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Pharmacological Actions of Glucagon-Like Peptide-1, Gastric Inhibitory Polypeptide, and Glucagon

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Abstract
Glucagon family of peptide hormones is a group of structurally related brain-gut peptides that exert their pleiotropic actions through interactions with unique members of class B1 G protein–coupled receptors (GPCRs). They are key regulators of hormonal homeostasis and are important drug targets for metabolic disorders such as type-2 diabetes mellitus (T2DM), obesity, and dysregulations of the nervous systems such as migraine, anxiety, depression, neurodegeneration, psychiatric disorders, and cardiovascular diseases. The current review aims to provide a detailed overview of the current understanding of the pharmacological actions and therapeutic advances of...
three members within this family including glucagon-like peptide-1 (GLP-1), gastric inhibitory polypeptide (GIP), and glucagon.

1. INTRODUCTION

Secretin family of hormones is a group of short brain–gut peptides named after the first hormone discovered among them. Members of this family are further classified into five subgroups based on their structures: (1) secretin (SCT) subfamily including SCT, pituitary adenylate cyclase-activating peptide (PACAP), vasoactive intestinal peptide (VIP), and growth hormone-releasing hormone (GHRH); (2) glucagon subfamily including glucagon, GLP-1, GLP-2, and GIP; (3) corticotrophin-releasing hormone (CRH) subfamily; (4) calcitonin (CCT) subfamily; and (5) parathyroid hormone (PTH) subfamily (Cardoso et al., 2010). These peptide hormones exert their effects through activation of class B secretin family of GPCRS comprising at least 15 members (Harmar, 2001; Laburthe et al., 1996) also divided into 5 subgroups similar to their ligands. Recent pharmacological interest in the development of ligand targets for these receptors has opened novel strategies for therapeutic intervention of many pathophysiological conditions including type-2 diabetes mellitus (T2DM) (GLP-1 receptor agonist) and depression (CRH receptor agonist) (Archbold et al., 2011; Furness et al., 2012; Poyner and Hay, 2012; Watkins et al., 2012). Initially, pharmaceutical industries struggled to develop agents that act on family B GPCRs partly due to the fact that cognate receptor ligands are not useful templates for drug development. Recent identification of high-affinity small molecular compounds has changed the situation and currently there are marketed therapies targeting this class of receptors at different stages of clinical trials (Archbold et al., 2011; Dunworth and Caron, 2009; Kadmiel et al., 2011). In addition, the N-terminal crystal structures of several members, with or without ligand binding, have provided novel information into drug design and screening of small chemicals (Grace et al., 2004, 2010; Kusano et al., 2012; Pal et al., 2010; Parthier et al., 2007; Pioszak and Xu, 2008; Runge et al., 2008; ter Haar et al., 2010). More importantly, the recently published crystal structures including the transmembrane domains of the human glucagon receptor (Siu et al., 2013) and human CRH receptor (Hollenstein et al., 2013) have opened up new opportunities by homology modeling to hypothesize structures of all class B GPCRs for virtual drug screening (Singh et al., 2016).
2. GLUCAGON-LIKE PEPTIDE-1

Synthesized and secreted from the enteroendocrine L cells, glucagon-like peptide-1 (GLP-1) is a naturally occurring 30-amino acid peptide hormone. GLP-1 is produced after posttranslational processing of proglucagon by prohormone convertase 1/3 (PC1/3) and its physiological effects are of great research interest taking into consideration its potential clinical relevance (Habib et al., 2012). Expressed in the intestinal L cells, pancreatic islet α-cells, and the brain, proglucagon gene encodes a 160-amino acid precursor, containing sequences for glicentin-related pancreatic polypeptide (GRPR), glucagon, GLP-1 and GLP-2. Cleavage of proglucagon in the pancreas results in GRPP, glucagon, an IP-1, and a major proglucagon fragment containing both GLP-1 and GLP-2, while in the gut and brain, cleavage of proglucagon results in glicentin, GLP-1, IP-2, and GLP-2. The glicentin moiety may be further processed into GRPP and oxyntomodulin (glucagon with IP-1) (Barrera, 2009). GLP-1 is released from the gut after nutrient ingestion and is known to inhibit gastric acid and glucagon secretion along with its effect on gut motility. Well-characterized as an incretin hormone, its most important physiological effect remains the augmentation of glucose-stimulated insulin release. It exerts its insulinotropic effect through GLP-1 receptor (GLP-1R) that is highly expressed on islet-β-cells (Drucker and Nauck, 2006). GLP-1 has also been found to be a central hormone produced mainly in the brain stem of the CNS from where it is transported to other brain areas to induce neuroprotective, cardiovascular, and metabolic actions (Kastin et al., 2002). Of particular interest is its satiety-inducing effect that leads to weight reduction (Turton et al., 1996). Activation of GLP-1R signaling pathways in the brain further leads to other physiological actions in the periphery including reduced lipogenesis in white adipose tissue, increased thermogenesis in brown fat, reduced lipid and glucose output from the liver, and reduced glucose utilization in the liver (Campbell and Drucker, 2013). Fig. 1 is a diagrammatic representation of pleiotropic physiological actions of GLP-1.

