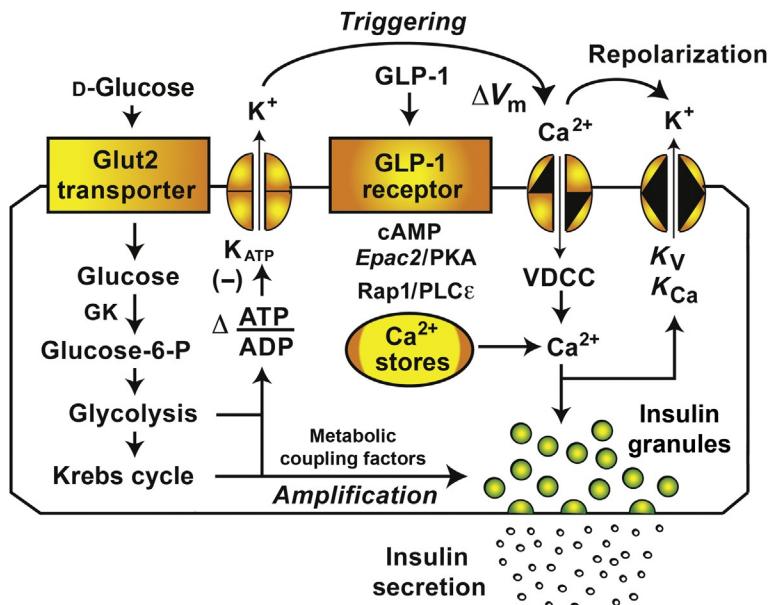
Figure 2.1 Role of GLP-1 in β-cell stimulus-secretion coupling. GLP-1 binds to its GPCR in order to stimulate cAMP production and to potentiate GSIS. One cAMP-dependent action of GLP-1 is mediated by PKA that phosphorylates secretory granule-associated proteins (e.g., Snapin) in order to facilitate Ca^{2+}-dependent exocytosis of insulin. The PKA-independent action of GLP-1 is mediated by the cAMP-regulated guanine nucleotide exchange factor Epac2. Binding of cAMP to Epac2 results in sequential activation of Rap1 GTPase and PLCε, thereby promoting PIP_2 hydrolysis and intracellular Ca^{2+} mobilization. GLP-1 also exerts PKA and Epac2-mediated actions to enhance glucose-dependent K_{ATP} channel closure, thereby promoting Ca^{2+} influx through VDCCs. The primary role of GLP-1 relevant to insulin secretion is to act as a β-cell glucose sensitizer in order to enhance insulin exocytosis mediated by the triggering and amplification pathways of GSIS. Abbreviations: GK, glucokinase; $\triangle V_m$, depolarization; K_v, voltage-dependent K^+ channel, K_{Ca}, calcium-activated K^+ channel.

disturbed, so that glucose is fully capable of generating the cytosolic Ca^{2+} signal that stimulates insulin exocytosis. Importantly, single cell studies demonstrate that this Ca^{2+} signal is a more efficient stimulus for insulin exocytosis under conditions in which β–cells are treated with GLP–1.[91] Such a facilitation of exocytosis by GLP–1 is explained by its PKA– and Epac2–mediated actions that occur at "late" steps of β–cell stimulus–secretion coupling and that promote Ca^{2+}-dependent fusion of secretory granules with the plasma membrane (Fig. 2.1).

Since a K_{ATP} channel–dependent action of GLP–1 is likely to explain insulin secretagogue properties of GLP–1 in patients with T2DM, it is useful

to summarize what is known concerning this effect. Restoration of K_{ATP} channel closure by GLP-1 is measurable under conditions in which rat β-cells are initially exposed to a glucose-free solution that depletes intracellular ATP.[85] Transient reintroduction of glucose weakly inhibits K_{ATP} channel activity, and this action of glucose is greatly potentiated by GLP-1. Such a restorative action of GLP-1 reflects its ability to alter the adenine nucleotide sensitivity of K_{ATP} channels so that these channels close more efficiently in response to the increase of cytosolic ATP/ADP concentration ratio that glucose metabolism produces. In fact, PKA reduces the stimulatory action of Mg-ADP at SUR1,[92] whereas Epac2 enhances the inhibitory action of ATP at Kir6.2.[93,94] These dual mechanisms of K_{ATP} channel modulation underlie the ability of GLP-1 to act as a β-cell glucose sensitizer so that it may facilitate glucose metabolism-dependent depolarization of β-cells.[91]

Studies of mice lacking the sulfonylurea receptor-1 (SUR1) and pore-forming Kir6.2 subunits of K_{ATP} channels provide additional evidence for a K_{ATP} channel-dependent action of GLP-1 to stimulate insulin secretion. In these SUR1 and Kir6.2 KO mice, potentiation of GSIS by GLP-1 is absent[95,96] or reduced.[97] Furthermore, in mice harboring a tyrosine to stop codon (Y12STOP) mutation in the gene coding for Kir6.2, K_{ATP} channel expression and GLP-1-stimulated insulin secretion are absent.[98] Important findings are also provided by a study of patients with neonatal diabetes mellitus (NDM) owing to gain-of-function mutations (C435R; R1380H) in the gene coding for SUR1.[99] These mutations lead to overactive K_{ATP} channels and a consequent reduction of GSIS. Remarkably, administration of a GLP-1R agonist restores insulin secretion in these patients.
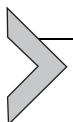
6. ALTERED GLP-1 ACTION IN A RODENT MODEL OF INSULIN RESISTANCE