2.1 Therapeutic Potency

2.1.1 Type-2 Diabetes Mellitus

A combination of Metformin and lifestyle alterations is being used in the intervention of the complex and multifaceted pathophysiology of T2DM, and yet the progressive nature of the disorder inevitably demands other
supplementary therapies. This has given birth to incretin-based therapy approach targeting to alleviate the dysregulations in the incretin system (Tuomilehto et al., 2001). Incretin effect is designated as the amplification of insulin biosynthesis elicited by two key gastrointestinal hormones, GIP and GLP-1, both of which are released upon oral glucose intake. Both GIP and GLP-1 have short half-lives due to rapid enzymatic inactivation particularly by dipeptidyl peptidase-4 (DPP-4). While GLP-1 extenuates postprandial glucagon release, GIP augments it. In the case of T2DM, the insulinotropic effect of GIP is negligible when compared relative to that of GLP-1 (Baggio and Drucker, 2007). Therefore, much of the research efforts to modulate the incretin system are being directed toward GLP-1. The strongest known genetic risk factors for T2DM and \( \beta \)-cell dysfunction remain as variations in Tcf7l2 gene while the gene product TCF7L2 is a transcription factor playing a key role in \( \beta \)-cell physiology and is activated by the Wnt/\( \beta \)-catenin pathway (Grant et al., 2006). GLP-1R activation stimulates Wnt signaling in isolated mouse islets and INS-1 cells by increasing the phosphorylation and stabilization of \( \beta \)-catenin via a cAMP/PKA dependent mechanism involving AKT and ERK1/2 (Heller et al., 2011; Sonoda et al., 2008). Pancreas-specific knockdown of Tcf7l2 resulted in reduced GLP-1R expression in the islet and reduced insulin release in response to GLP-1 stimulation in isolated islets (da Silva Xavier et al., 2012).
β-arrestin-1 has also been shown to be important in GLP-1R signaling as it associates with the GLP-1R in the presence of GLP-1 (Sonoda et al., 2008). Knockdown of β-arrestin-1 reduced the stimulation of cAMP by GLP-1. While β-arrestin-1 is important for internalization and desensitization of the membrane GLP-1R, it does not modulate the surface expression of GLP-1R (Quoyer et al., 2010; Sonoda et al., 2008). Modulation of the incretin system therefore provides a viable option for treatment of T2DM. Two therapeutic strategies, GLP-1R agonist and DPP-4 inhibitors are currently used and have had some success in alleviating the symptoms of T2DM.

2.1.1.1 GLP-1R Agonists

Native GLP-1 has a very short half-life of about 2 min due to rapid degradation by DPP-4 (Baggio and Drucker, 2007; Vilsbøll et al., 2003). The challenge therefore is to develop GLP-1R agonists that can be resistant to inactivation by DPP-4. GLP-1R agonists have since been developed based on either amino-acid alterations of the native GLP-1 peptide or on naturally occurring protein (exendin-4) that can effectively activate GLP-1R. However, the commercially available GLP-1R agonists can be broadly classified into either short acting or continuous acting. Although the short-acting GLP-1R agonists (lixisenatide and exenatide) are resistant to degradation by DPP-4, they are subjected to renal elimination that renders them short acting to have only 2–4 h approximate half-life (Linnebjerg et al., 2007; Ratner et al., 2010). Due to this limitation, they have to be given once or twice on a daily basis. This gives rise to inconsistency in the plasma concentration of the GLP-1R agonist, which eventually gives rise to fluctuations in GLP-1R activation. To overcome this drawback, modifications have been done to produce continuous-acting peptides that act for a longer period with minimal alteration in its efficacy to stimulate the GLP-1R. For example, albiglutide is a continuous-acting peptide obtained by fusion with larger carrier molecule (albumin) while dulaglutide is obtained by fusion with immunoglobulin G’s Fc fragments. Liraglutide and semaglutide are obtained by inclusion of fatty-acid side-chain that paves way for reversible binding to albumin. These longer-acting peptides provide more convenience to the patient as they could be administered at much larger intervals while they reduce the fluctuations in activation of GLP-1R and help in maintaining consistent blood glucose levels. The tolerability and efficacy of the different GLP-1R agonists depends on their pharmacokinetics, structure, and size. A summary of characteristics of GLP-1R agonists is given in Table 1 and a summary of clinical trial programmes on GLP-1R agonists is given in Table 2.
### Table 1 Characteristics of GLP-1R agonists.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Chemistry</th>
<th>Compound half life (h)</th>
<th>Trade name (company)</th>
<th>Dosing (daily)</th>
<th>Elimination</th>
<th>Common adverse reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exenatide</td>
<td>Synthetic form of a naturally occurring parent compound exendin-4</td>
<td>2–3</td>
<td>Byetta (Bristol Myers Squibb-AstraZeneca)</td>
<td>10 μg (twice)</td>
<td>Renal glomerular filtration</td>
<td>Mild to moderate nausea, vomiting, and diarrhea</td>
</tr>
<tr>
<td>Lixisenatide</td>
<td>Based on exendin-4 with addition of six-lysine amino acids and deletion of proline at the C-terminus</td>
<td>2–3</td>
<td>Lyxumia (Sanofi)</td>
<td>20 μg (once)</td>
<td>Renal</td>
<td>Nausea and diarrhea related to the GI system</td>
</tr>
<tr>
<td>Liraglutide</td>
<td>Synthetic molecule with 97% homology with native GLP-1 due to addition of a 16 carbon fatty-acid side-chain at Lys26 and an Arg34Lys substitution</td>
<td>11–15</td>
<td>Victoza (Novo Nordisk)</td>
<td>1.2 mg (once)</td>
<td>General proteolysis, nonrenal</td>
<td>Nausea, vomiting, and diarrhea</td>
</tr>
<tr>
<td>Exenatide once weekly</td>
<td>Exenatide molecules captured in injectable microspheres to be delivered in a slow and continuous manner by diffusion and erosion of the microspheres</td>
<td>—</td>
<td>Bydureon (Bristol Myers Squibb-AstraZeneca Lilly)</td>
<td>2 mg (once weekly)</td>
<td>Renal glomerular filtration</td>
<td>Fewer incidence of nausea, diarrhoea, and vomiting</td>
</tr>
</tbody>
</table>
### Table 2 Clinical trial programmes on GLP-1R agonists.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Clinical trial programme name</th>
<th>Period (weeks)</th>
<th>Reduction in HbA1C baseline (%)</th>
<th>Reduction in FPG (mM)</th>
<th>Reduction in weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exenatide</td>
<td>AMIGO (Buse et al., 2004; DeFronzo et al., 2005; Kendall et al., 2005)</td>
<td>30</td>
<td>1.0–1.2</td>
<td>1.0–1.4</td>
<td>1.6</td>
</tr>
<tr>
<td>Lixisenatide</td>
<td>GetGoal (Ahre´n et al., 2013; Bolli et al., 2014; Fonseca et al., 2012; Pinget et al., 2013; Ratner et al., 2010; Riddle et al., 2013; Rosenstock et al., 2013; Seino et al., 2012)</td>
<td>24</td>
<td>0.5–0.9</td>
<td>0.8–1.2</td>
<td>1–3</td>
</tr>
<tr>
<td>Liraglutide</td>
<td>LEAD (Buse et al., 2009, 2010b; Garber et al., 2009; Marre et al., 2009; Nauck et al., 2009; Russell-Jones et al., 2009; Zinman et al., 2009)</td>
<td>26</td>
<td>0.8–1.5</td>
<td>2.6</td>
<td>2–3</td>
</tr>
<tr>
<td>Exenatide once weekly</td>
<td>DURATION (Bergenstal et al., 2010; Blevins et al., 2011; Buse et al., 2010a, 2013; Diamant et al., 2010)</td>
<td>24</td>
<td>1.9</td>
<td>2.3</td>
<td>1.6</td>
</tr>
</tbody>
</table>