It is interesting to note that expression of Epac2 within β-cells is of critical importance to β-cell compensation that occurs in mice fed with a high-fat diet (HFD).[54] The HFD induces insulin resistance, and under these conditions, GSIS is enhanced in order to compensate for insulin resistance.[100] When comparing wild-type (WT) and Epac2 KO mice fed with the HFD, it is possible to demonstrate that compensatory GSIS is lost in Epac2 KO mice.[54] This unexpected role of Epac2 to *enable* GSIS is measurable in isolated islets and it does not require treatment of islets with GLP-1R agonists. Furthermore, this compensation under conditions

of the HFD results from alterations of β-cell Ca^{2+} handling such that there is enhanced glucose-dependent Ca^{2+} influx and Ca^{2+} mobilization.[54] Since Epac2 mediates stimulatory effects of GLP-1 on Ca^{2+} influx and mobilization,[49,91,101–103] it appears that "plasticity" exists in the β-cell cAMP signaling network such that the HFD leads to an unexpected coupling of glucose metabolism to Epac2 activation and insulin secretion.[54,104]

Since GLP-1R expression is elevated in islets of mice fed with the HFD,[105] it is evident that GLP-1 also participates in the functional adaptation of islets to diet–induced insulin resistance. In this regard, it is interesting to note that under conditions of the HFD, *in vivo* administration of a GLP-1R agonist leads to an additional compensatory increase of GSIS that is "durable" in that it is measurable in isolated islets in the complete absence of *in vitro* GLP-1R stimulation.[106] This finding indicates that a GLP-1R agonist has the capacity to upregulate the expression and functionality of key components of the β-cell stimulus–secretion coupling mechanism, most likely including glucose-sensing, oxidative glucose metabolism, ion channel regulation, and Ca^{2+}–dependent exocytosis.

When considering how the HFD also induces islet hyperplasia with a compensatory increase of β-cell mass, it could be that increased GLP-1R expression on β-cells plays a role. Thus, increased GLP-1R expression might favor increased β-cell sensitivity to circulating GLP-1, thereby allowing GLP-1 to efficiently signal through cAMP, PKA, and Epac2 to upregulate β-cell proliferation and survival. Although this is an attractive hypothesis, recent studies indicate that in mice fed with a normal diet, enhanced PKA activity per se does not increase β-cell mass.[50,107] Furthermore, a KO of Epac2 expression does not lead to decrease of β-cell mass in mice fed with a normal diet.[54] However, it could be that in mice fed with an HFD, a role for PKA and Epac2 in the induction of islet hyperplasia might be revealed.

Since cAMP-independent actions of GLP-1 exist, such actions might also play a role in promoting adaptive responses of β-cells under conditions of the HFD. Thus, it is of interest to summarize what is known concerning cAMP-independent actions of GLP-1 that allow it to act as a β-cell trophic factor. Studies performed primarily with β-cell lines or mouse β-cells demonstrate cAMP-independent actions of GLP-1R agonists to counteract endoplasmic reticulum stress[108] and to signal via the GLP-1R through β-arrestin[109,110] and epidermal growth factor (EGF) receptor trans-activation[111] in order to downregulate the activities of proapoptotic protein

BAD,[112] the SirT1 deacetylase,[113] and transcription factor FoxO1.[114] GLP-1 also upregulates the activities of c-Src kinase,[115] phosphatidylinositol 3-kinase (PI-3-kinase),[116] protein kinase B (PKB),[117] protein kinase c-zeta (PKC-ζ),[118] and extracellular signal–regulated protein kinases (ERK1/2).[119] Conceivably, these growth factor-like signaling pathways might be selectively activated by allosteric GLP-1R agonists that have "biased" signal transduction properties and that bind the GLP-1R in order to promote β-cell compensation under conditions of the HFD.
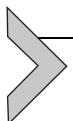
7. GLUCOREGULATORY PROPERTIES OF GLP-1 MEDIATED BY THE NERVOUS SYSTEM

A high-profile area of research concerns GLP-1 action in the nervous system, and it is recently appreciated that such actions of GLP-1 are of importance to glucoregulation. Vagal–vagal reflexes are activated by injection of a GLP-1R agonist into the hepatic portal vein, and they are measurable as increased electrical activity in vagal sensory afferent neurons and also in vagal efferent neurons.[120] By measuring insulin secretion induced by intraportal administration of glucose, it is also possible to demonstrate that GSIS is potentiated by coadministration of glucose with GLP-1.[121] This action of GLP-1 to potentiate GSIS is reduced by a ganglionic blocker,[121] as expected if vagal–vagal reflexes activate neurons within pancreatic ganglia in order to stimulate insulin secretion. Vagal sensory neurons express GLP-1 receptors,[122–124] and direct application of GLP-1 to vagal afferent neuron cell bodies within the nodose ganglion leads to action potential generation.[125]