2.1.1.1.1 Short-Acting GLP-1R Agonists

2.1.1.1.1.1 Exenatide—Twice Daily

Exenatide, under the trade name Byetta (Bristol Myers Squibb–AstraZeneca), was the first GLP-1R agonist approved by the US Food and Drug Administration (FDA) in 2005 (Davidson et al., 2005) and European Medications Agency (EMA) in 2007 while recommended by the National Institute for Health and Clinical Excellence (NICE) in 2008. Exenatide (AC2993) is the synthetic form of a naturally occurring parent compound exendin-4, a 39-amino acid-peptide secreted by the salivary glands of the desert Gila monster (Heloderma suspectum). While it is a potent agonist of mammalian GLP-1R, it shares a merely 53% sequence homology with human GLP-1 (Eng et al., 1992). Exenatide is resistant to DPP-4 degradation and hence has a longer half-life when compared to GLP-1. Half-life of exenatide after subcutaneous (s.c.) injection is approximately 2–3 h and is detectable in plasma up to 10 h after injection (Bray, 2006). Usual recommendation for exenatide is to be administered subcutaneously 5-μg twice daily that may be titrated up to 10-μg twice daily after an initial 1 month if well tolerated by the patient. Diabetes Management for improving Glucose Outcome (AMIGO) investigated the effects of exenatide twice daily (Buse et al., 2004; DeFronzo et al., 2005; Kendall et al., 2005). Significant reductions in glycated haemoglobin (HbA1c) of around 1.0–1.2% were observed in these trials when compared to placebo along with a modest 1.0–1.4 mM reduction in fasting plasma glucose (FPG) levels compared to placebo. Additionally, exenatide treated groups were found to have an average weight loss of 1.6 kg together with significant reduction of approximately 2.8 mmHg in systolic blood pressure when compared to placebo (Okerson et al., 2010). Interestingly, exenatide treatment group exhibited blunted postprandial glucose (PPG) excursions mainly due to significant deceleration in gastric emptying. This effect was evident only during meals with parallel drug administration and has been observed over a 30-week treatment period (DeFronzo et al., 2005). Glucose-dependent insulinotropic and glucagonostatic effects of exenatide reduce the risk of hypoglycemia during the usage of this drug (MacConell et al., 2012). Mild to moderate nausea, vomiting, and diarrhea are the main side effects of exenatide administration that often are less pronounced over time (MacConell et al., 2012). The use of exenatide twice daily is advocated by the NICE guidelines as an adjunctive in combination as a triple therapy with oral hypoglycemic agents, metformin, and either thiazolidinedione or sulfonylurea in diabetic patients whose glycemic control could not be achieved (NCCCC, 2008).
Lixisenatide has been approved for treatment of T2DM under the trade name Lyxumia (Sanofi) on Feb. 2013 (Elkinson and Keating, 2013; Palczewski et al., 2000). Similar to exenatide, lixisenatide is based on exendin-4 with an addition of six lysines and a deletion of a proline at the C-terminus (Christensen et al., 2011). Although these modifications result in a 2–3-h half-life after s.c. injection that suggest a twice-daily administration regimen, an acceptable tolerability and effect was observed from the clinical trials with a dose of 20-μg once daily (Ratner et al., 2010). A clinical trial programme named GetGoal tested the effectiveness of Lixisenatide (Ahren et al., 2013; Bolli et al., 2014; Fonseca et al., 2012; Pinget et al., 2013; Ratner et al., 2010; Riddle et al., 2013; Rosenstock et al., 2013; Seino et al., 2012). A significant reduction of approximately 0.5–0.9% in mean HbA1c levels and a moderate reduction of approximately 0.8–1.2-mM FPG was observed in lixisenatide treatment groups compared to placebo. A standardized meal test revealed a prominent 75% reduction in PPG excursion by lixisenatide presumably due to its pronounced decelerating effect on gastric emptying which is sustained even after 4 weeks of treatment (Amori et al., 2007; Lorenz et al., 2013; Okerson et al., 2010). Approximately 1–3 kg reduction in bodyweight was also observed after lixisenatide treatment (Barnett, 2011). Like other GLP-1R agonists, lixisenatide’s most prominent side effects are nausea and diarrhea. Head-to-head comparison with exenatide treatment revealed that lixisenatide is not inferior with respect to its effect on HbA1c while it has a better tolerance as seen by a lower incidence of vomiting, nausea, diarrhea, and hypoglycemia when compared to exenatide (Ratner et al., 2010), although its effect on weight reduction was less prominent (Rosenstock et al., 2013). An approval for lixisenatide was sought from the US-FDA on Feb. 2013 but was retrieved in Sep. 2013 as FDA wanted to review the data from ELIXA, an ongoing cardiovascular endpoint trial (Lagerstrom and Schioth, 2008).

Liraglutide, the second GLP-1 analog approved in 2009 in Europe and 2010 in USA under the trade name Victoza (Novo Nordisk). Liraglutide is a synthetic molecule produced using DNA recombinant technology. Compared to native GLP-1, it has an addition of a 16 carbon fatty-acid side-chain at Lys26 and an Arg34Lys substitution (Neumiller et al., 2010). After s.c. injection, only 1–2% of liraglutide circulates as free peptide in the plasma as the rest is noncovalently bound to albumin as ensured by the fatty acid side-chain (Zhang et al., 2012). This
prolongs the half-life of liraglutide to 11–15 h approximately and hence administration once daily is needed. A starting dose of 0.6 mg/dose is recommended for one week, and if well tolerated, it is increased to 1.2 mg/dose. The dosage can further be increased to 1.8 mg/dose if required. A clinical trial programme called Liraglutide Effect and Action in Diabetes (LEAD) studied the effectiveness of liraglutide (Buse et al., 2009, 2010b; Garber et al., 2009; Marre et al., 2009; Nauck et al., 2009; Russell-Jones et al., 2009; Zinman et al., 2009). HbA1c, FPG, and body weight levels were significantly reduced by 0.8–1.5%, 2.6 mM, and and 2–3 kg, respectively, in Liraglutide groups when compared to placebo. 1.7–2.5 mM reduction in PPG excursions in liraglutide treatment groups is attributed to its effect on gastric emptying (Degn et al., 2004). Moreover, occurrence of hypoglycemia was low. A 26-week randomized, multinational, open-label, parallel group study was conducted by Buse and coworkers, which compared the effect of liraglutide on a head-to-head trial with that of exenatide and Lixisenatide (Buse et al., 2009; Kapitza et al., 2013). Liraglutide brought about more reductions in HbA1c levels compared to that of exenatide (1.1% vs 0.8%) along with better control in FPG (1.6 vs 0.6 mM) (Buse et al., 2009). Exenatide on the other hand had better control in PPG after breakfast and dinner compared to liraglutide (1.9 vs 1.3 mM). Both exenatide and liraglutide performed equally in body weight reduction. When compared with lixisenatide, liraglutide performed better in reducing HbA1c level (0.3% vs 0.5%) and FPG level (0.3 vs 1.3 mM) while lixisenatide performed better in reducing PPG excursion after breakfast. Liraglutide brought about significant reductions in body weight reduction (2.4 vs 1.6 kg) as well when compared to lixisenatide (Kapitza et al., 2013).

2.1.1.2.2 Exenatide—Once Weekly Approved by the EMA in 2011 and US-FDA in 2012 under the trade name Bydureon (Bristol Myers Squibb-AstraZeneca), exenatide was modified to an extended-release preparation. Captured in injectable microspheres, exenatide molecules in this compound are delivered in a slow and continuous manner by diffusion and erosion of the microspheres. Administered once a week at a dose of 2 mg, therapeutic levels of plasma exenatide concentrations are obtained in 2–4 weeks of treatment while steady state level is reached at 6–7 weeks after treatment. A clinical trial programme called “Diabetes therapy Utilization: Researching changes in HbA1c weight and other factors Through Intervention with exenatide Once-weekly” (DURATION) examined the effectiveness of exenatide once-weekly formulation (Bergenstal et al., 2010; Blevins et al., 2011;
Buse et al., 2010a, 2013; Diamant et al., 2010). This programme had exenatide once-weekly formulation compared with exenatide twice daily in two head-to-head trials. Exenatide once weekly was found more effective compared to exenatide twice daily in reducing HbA1c level (1.9% vs 1.5%) and FPG level (2.3 vs 1.4 mM). Reductions in PPG excursions were greater in twice daily after breakfast and dinner that is attributed to a greater effectiveness of short-acting compound in deceleration of gastric emptying (Buse et al., 2013; Kim et al., 2007). Both compounds were equally effective in body weight reduction although comparatively higher incidence of nausea occurred in twice daily than once weekly (35% vs 14%). However, antiexenatide antibody formation (73% vs 51%) and injection site related adversities (13% vs 10%) were higher in once weekly. A head-to-head trial comparison was made between exenatide once weekly and liraglutide. In this study, while liraglutide had greater effectiveness in HbA1c reductions (1.5% vs 1.3%) and in weight reduction (3.9 vs 2.7 kg), exenatide once weekly was better tolerated with fewer incidence of nausea (9% vs 2.1%), diarrhea (6% vs 13%), and vomiting (4% vs 11%).

2.1.1.1.2.3 Albiglutide In late clinical development by GlaxoSmithKline, albiglutide is developed as continuous-acting peptide by covalent binding of DPP-4 resistant-GLP-1 analog to human albumin that leads to a reduced clearance by kidney. Half-life of the molecule is 5–8 days and once weekly dosage of 30 mg/dose is recommended (Bush et al., 2009; Matthews et al., 2008; Seino et al., 2009). When compared to exenatide twice daily by a clinical phase-2 study (Rosenstock et al., 2009), albiglutide was found to bring about greater reduction in HbA1c (0.9% vs 0.5%) and FPG (1.4 vs 0.8 mM) but less effective in bodyweight reduction (1.4 vs 2.4 kg). A clinical head-to-head trial programme called HARMONY compared the effectiveness of albiglutide and liraglutide (Pratley et al., 2012). Liraglutide was found to be more effective in reducing HbA1c level (1.0 vs 0.8 mM), FPG level (1.7 vs 1.2 mM), and body weight (2.2 vs 0.6 kg). However, tolerance level of albiglutide was better than liraglutide as seen by lower incidence of nausea (10% vs 29%), vomiting (5% vs 9%), and hypoglycemia (16% vs 21%) (Rosenstock et al., 2009).

2.1.1.1.2.4 Other GLP-1R Agonists Among the other emerging GLP-1R agonists are dulaglutide and semaglutide. In late clinical development by Eli Lilly, dulaglutide consists of two DPP-4 resistant GLP-1 molecules covalently bound to a modified IgG4 Fc fragment resulting in a 90-h
half-life \cite{Barrington2011,Glaesner2010,Grunberger2012}. Developed by Novo Nordisk, semaglutide is a compound that is structurally similar to liraglutide but with a larger linker molecule comprising of increased length of fatty acid derivative. Along with this, alanine in position 8 is modified to amino-isobutyric acid, and these modifications increases the albumin-binding efficiency and stability resulting in a half-life of around 160 h \cite{Kapitza2012,Nauck2012}. A phase 3 head-to-head clinical trial programme called SUSTAIN has been initiated to compare the efficiency of semaglutide and exenatide once weekly \cite{Nauck2012}.