Vagal sensory neurons activated by GLP-1 project to the brainstem and hypothalamus, and under conditions in which the GLP-1R agonist exendin-4 is administered intraperitoneally, a surgical subdiaphragmatic vagotomy blunts activation of neurons located within the hypothalamic and paraventricular nuclei.[126] Such findings obtained with rats indicate that peripherally administered exendin-4 acts via the vagus nerve to stimulate neural activity within the brain and that this effect of exendin-4 complements its more direct action to cross the blood–brain barrier in order to activate CNS GLP-1 receptors.[127–129] A vagus nerve-mediated action of GLP-1R agonists also occurs in humans since in vagotomized patients treated for pyloroplasty, there is a reduced ability of intravenously infused GLP-1 to suppress appetite, to slow gastric emptying, to stimulate insulin secretion, and to suppress glucagon secretion.[130]

GLP-1 receptors are widely expressed within the brain where they are activated by neuronally released GLP-1. Thus, GLP-1 is a neuropeptide, and neuroanatomical studies demonstrate that it is contained within neuronal cell bodies located in the medullary caudal nucleus tractus solitarius (NTS), the raphe obscurus, and the intermediate reticular nucleus.[131,132] Axons of these neurons project to regions of the brain that are involved in the control of appetite, metabolism, water intake, stress, and cardiovascular functions.[133–136] These regions include the dorsal vagal nucleus, dorsomedial and paraventricular hypothalamic nuclei, ventrolateral periaqueductal gray, and thalamic paraventricular nucleus.[131,132] GLP-1-containing neurons project to the brainstem where they synapse on cholinergic vagal motor neurons, some of which project to the pancreas.[137,138] Collectively, these findings indicate three mechanisms by which GLP-1 controls vagal efferent activity: (1) vagal–vagal reflexes in which GLP-1 initially activates the GLP-1R located on vagal sensory nerve terminals, (2) an action of circulating GLP-1 that requires its action at, or transit across, the blood–brain barrier in order to activate brainstem neural circuits, and (3) direct or indirect synaptic relays in which GLP-1 released within the brain activates vagal motor neurons.

When considering the physiological significance of such neural influences of GLP-1, it is important to note that neural control of insulin secretion is not an absolute requirement in order to measure an insulin secretagogue action of a GLP-1R agonist. This fact is demonstrated in studies of glucoregulation using Pdx1-hGLP1R:Glp1r−/− mice administered with the DPP-IV-resistant GLP-1R agonist exendin-4.[139] These engineered mice do not express the mouse GLP-1R in any tissue, whereas they express recombinant human GLP-1 receptors only in the pancreas. In such mice, exendin-4 exerts its normal action to potentiate GSIS and to improve glucose tolerance in the absence of neural GLP-1R activation.[139]

Despite the fact that GLP-1R agonist action is preserved in Pdx1-hGLP1R:Glp1r−/− mice, there is reason to believe that the nervous system does in fact mediate important glucoregulatory actions of GLP-1.[140–145] For example, under conditions in which pancreatic insulin secretion is induced by intragastric infusion of mice with glucose, an intracerebroventricular (i.c.v.) injection of GLP-1R antagonist exendin(9–39) results in less insulin being secreted.[142,146] Furthermore, glucose uptake and glycogen synthesis within skeletal muscle are enhanced by intragastric infusion of glucose, and this effect of glucose is blocked by exendin(9–39) delivered by the i.c.v. route.[142,146] Such findings indicate that during the initial prandial state

of intestinal glucose absorption, GLP-1 "primes" whole-body metabolism by acting within the brain to facilitate pancreatic insulin secretion while also enhancing skeletal muscle glucose disposal.

Interestingly, the neurally mediated action of GLP-1 that is important to glucoregulation may be different under conditions that mimic the postprandial state when levels of blood glucose are rising. In studies of mice using infusion clamp techniques that elevate levels of blood glucose and insulin, it is reported that i.c.v. administration of GLP-1R agonist exendin-4 reduces blood flow and glucose uptake within skeletal muscle.[142,146] Simultaneously, insulin secretion is stimulated in order to enhance insulin-dependent hepatic glucose uptake.[142,146] Thus, in contrast to the initial prandial state of glucose absorption described earlier, GLP-1 acts in the postprandial state to shift glucose disposal from muscle to liver. Resultant hepatic glycogen synthesis allows for sufficient glycogen mobilization and hepatic glucose production during the subsequent fasting state. What remains to be demonstrated is that such neurally mediated effects of GLP-1 occur in healthy humans and/or patients with T2DM.

It is also recognized that GLP-1 receptors located on neurons within the arcuate nucleus (Arc) are activated in order for GLP-1 to stimulate insulin secretion while also suppressing hepatic glucose production.[147–149] One mechanism that may explain how GLP-1 alters neural function in the Arc is provided by the finding that transmission in these neural circuits is modulated by neuropeptides, nutrients, and hormones that control K_{ATP} channels.[149–151] In this regard, GLP-1 may inhibit K_{ATP} channel activity within the Arc in order to regulate blood glucose homeostasis. It will be interesting to assess whether this action of GLP-1 is selective for glucose-responsive neurons in the Arc and whether the inhibition of K_{ATP} channel activity results from Epac2 activation, as described for β-cells. Furthermore, since leptin activates K_{ATP} channels in the Arc,[150,151] it could be that GLP-1 and leptin act as counterregulatory hormones to control Arc circuits important to glucoregulation.

Finally, it is interesting to note that GLP-1R agonists are under evaluation for use in the treatment of neurological disorders.[152] In an *in vitro* model of Alzheimer's disease, GLP-1 protects hippocampal neurons from cytotoxicity induced by amyloid-beta peptide.[153] Also surprising is the report that such neuroprotection is conferred by GLP-1(9–36)amide, which is the metabolite generated by DPP-IV-catalyzed degradation of GLP-1 (7–36)amide.[154] This finding suggests the existence of a nonconventional GLP-1R, although its identity remains unknown. Just as interesting, there

is a potential usefulness of GLP-1R agonists to treat Parkinson's disease.[155,156] Collectively, the available evidence suggests that these neuroprotective actions of GLP-1 might be secondary to its ability to alter glucose homeostasis in the brain. For example, under conditions of hyperglycemia, peripherally administered GLP-1 increases the phosphorylation velocity (V_{max}) of neuronal hexokinase while also increasing blood–brain glucose transport capacity (T_{max}).[157,158]

8. GLP-1 RECEPTOR AGONISTS

Drug development strategies have led to the identification of GLP-1R agonists that are either peptide-based or small molecule-based. For peptide-based GLP-1R agonists, a further subdivision exists in order to classify "incretin mimetics" or "GLP-1 analogs," as summarized in Table 2.1. Exenatide, also known as Byetta, is the prototypical incretin mimetic and it is the synthetic form of exendin-4 (Ex-4). In contrast, the prototypical GLP-1 analog is liraglutide, also known as Victoza. Liraglutide is structurally equivalent to GLP-1(7–37) except that lysine residue 26 is acylated by its conjugation to a hexadecanoyl (C16) side chain, whereas residue 34 contains arginine rather than the lysine residue found within native GLP-1. Exenatide and liraglutide are both high-affinity agonists at the GLP-1R, yet they are relatively resistant to hydrolysis by DPP-IV. For example, after intravenous administration, the half-life of circulating GLP-1 is only 1.5–5.0 min, whereas the half-lives of exenatide and liraglutide are 26 min and 8 h, respectively. Thus, exenatide and liraglutide exert prolonged blood glucose-lowering actions when they are administered by subcutaneous injection to patients with T2DM.

Mechanistically, the hexadecanoyl side chain of liraglutide allows this peptide to bind to plasma albumin via hydrophobic interactions, thereby minimizing hydrolysis by DPP-IV. In the GLP-1 analog albiglutide, two molecules of GLP-1(7–36) are fused in tandem, and the tandem is covalently conjugated to recombinant human albumin in order to achieve DPP-IV resistance. Simultaneously, a glycine substitution is introduced at residue 8 in order to improve DPP-IV resistance. In dulaglutide, a different approach is taken in which GLP-1(7–36) is fused to human immunoglobulin heavy constant γ4 chain (IgGγ4-Fc) to create a monomer that then dimerizes with itself in order to generate the DPP-IV-resistant GLP-1R agonist.

Table 2.1 Pharmacological properties of GLP-1R agonists currently in use or under study for the treatment of T2DM

GLP-1R agonist	Parental peptide	Modifications	Half-life	Route of administration	HbA1c reduction	Weight reduction	Refs.
Exenatide BID	Ex-4	None	2.4 h	SC 5 or 10 μg BID	0.7–0.9%	2.8–3.1 kg	159
Lixisenatide	Ex-4	Proresidue deleted from C–terminus, six Lys residues added to C–terminus	3 h	SC 20 μg QD	0.8–0.9%	1.8–3.0 kg	160
Exenatide LAR	Ex-4	Injectable microspheres of biopolymer with entrapped exenatide	5–6 d	SC 2 mg QW	1.3–1.9%	3.6 kg	161,162
Liraglutide	GLP-1	Palmitic acid conjugated to Lys-26, Lys-34/Arg substitution	11–13 h	SC 1.2 mg QD	1.1–1.8%	2.0–3.0 kg	163,164
Semaglutide	GLP-1	Palmitic acid conjugated to Lys-26, Gly-8/aminoisobutyric acid, and Lys-34/Arg substitutions	6–7 d	SC 0.1–1.6 mg QW	1.7%	4.8 kg	165
Albiglutide	GLP-1	Two molecules of GLP-1 fused as a tandem and conjugated to albumin; Ala-2/Gly substitution	6–8 d	SC 50 mg QW	0.8%	0.6 kg	166–168
CJC-1134-PC	Ex-4	Peptide coupled to albumin by a linker	8 d	SC 2 mg QW	1.4%		169
Dulaglutide	GLP-1	Two molecules of GLP-1 covalently linked to a IgG4-Fc heavy chain; Ala-8/ Gly,Gly-26/Glu, Arg-36/Gly substitutions	4 d	SC 1.5 mg QW	1.5%	NS	170,171
Langlenatide	Ex-4	Peptide fused to Fc region	6 d	SC 1–4 mg QW SC 8–16 mg QMT	NDA	NDA	172
VRS-859	Ex-4	Peptide fused to Xten protein	3 d	SC 200 mg QMT	NDA	NDA	173

Ex-4, exendin-4; BID, twice daily dosing; QD, once daily dosing; QW, once weekly dosing; QMT, once monthly dosing; SC, subcutaneous administration; HbA1c, hemoglobin A1c.