2.1.1.2 DPP-4 Inhibitors

DPP-4 inhibitors comprise a diverse group of compounds that can be broadly divided into two categories; ones that mimic the structure of DPP-4 substrates and ones that are nonpeptidomimetic. Former class includes sitagliptin \cite{Kim2005,Vincent2007}, vildagliptin \cite{He2009,Villhauer2003}, and saxagliptin \cite{Augeri2005}, while the latter class includes alogliptin \cite{Augeri2005,Karim2007} and linagliptin \cite{Blech2010,Eckhardt2007}. Developed for therapeutic use, these compounds are competitive reversible inhibitors displaying high affinities for DPP-4, with inhibition constants ($K_i$) in low nanomolar range \cite{Augeri2005,Burkey2008,Kirby2008,Thomas2008}. The mode of interaction with the enzyme, however, is different between the compounds. Alogliptin, linagliptin, and sitagliptin form noncovalent interactions with residues in the catalytic site to inhibit the function of DPP-4 \cite{Eckhardt2007,Feng2007,Kim2005}. Saxagliptin and vildagliptin inhibit DPP-4 in a two-step process, the first step involving a slow rate of inhibitor binding by the formation of a reversible covalent enzyme-inhibitor complex and the second step involving a slow rate of inhibitor dissociation so that the enzyme slowly equilibrates between the active and inactive forms \cite{Brandt2005,Burkey2006,Kim2006}. The catalytic activity of the enzyme is inhibited even after the clearance of the drug from the circulation and this explains the inhibition of DPP-4 activity by vildagliptin and saxagliptin even beyond their short half-lives. A summary of characteristics of DPP-4 inhibitors is given in Table 3.

2.1.1.2.1 Sitagliptin

Sitagliptin under the trade name Januvia (Merck) was the first agent developed in the class of DPP-4 inhibitor
<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Chemistry</th>
<th>Compound half life (h)</th>
<th>Trade name (company)</th>
<th>Dosing (daily)</th>
<th>Metabolism</th>
<th>Elimination</th>
<th>Common adverse reaction</th>
<th>Elimination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitagliptin</td>
<td>β-Amino acid based</td>
<td>8–24</td>
<td>Januvia (Merck)</td>
<td>100 mg (once)</td>
<td>Not significantly metabolized</td>
<td>Renal (~80% unchanged as parent)</td>
<td>Upper respiratory tract infection, nasopharyngitis, headache</td>
<td>Renal (~80% unchanged as parent)</td>
</tr>
<tr>
<td>Saxagliptin</td>
<td>Cyanopyrrolidine</td>
<td>2–4 (parent) 3–7 (metabolite)</td>
<td>Onglyza (Bristol-Myers Squibb/AstraZeneca)</td>
<td>5 mg (once)</td>
<td>Hepatic metabolism to active metabolite (via P450 3A4/5)</td>
<td>Renal (12–29% as parent, 21–52% as active metabolite)</td>
<td>Upper respiratory tract infection, urinary tract infection, headache</td>
<td>Renal (12–29% as parent, 21–52% as active metabolite)</td>
</tr>
<tr>
<td>Vildagliptin</td>
<td>Cyanopyrrolidine</td>
<td>1.5–4.5</td>
<td>Galvus (Novartis)</td>
<td>50 mg (twice)</td>
<td>Inactive metabolite formed by hydrolyzation (P450 independent)</td>
<td>Renal (22% as parent, 55% as primary metabolite)</td>
<td>Nasopharyngitis, dizziness, headache, back pain, diarrhoea, and upper respiratory tract infection</td>
<td>Renal (22% as parent, 55% as primary metabolite)</td>
</tr>
<tr>
<td>Linagliptin</td>
<td>Xanthine based</td>
<td>10–40</td>
<td>Tradjenta (Boehringer Ingelheim Pharmaceuticals and Eli Lilly)</td>
<td>5 mg (once)</td>
<td>Not significantly metabolized</td>
<td>Biliary (&gt;70% unchanged as parent); &lt;6% through kidney</td>
<td>Nasopharyngitis, cough, hyperlipidemia, hypertriglyceridemia, and weight increase (when administered in combination with pioglitazone)</td>
<td>Biliary (&gt;70% unchanged as parent); &lt;6% through kidney</td>
</tr>
</tbody>
</table>
approved by FDA in Oct. 2006. It is available in 25-, 50-, and 100-mg tablets. It has been used as a monotherapy or in combination with other diabetic agents such as metformin, sulfonylureas, and pioglitazone. A sitagliptin/metformin combination was approved by the FDA in Apr. 2007 and is available under the trade name Janumet (Merck) in doses of 50/500 mg or 50/1000 mg. In a monotherapy trial, 743 diabetic patients were randomized to receive 5, 12, 25, or 50 mg of sitagliptin twice daily (Scott et al., 2007). After 12 weeks of treatment, all doses of sitagliptin resulted in a strong reduction in HbA1c levels ranging from 0.38% to 0.77% with 50-mg twice daily producing the greatest effect. The effect of sitagliptin on body weight was neutral and with very low incidence of hypoglycemia. Common adverse reactions are upper respiratory tract infection, nasopharyngitis, and headache. Since the renal and hepatic pathways are involved in the elimination of oral doses of sitagliptin, it has also been tested in patients with renal and hepatic insufficiency. After ingestion of carbon-14 labeled sitagliptin, approximately 13% was found in feces and 87% in urine. Of the 87% in urine, 24% was unchanged structurally while 36% was active metabolites of the parental compound (Merck and Co, 2010). Half-life of saxagliptin is 12.4 h (Merck and Co, 2010). Evaluated in patients with various degrees of renal insufficiency: mild (CL_{CR}, 50–80 mL/min), moderate (CL_{CR}, 30–50 mL/min), and severe (CL_{CR}, <30 mL/min) and end-stage renal disease, sitagliptin levels in plasma were found to be twofold increased in patients with mild renal insufficiency and 2.3–4.5-fold increased in patients with moderate to severe renal insufficiency (Bergman et al., 2007). A double-blind, randomized, crossover, multicenter study was conducted by DeFronzo and coworkers to compare the effectiveness of exenatide versus sitagliptin in subjects with T2DM currently receiving metformin therapy (DeFronzo et al., 2008). Exenatide 5-μg twice daily was administered to patients for a week after which 10-μg twice daily was given for another week. Following this, patients were crossed over to alternate therapy. From the results, it was observed that in patients receiving exenatide, 2-h PPG levels were significantly reduced compared to subjects receiving sitagliptin (133 vs 208 mg/dL) Switching to sitagliptin from exenatide produced an increase of 73 mg/dL in 2-h PPG level during the crossover period. Vice versa, switching from sitagliptin to exenatide produced a decrease of 73 mg/dL in 2-h PPG level. Similar reductions in FPF levels (15 vs 19 mg/dL) were found in exenatide and sitagliptin treatment. Exenatide, however, reduced the caloric intake to a greater extent (−134 vs +130 kcal) and had a significantly greater effect on the
insulinogenic index and reduction in glucagon content. The advantages exhibited by exenatide had significant clinical implications. A similar multicenter open-label, parallel group study was conducted by Pratley and coworkers to compare the effectiveness of liraglutide and sitagliptin in T2DM patients currently on metformin therapy (Pratley et al., 2010). Subjects were randomized to receive 100-mg oral sitagliptin once daily or 1.2- or 1.8-mg liraglutide once daily. Results provided evidence that compared to sitagliptin, both doses of liraglutide exhibited superior effectiveness in glycemic control (−0.9, −1.5, and −1.24%, respectively). All treatment groups had low incidence of hypoglycemia. However, 1.8 mg (27%) and 1.2 mg (21%) liraglutide treatment group had higher incidence of nausea than 100 mg sitagliptin (5%). The very small number of adverse side effects in subjects with sitagliptin treatment provides evidence for their potential space in clinical usage.

2.1.1.2.2 Saxagliptin Approved by the FDA in 2009 under the trade name Onglyza (Bristol-Myers Squibb/AstraZeneca), saxagliptin is the second DPP-4 inhibitor in the market. It is taken in the dosage of 2.5–5 mg/day orally. Peak level of saxagliptin in plasma occurs within 2 h following ingestion, with a 4-h peak for its active metabolite (Squibb, 2009). Half-life of saxagliptin is 2.5 h while its active metabolite has a half-life of 3.1 h. The active metabolite, BMS-510849, retains 50% activity of its parent compound (Neumiller, 2011). Since its metabolism is mediated by the cytochrome P450 (CYP 450) 3A4/5 system, their inhibitors and inducers are known to affect the concentration of saxagliptin (Augeri et al., 2005; Squibb, 2009). As the renal/hepatic systems are involved in the elimination of saxagliptin, it has been tested in patients with renal and hepatic insufficiency. $C_{\text{max}}$ of saxagliptin was unaffected in patients with different levels of renal insufficiency. Plasma area under curve (AUC) levels of saxagliptin and its metabolite were increased 20 and 70%, respectively, in subjects with mild renal insufficiency and this increase was not considered clinically relevant. In subjects with moderate to severe renal impairment, a 2.1- and 4.5-fold higher plasma AUC levels were observed (Squibb, 2009). Given these high values, dosage has to be adjusted in patients with moderate to severe renal insufficiency. A randomized placebo control study was conducted in 743 patients with T2DM to receive 2.5-, 5-, or 10-mg saxagliptin once daily in addition to 1500–2550 mg/dL metformin (Defronzo et al., 2007). After 24 weeks, subjects receiving 2.5-, 5-, or 10-mg saxagliptin once daily plus metformin exhibited significant decrease in
HbA$_{1C}$ (0.73, 0.83, and 0.71%, respectively) compared with placebo. Decreased postprandial glucagon level, increased postprandial insulin, and C-peptide level were found after saxagliptin treatment, while no change in hypoglycemia and weight were observed (Defronzo et al., 2007). A 18-week double-blind, randomized, multicenter, noninferiority trial was conducted by Scheen and coworkers to analyze the effectiveness of sitagliptin and saxagliptin as add-on therapies to metformin in patients whose T2DM was inadequately controlled (Scheen, 2012). In addition to metformin, 5-mg saxagliptin or 100-mg sitagliptin was used. HbA$_{1C}$ levels were reduced by −0.52 and −0.62% from baseline with saxagliptin or sitagliptin, respectively. Saxagliptin and sitagliptin are similar in their effects in glycemic control.