Attempts to identify small molecule GLP-1R agonists are complicated by the complex ligand–receptor interactions that are characteristic of group B GPCRs.[46,174] Despite this complication, new "ago-allosteric" modulators of the GLP-1R are described. These small molecules not only act as GLP-1R agonists (ago control) but also modify the ability of GLP-1 itself to activate the GLP-1R (allosteric control). Synthetic ago-allosteric modulators currently under preclinical investigation include substituted quinoxaline[76,175–177] and cyclobutane derivatives.[178–180] A substituted quinoxaline designated as compound 2 acts as a partial agonist at the GLP-1R, but it is particularly revealing that the efficacy of compound 2 as a cAMP-elevating agent is enhanced rather than reduced by GLP-1R antagonist exendin(9–39). This finding is consistent with the concept that allostery results from binding of compound 2 to a site on the GLP-1R that is not recognized by peptide-based agonists and antagonists.[181] Expanding on these findings, it is reported that novel substituted pyrimidines also act as GLP-1R agonists and that they do not compete with radiolabeled GLP-1 for binding to the GLP-1R.[182]

GLP-1R activation by the quinoxaline compound 3 is strongly influenced by mutations introduced into transmembrane α-helices 2 and 7, whereas such mutations do not alter the action of GLP-1.[183] These findings indicate that small molecule agonists activate or modulate the GLP-1R in a manner that is distinct from that of GLP-1. In fact, a quinoxaline (compound 2) and a pyrimidine (compound B) act in an additive manner to activate the GLP-1R under conditions in which the receptor is truncated to remove the N-terminal extracellular domain at which the C-terminus of GLP-1 binds.[184] Just as intriguing, GLP-1R-mediated signaling properties of ago-allosteric modulators are not identical, thereby suggesting that such agonists could be used in order to achieve signal transduction bias.[77,184–187]

9. DPP-IV INHIBITORS

DPP-IV encoded by the *DPP4* gene is a member of the prolyl oligopeptidase family of serine proteases, and it plays a role in the control of immune function and is a key determinant of incretin hormone action. DPP-IV exists as a soluble circulating form[188] or as a type II transmembrane serine exopeptidase.[189] Both forms of the enzyme catalyze the cleavage of dipeptides from the N-terminus of peptide substrates that contain on average 30-amino-acid residues and that have a proline or alanine residue in the penultimate position.[189] These substrates include chemokines (CCL5),

neuropeptides (PYY and NPY), and hormones (GLP-1 and GIP).[190] Terminology exists in which DPP-IV is also known as adenosine deaminase complexing protein 2 (ADCP 2) or as the T-cell activation antigen CD26. DPP-IV is highly expressed on endothelial cells, differentiated epithelial cells, and lymphocytes. In the immune system, DPP-IV exists as an integral membrane glycoprotein in which it acts as a cofactor to control intracellular signaling pathways that are of importance to T-cell proliferation and T-cell activation.[191]

Crystallographic analysis combined with molecular modeling reveals that the 766-amino-acid residue DPP-IV contains an N-terminal β-propeller domain and a C-terminal α/β hydrolase domain that together form a cavity in which the enzyme's active site is located.[192,193] A distinguishing feature of DPP-IV is that the enzyme's α/β hydrolase domain contains a serine–aspartate–histidine catalytic triad, whereas the β-propeller domain contains two glutamate residues that are necessary for catalytic function and that align the substrate peptide so that only the penultimate proline or alanine residues may engage the active site. This structural feature of DPP-IV explains its substrate specificity in which it hydrolyzes peptides with N-terminal X–proline or X–alanine residues.[194]

Summarized in Table 2.2 are the pharmacological properties of small molecule DPP-IV inhibitors now in use for the treatment of T2DM. The xanthine class of DPP-IV inhibitors includes sitagliptin, linagliptin, and alogliptin, whereas vildagliptin and saxagliptin are members of the cyanopyrrolidine class of DPP-IV inhibitors. Inhibition of DPP-IV activity by sitagliptin is achieved by its noncovalent binding to the conserved glutamate residues 205 and 206 located within the enzyme's β-propeller, whereas saxagliptin binds not only to these glutamate residues but also to the serine residue located within the catalytic triad of the α/β hydroxylase domain.[190] In general, cyanopyrrolidines such as saxagliptin are competitive inhibitors rather than noncompetitive inhibitors of DPP-IV enzymatic activity since they form reversible covalent bonds with serine residue 630 located within the enzyme's active site.[190]

DPP-IV catalyzes the hydrolysis of GLP-1(7–36)amide to generate GLP-1(9–36)amide and the N-terminal histidine–alanine dipeptide. Therefore, DPP-IV inhibitors raise levels of endogenous GLP-1(7–36)amide, and it could be that at clinically relevant doses, this action of DPP-IV inhibitors produces a relatively selective increase of GLP-1(7–36)amide in the hepato-portal circulation or at the interface of L cells and vagal sensory nerve terminals. Since DPP-IV inhibitors suppress enzymatic production

Table 2.2 Pharmacological properties of DPP-IV inhibitors currently in use or under study for the treatment of T2DM