2.1.1.2.3 Vildagliptin

Vildagliptin available under the trade name Galvus (Novartis) in the class of DPP-4 inhibitor is approved for use as monotherapy or combination therapy for treatment of T2DM. Also available is a fixed-dose combination of vildagliptin/metformin (Eucreas). Generally used dosage is 50-mg once or twice daily (Boji et al., 2007; Garber et al., 2007, 2008). Vildagliptin is a potent, selective inhibitor of DPP-4 and binds covalently to the catalytic site of DPP-4 (Ahren et al., 2011; He, 2012). Binding of vildagliptin to DPP-4 active site is a two-step, reversible, competitive process involving a rapid binding followed by a slow dissociation (tight-binding phase) while sitagliptin binds noncovalently and reversibly as a simple competitive inhibitor leading to a rapid binding and dissociation from DPP-4. A 50% inhibition of DPP-4 activity was achieved by using vildagliptin concentration of 4.5 nmol/L (He et al., 2007c). A single dose of 50-mg vildagliptin to healthy subjects resulted in a mean maximum plasma concentration (C$_{max}$) of 245 ng/mL reached in a median time (t$_{max}$) of 1.5 h (He et al., 2007b). Hydrolysis by multiple organs is the main elimination pathway for vildagliptin. Hydrolysis of the cyano group of the parent drug results in formation of LAY151 (M20.7) that is the major pharmacologically inactive carboxylic acid metabolite (EMA, 2013; He et al., 2009). LAY151 accounted for 55% of circulating radioactivity while 26% of unchanged drug was observed in the circulation (He et al., 2009). Vildagliptin does not interact with cytochrome P450 enzyme and is not metabolized by the same (EMA, 2013). Vildagliptin’s main mode of elimination is via urinary excretion as LAY151 (He et al., 2009). After 168 h following oral administration of radiolabeled vildagliptin, an average of 85 and 15% of radioactivity was recovered in the urine and feces, respectively, with >90% recovered in the first 48 h (He et al., 2009). The elimination
The half-life of oral vildagliptin was approximately 2–3 h (He et al., 2007b). The steady-state vildagliptin AUC levels from time 0 to 24 h was observed to be increased by 40, 71, and 100% in patients with mild, moderate, or severe renal impairment, respectively (He et al., 2013). EU SPC recommends 50-mg once daily in patients with moderate or severe renal impairment while vildagliptin/metformin single-pill is not recommended for usage in patients with creatinine clearance (CLCR) of <60 mL/min as metformin is not suitable for this patient population (EMA, 2013). Monotherapy with 50-mg twice-daily vildagliptin significantly reduced the mean HbA1C baseline (−0.7 to −0.9%) and FPG levels (−1.4 vs 0.1 mmol/L) compared to placebo (Dejager et al., 2007; Kikuchi et al., 2009; Pi-Sunyer et al., 2007). Randomized, double-blind, multicenter trials of 12 weeks to 2 years were performed to compare the efficacy of vildagliptin with other drugs such as metformin (Schweizer et al., 2007), gliclazide (Foley and Sreenan, 2009), voglibose (Iwamoto et al., 2010), and acarbose (Pan et al., 2008). Based on the reduction of HbA1C baseline, metformin performed better than vildagliptin while there were no significant differences in HbA1C levels between vildagliptin and gliclazide or acarbose recipients while voglibose was found less effective.

2.1.1.2.4 Linagliptin

Approved by the FDA in 2011, linagliptin is available under the trade name Tradjenta (Boehringer Ingelheim Pharmaceuticals and Eli Lilly). Linagliptin is a xanthine-derived competitive, reversible DPP-4 inhibitor (Eckhardt et al., 2007; Thomas et al., 2008). Compared with IC50 values of 19, 50, and 62 nmol/L for sitagliptin, saxagliptin, and vildagliptin, respectively, the reported value for linagliptin is 1 nmol/L (Thomas et al., 2008). A sustained inhibition of DPP-4 is obtained with once-daily oral doses of 5–10-mg linagliptin (Heise et al., 2009) with a half-life (t1/2) of 131 h (Hüttner et al., 2008) and the steady state concentration can be achieved after 3 doses of 5 mg/day (Boehringer Ingelheim Pharmaceuticals, 2011). The long half-life is attributed to linagliptin’s extensive binding to plasma proteins and its high affinity binding to DPP-4 enzyme resulting in a nonlinear pharmacokinetic profile (Fuchs et al., 2009; Heise et al., 2009). Oral bioavailability of the drug is approximately 30% (Reltich et al., 2010). Elimination of linagliptin is primarily hepatic (85%) and some in the kidney (5%) (Blech et al., 2010; Hüttner et al., 2008). The main metabolite (CD1790) is pharmacologically inactive (Blech et al., 2010) and no dosage adjustment has been recommended for subjects with hepatic (Boehringer Ingelheim Pharmaceuticals, 2011) or renal impairment.
A multicenter, randomized, parallel-group, placebo-controlled clinical trial was conducted to assess the efficacy and tolerability of 5-mg/day linagliptin for over 24 weeks (Del Prato et al., 2011). The end-point changes in HbA1C levels from baseline were −0.5% which was significantly reduced from that of placebo (+8%). Significant reductions in FPG and PPG levels with adjusted mean differences of −23.4 and −57.7 mg/dL were observed in linagliptin treatment group compared to placebo. A double blind, 104-week trial compared the efficacy of linagliptin and glimepiride as a treatment adjunctive to metformin in T2DM patients whose blood glucose were inadequately controlled and both linagliptin and glimepiride were found to be equally effective (Gallwitz et al., 2011). As indicated in the package insert, nasopharyngitis, cough, hyperlipidemia, hypertriglyceridemia, and weight increase (when administered in combination with pioglitazone) are the adverse side effects in more than 2% of study participants treated with linagliptin.

**2.1.2 Weight Reduction and Energy Intake**

It is a well-established fact that increased weight is associated with increased incidence of T2DM and increase in mortality and morbidity in patients with T2DM. Merely a 1-kg weight loss has been shown to be significantly beneficial in the systematic review by Ross and coworkers who concludes that an appropriate antidiabetic therapy should consider weight management as an essential factor (Ross et al., 2011). Several systematic reviews and metaanalyses have confirmed the ability of GLP-1R agonists to produce significant weight loss (Aroda et al., 2012; Shyangdan et al., 2010) as aforementioned in Section 1.1.1. It could be noted that DPP-4 inhibitors are weight neutral (Karagiannis et al., 2012) as discussed in Section 1.1.2. Along with glycemic control, GLP-1 receptor agonists therefore are useful also in bringing about weight loss in T2DM patients (Madsbad, 2012). GLP-1 physiologically functions to inhibit intestinal motility and reduces gastric emptying. Importantly, while central GLP-1 has been found to act through arcuate nucleus to induce satiety, infusions of peripheral GLP-1 has been shown to dose-dependently bring about a significant reduction in food intake in obese subjects with or without T2DM.