Inhibitor	Half-life	Route of administration	Plasma DPP-IV inhibition	Plasma GLP-1 increase	HbA1c reduction	Route of elimination	Refs.
Sitagliptin	11–13 h	PO 25–200 mg QD	80% with 50 mg	2 times	0.6–0.8%	Mostly renal	195–197
Vildagliptin	1.7–3 h	PO 25–200 mg QD or BID	80–90%	2–3 times	0.5–1.5%	Mostly renal	197–199
Saxagliptin	2.2–3.8 h 3.0–7.4 h	PO 2.5–50 mg QD	70%	1.5–2 times	0.5–0.9%	Mostly renal	23,197,200,201
Linagliptin	113–260 h	PO 0.5–10 mg QD	46% with 0.5 mg 78% with 2.5 mg 90% with 10 mg	2 times (0.5 mg) 3 times (2.5 mg) 4 times (10 mg)	0.4–0.8%	Hepatic (biliary excretion)	197,202,203
Alogliptin	12–21 h	PO 25–800 mg QD	74–97%	2–4 times	0.5–0.9%	Mostly renal	197,204,205

of GLP-1(9–36)amide, while also preventing release of the histidine–alanine dipeptide, it is of concern that these two metabolites might have important biological actions that would be missing in patients administered with DPP-IV inhibitors. In fact, GLP-1(9–36)amide exerts prosurvival actions in neurons and cardiomyocytes[154,206] while also suppressing hepatic glucose production in obese patients.[207,208] Furthermore, the histidine–alanine dipeptide is reported to influence glucose tolerance and insulin secretion in mice.[209]
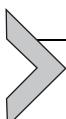
10. GLP-1-BASED STRATEGIES FOR THE TREATMENT OF T2DM

A GLP-1-based strategy for the treatment of T2DM is indicated in view of the fact that GLP-1R agonists and DPP-IV inhibitors exert a beneficial constellation of physiological effects that include (1) glucose-dependent stimulation of insulin secretion, (2) suppression of glucagon secretion, (3) normalization of blood glucose without an attendant risk of hypoglycemia, (4) slowing of gastric emptying, (5) appetite suppression, and (6) weight loss. Potential additional benefits are actions to promote β-cell survival by slowing apoptosis or to promote β-cell regeneration by stimulating β-cell proliferation. Thus, it was originally anticipated that such a GLP-1-based therapy might lead to a long-term remission and possibly a cure for T2DM.[210] Since the notion of a GLP-1-based therapy has led to the term "incretin therapy," it is important to note that when considering the use of incretins for the treatment of T2DM, only GLP-1 is effective, whereas GIP is ineffective.[8,211]

Presently available GLP-1R agonists include exenatide (approved in the United States in 2005) and liraglutide (approved in the United States in 2010), both of which are administered by subcutaneous injection. Exenatide is approved for use twice a day, and liraglutide is approved for use once a day. An extended release (ER) formulation of exenatide is intended for use once a week. Additional long-acting formulations are under investigation including one depot preparation of exenatide that can be given once every 6 months. In contrast to exenatide and liraglutide, the DPP-IV inhibitors are orally administrable and are therefore a more convenient means by which to treat T2DM. Currently, in the United States, there are four approved drugs of this class. Sitagliptin was first approved in 2006, and since then, three additional DPP-IV inhibitors have been approved. They are saxagliptin, linagliptin, and alogliptin. In addition, vildagliptin and gemigliptin are available in other countries.

When considering the use of GLP-1R agonists or DPP-IV inhibitors for the treatment of T2DM, it is important to note that DPP-IV inhibitors raise

levels of circulating GLP-1 by approximately twofold, whereas GLP-1R agonists exert a dose-dependent pharmacological effect that is considerably more potent since their circulating levels easily exceed endogenous GLP-1 levels by eightfold.[212] These pharmacological differences may explain why GLP-1R agonists are more effective inhibitors of gastric emptying, while also suppressing appetite and promoting weight loss. In fact, in some patients, the high potency of GLP-1R agonists can lead to adverse side effects of nausea and vomiting.[213]

GLP-1R agonists and DPP-IV inhibitors are approved for use in patients with T2DM, typically as adjuncts to diet and exercise and as either a monotherapy or a combination therapy with other antidiabetic medications.[214–217] Exenatide ER and liraglutide are not recommended as first-line therapies although they may be considered for monotherapy in patients who are unable to use other first-line therapies because of a lack of efficacy or due to contraindications such as allergic hypersensitivity, end-stage renal disease, and gastrointestinal diseases. DPP-IV inhibitors are also contraindicated in patients with hypersensitivity reactions such as urticaria, angioedema, or bronchial hypersensitivity. In addition to a history of serious hypersensitivity as a contraindication, exenatide ER and liraglutide are also contraindicated in patients with a personal or family history of medullary thyroid cancer or with a history of multiple endocrine neoplasia syndrome type 2 (MEN2). Prescribing information, warning labels, and precaution sections for exenatide and liraglutide or various DPP-IV inhibitors also list pancreatitis as a potential adverse side effect of their use.[214–217]

A review of the clinical DPP-IV literature concerning monotherapy for the treatment of T2DM indicates 25 randomized control trials (RCTs) in adult patients with trial durations of at least 12 weeks.[218] Sitagliptin and vildagliptin therapy results in an HbA1c reduction of 0.7% and 0.6%, respectively. In another review that includes 17 RCTs of 8 weeks minimum duration, monotherapy with GLP-1R agonists results in reductions of HbA1c of ca. 1%.[219] Although β-cell function improves with GLP-1R agonist treatment, it is interesting to note that a rapid deterioration of glucoregulation can occur after withdrawal of these medications. Thus, unlike the situation reported for mice administered with a GLP-1R agonist,[106] a "durable" effect of GLP-1R agonists is not so obvious in humans. This finding seems to argue that in humans, the primary effect of GLP-1R agonists is to exert an acute stimulatory effect on β-cell insulin secretion, rather than acting long term to alter β-cell gene expression.

GLP-1R agonists and DPP-IV inhibitors are also under study for use in combination with non-GLP-1-based medications such as insulin,

sulfonylureas, the biguanide metformin, and the thiazolidinedione pioglitazone.[220–225] Especially noteworthy is the 2011 approval in the United States of exenatide as an add-on therapy to basal insulin analog glargine for patients with T2DM who are not achieving adequate glycemic control using glargine alone. Although GLP-1R agonists and DPP-IV inhibitors can be used in combination with sulfonylureas, there is an increased risk of hypoglycemia so that caution should be exercised and pre-emptive dose reduction should be implemented. In this regard, an attractive alternative therapy is based on the use of metformin in combination with a GLP-1R agonist or DPP-IV inhibitor. This combination therapy has a reduced risk hypoglycemia, yet it still promotes beneficial weight loss in patients with T2DM.

In the DURATION clinical trial series,[161,226–229] T2DM patients are reported to lose an average 2–4 kg body weight when treated with exenatide ER (2 mg per weekly as a single injection). The weight loss achieved with exenatide ER is similar to that observed in patients administered with non-ER exenatide twice daily (5–10 mcg per single injection). Importantly, body weight reduction is significantly larger for patients administered with exenatide ER in comparison to administered sitagliptin (-2.3 vs. -0.8 kg, respectively).[226] The LEAD (Liraglutide Effect and Action in Diabetes) trial also reveals significant body weight reduction with liraglutide mon-otherapy.[230] This weight loss is primarily due to reduced fat mass, mainly visceral adipose tissue.[231]

As summarized in Table 2.3, a GLP-1-based therapy for the treatment of T2DM is particularly attractive since it not only normalizes glycemia while reducing body weight but also improves cardiovascular function.[241,242] GLP-1R agonist treatment has positive effects on cardiovascular risk factors such as diabetes, hypertension, hyperlipidemia, and obesity. A pooled data analysis from six clinical trials investigating the outcomes of 6-month exenatide treatment in 2171 T2DM patients reveals significantly greater reductions in systolic blood pressure compared with placebo.[243] Mechanis-tically, such a reduction of blood pressure is consistent with the report that liraglutide exerts an action in the mouse atrial myocardium to stimulate the release of atrial natriuretic factor (ANF) that then acts to relax vascular smooth muscle while also promoting renal excretion of sodium ion.[244] GLP-1R agonist therapy also results in favorable changes in circulating lipids, which are another important cardiovascular risk factor. Meta-analysis demonstrates that liraglutide lowers blood levels of total cholesterol, low-density lipoproteins, free fatty acids, and triglycerides.[245]

Table 2.3 Cardiovascular actions of GLP-1-based therapeutics

GLP-1R agonist	Experimental/ clinical setting	Effect of GLP-1R agonist	Refs.
Exenatide	TG9 mice (murine DCM model)	Improvement of glucose tolerance; increase 2-deoxyglucose uptake and GLUT4 expression in myocardium	232
GLP-1	Dogs with pacing-induced DCM	Increase insulin sensitivity, basal and insulin–stimulated glucose extraction, and uptake in myocardium, and decrease plasma glucagon	233
GLP-1 GLP-1 (9–36)	Dogs with pacing-induced DCM	Both peptides increase insulin sensitivity and basal and insulin–stimulated glucose uptake in myocardium, and decrease plasma glucagon	234
Exenatide	Diabetic (STZ-induced) rats	Increase myocardial glucose uptake	235
Vildagliptin	Model of murine heart failure	Increase plasma GLP-1, improvement of glucose tolerance	236
Liraglutide	Mice on HFD	Decrease insulin resistance	237
GLP-1	Patients before, during, and after CABG	Decrease pre- and perioperative plasma glucose, decrease postoperative plasma glucagon, decrease postoperative insulin infusion required, decrease pharmacological or mechanical support to achieve hemodynamic stability in postoperative period	238
GLP-1	T2DM patients after CABG	Decrease postoperative insulin infusion required Decrease dobutamine infusion required	239
GLP-1	T1D patients	Decrease hyperglycemia- or hypoglycemia–induced oxidative stress, inflammation, and endothelial dysfunction	240

DCM, dilated cardiomyopathy; STZ, streptozotocin; HFD, high-fat diet; CABG, coronary artery bypass grafting.
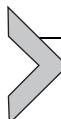
11. IMPROVED GLUCOREGULATION AFTER BARIATRIC SURGERY

There is evidence that intestinally released GLP-1 might mediate the beneficial outcomes of bariatric surgery in which a Roux-en-Y gastric bypass (RYGB) leads to weight loss that is accompanied by elevated levels

of plasma GLP-1 and improved glucoregulation in patients with T2DM.[246–252] This is a clinically important issue to address since RYGB surgery results in weight loss in 30–40% of obese patients, whereas improved glucoregulation is observed in about 80% of T2DM patients.[253–255] Unfortunately, our understanding how these beneficial outcomes of RYGB surgery are achieved is complicated by weaknesses in the experimental designs of published studies.[256] In fact, the role of elevated plasma GLP-1 as a determining factor in the remission of T2DM is disputed,[257] and it is instead reported that β-cells are rendered more sensitive to circulating GLP-1 after RYGB surgery.[258] Evidently, RYGB induces compensatory changes in β-cells that lead to improved blood glucose control.[258]

Additional clinical observations reveal that levels of blood glucose are quickly normalized after RYGB surgery, even before significant weight loss is achieved.[259] Though decreased caloric intake or reduced intestinal nutrient absorption is an obvious cause for the weight loss, there appear to be additional important factors that explain a remission of T2DM after RYGB surgery. This conclusion is supported by the following observations: (1) Remission occurs in the immediate postoperative period before any weight loss occurs, (2) remission is more pronounced after RYGB surgery as compared with outcomes achieved by dieting in order to achieve comparable weight loss, and (3) remission is more pronounced after RYGB surgery as compared with other forms of bariatric surgery (sleeve gastrectomy and gastric banding).

Differences in the outcomes achieved following RYGB or dieting are clearly evident since RYGB, but not dieting, leads to enhanced postprandial release of GLP-1, thereby restoring the incretin effect in patients with T2DM.[248] Under postoperative conditions of RYGB in T2DM, there is also a restoration of the missing first phase kinetic component of GSIS, and there is an accompanying improvement of oral glucose tolerance.[248] Unfortunately, the physiological basis for immediate or long-term endocrine and metabolic changes after RYGB is not fully elucidated. Changes in the rate of eating, gastric emptying, nutrient absorption and sensing, incretin hormone release, bile acid metabolism, and intestinal microbiota composition may all be important.[260–263]

Increased intestinal GLP-1 secretion after gastric bypass surgery appears to be sustained and can potentially have beneficial effects in terms of weight loss and long-term remission of T2DM. In addition to this surgery's acute stimulatory effect on insulin secretion, it could be that the sustained elevation of blood GLP-1 might regenerate β-cells. However, a potential

drawback to surgery is that it is not yet clear whether a postoperative remission of T2DM is permanent or only temporary.[256,264–267] Furthermore, this gastric bypass surgery can lead to hyperinsulinemic hypoglycemia, thereby necessitating pancreatectomy.[268,269] Interestingly, the hyperinsulinemia in some patients who have had gastric bypass surgery does not appear to be secondary to an increase of β–cell mass, as might be expected if bypass surgery upregulates long-term actions of GLP-1 to stimulate β–cell proliferation. In one study of gastric bypass patients undergoing partial pancreatectomy to correct for hyperinsulinemia, histological analyses of pancreatic sections reveal no change in β–cell mass, proliferation, neogenesis, or apoptosis.[269] Thus, the nature of the adaptive change that underlies remission of T2DM after gastric bypass surgery remains to be determined.

12. SAFETY CONSIDERATIONS FOR GLP-1-BASED THERAPEUTICS

An ongoing controversy concerns whether the use of GLP-1–based therapeutics predisposes to unexpected side effects including inflammation of the pancreas (pancreatitis) or even pancreatic cancer in patients with T2DM.[270–274] Furthermore, postmortem histological analyses of pancreatic tissue from patients treated with a GLP-1R agonist or DPP–IV inhibitors provide evidence for an increased incidence of pancreatic exocrine cell dysplasia accompanied by hyperplasia of glucagon–secreting α–cells of the endocrine pancreas.[79] These findings have raised the specter that chronic GLP-1R activation in humans might lead to the appearance of exocrine cell adenocarcinomas or neuroendocrine tumors such as glucagonomas or insulinomas. Additional studies of rodents indicate that chronic stimulation of GLP-1 receptors on calcitonin-secreting C cells of the thyroid can lead to C-cell hyperplasia with eventual medullary thyroid cancer,[275] although this outcome is not measurable in nonhuman primates[276] and has yet to be demonstrated for humans. Countering these findings, it is argued that the benefits of GLP-1–based therapeutics outweigh their risks when considering their usefulness for the treatment of T2DM.[277,278] Currently, these safety concerns remain debated, and it is pointed out that in the published literature, there is no direct demonstration of causality linking GLP-1–based therapeutics to human pancreatitis, pancreatic cancer, or thyroid cancer. Despite this fact, the Food and Drug Administration acted in 2007 to issue a safety alert concerning the potential for pancreatitis in patients treated with exenatide. Furthermore, a black box warning is now provided with

prescription information for both exenatide and liraglutide. In 2013, both the American Diabetes Association and the Endocrine Society called for independent review of findings relating to these potential adverse side effects of GLP-1R agonists and DPP-IV inhibitors.

13. CONCLUSION

Nearly 30 years of basic science and clinical research has culminated with the recognition that GLP-1-based therapies for the treatment of T2DM are highly effective. Unanticipated are the surprising beneficial cardiovascular and neuroprotective actions of this class of blood glucose-lowering agents. Since GLP-1R agonists also produce substantial weight loss in obese patients, it is clear that pharmacological GLP-1R activation can be particularly useful for treating or reversing the increasingly common metabolic syndrome of hyperglycemia, impaired cardiovascular function, excess weight, and neuropathology. Although safety concerns are increasingly debated, the general consensus at the present time is that additional clinical research is necessary in order to establish whether the use of GLP-1R agonists or DPP-IV inhibitors predisposes to pancreatitis or cancer. Looking to the future, it is anticipated that a new approach to drug development will be popularized in order to identify GLP-1R agonists that have a reduced propensity to promote cell growth while retaining their capacity to stimulate pancreatic insulin secretion. Particularly useful will be new approaches that allow oral delivery of GLP-1R agonists, either as synthetic small molecule compounds or as novel peptide conjugates.[279]

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