**2.1.3 Pancreatic β-Cell Pathogenesis**

Pathogenesis of T2DM is associated with a reduced β-cell mass thereby hindering the initial phase of insulin response. If the first and second line antidiabetic agents cannot effectively bring about glycemic control, insulin is given as the final therapy which is often time associated with the unwanted
hypoglycemia. The importance of an antidiabetic agent that simultaneously improves β-cell function is clearly evident. An initial study by Farilla and coworkers on the effects of GLP-1 in isolated human pancreatic islets concluded that GLP-1 improves β-cell morphology and function, as well as inhibits apoptosis (Farilla et al., 2003). Furthermore, it was found that GLP-1 enhances or restores in vivo glucose sensing and sulfonylurea sensitivity in human diabetic β-cells through unknown mechanisms (Gutniak et al., 1996). A diagrammatic representation of signaling pathways triggered by GLP-1 in pancreatic β-cell is provided in Fig. 2 (Campbell and Drucker, 2013). The robust stimulation of β-cell proliferation and protection by GLP-1R signaling in rodents have provoked interest in whether incretin therapy could be beneficial to T2DM patients in this aspect. An in vitro study showed the protective effects of GLP-1R agonists on rat β-cells (Cunha et al., 2009). Similarly, vildagliptin has been shown to improve β-cell function in vivo in rats (Duttaroy et al., 2011). Initial clinical studies to analyze effects of GLP-1R agonists on human β-cell function did not provide meaningful results (Gallwitz et al., 2012; Garber et al., 2011). Bunck and coworkers conducted a study in a small number of subjects randomly receiving intensive therapy with exenatide at higher than clinically approved doses, versus insulin glargine, for 3 years and compared the β-cell function by sequential glucose clamp studies (Bunck et al., 2011). No meaningful differences were
observed in β-cell function across different treatment groups. Exenatide-treated patients exhibited increased disposition index 4 weeks after cessation of therapy, yet it is unclear if this difference is a direct effect of exenatide on β-cell function or an indirect benefit effect secondary to its effect on weight loss as the patients had significant weight reduction. Hence, so far, the aforementioned studies cannot substantiate the hypothesis that GLP-1R agonists improve β-cell function.

### 2.1.4 Renal Impairment

T2DM patients also suffer from progressive deterioration of renal function leading to chronic kidney disease (CKD) (Levey et al., 2003). CKD is characterized by five stages depending on the glomerular filtration rate. To prevent further damage in CKD patients, it is of paramount importance to reach glycemic control. On the contrary, many antidiabetic agents require dosage adjustments depending on the degree of renal impairment (Cavanaugh, 2007). Even within the same class of drugs, there could be pharmacokinetic differences depending on their suitability to patients with renal damage. Exenatide and liraglutide are immensely different in their pharmacokinetic profiles as exenatide is eliminated primarily through kidneys while liraglutide undergoes proteolysis (Neumiller, 2011). Through studies in pigs, it has been shown that exenatide is eliminated solely by glomerular filtration (Simonsen et al., 2006). Evaluated in patients with various degrees of renal insufficiency: mild (CL_CR, 50–80 mL/min), moderate (CL_CR, 30–50 mL/min), and severe (CL_CR, <30 mL/min) and end-stage renal disease, exenatide was found to be well tolerated in patients with mild and moderate renal insufficiency (RI) but not in patients with severe and end-stage RI as there were adverse events of vomiting and nausea (Linnebjerg et al., 2007). The half-life of twice-daily 5-μg exenatide in healthy, mild, moderate, and end-stage RI patients was 1.5, 2.1, 3.2, and 6.0 h, respectively. Notably, mild and moderate RI patients were found to have systemic exposure of exenatide once weekly by 23 and 74%, respectively. Therefore, exenatide once weekly is suitable for patients with mild RI and is not to be prescribed for patients with moderate RI and above (EMA, 2012d). As opposed to exenatide, liraglutide undergoes proteolysis and is not removed through kidney. Through studies involving radiolabeled liraglutide (Malm-Erjefält et al., 2010; Neumiller, 2011), it was found that the drug is metabolized and cleaved by DPP-4 similar to endogenous GLP-1, but at a much slower rate. There is no excretion of the intact drug indicating that it is completely metabolized. Clinical studies have also shown that renal or
hepatic insufficiencies do not alter the pharmacokinetic properties of the drug. In a clinical study, liraglutide (0.75 mg) was administered subcutaneously to 30 subjects with varying degree of RI (Jacobsen et al., 2009), and there were no differences in AUC between patients with healthy renal function and severe RI. Decreased CL_{CR} had no effect on the pharmacokinetic profile of liraglutide. This study concluded that liraglutide is safe for patients with all degree of RI. The lack of long-term studies, however, has led to liraglutide being classified as not recommended for patients with severe RI (EMA, 2012f). Lixisenatide has recently been shown to be well tolerated and safe for patients with renal insufficiency (Fonseca et al., 2012) but similar to liraglutide, the lack of long-term study has also led to its classification as not recommended.

DPP-4 inhibitors vary hugely in their chemical structure and therefore have differences in their structure activity relationships and pharmacokinetic profiles (Neumiller, 2011). Sitagliptin is eliminated essentially by kidney, and in an open-label study involving 30 subjects with various degrees of RI, 50-mg oral dose of sitagliptin revealed a significant increase in AUC in patients with mild, moderate, severe, and end-stage RI (1.61-, 2.26-, 3.77-, and 4.5-fold, respectively) (Bergman et al., 2007). Hence, the recommended doses are 100-, 50-, and 25-mg daily in subjects with healthy renal function, moderate RI, and severe RI patients, respectively. Hepatic impairment, as would be predicted, did not alter the pharmacokinetic profile of sitagliptin (Migoya et al., 2009). On the contrary, saxagliptin is the only DPP-4 inhibitor that undergoes a significant hepatic biotransformation by cytochrome P450 (CYP)3A4 to form an active metabolite BMS-510849 (Neumiller, 2011). An open-label study in subjects with varying degree of hepatic insufficiency (HI) given 10 mg of saxagliptin daily revealed unfavorable Child Pugh scores (measure of HI) and reduced saxagliptin clearance (Boulton et al., 2011). An increase in AUC values of saxagliptin from +10% to +77% with a corresponding decrease in BMS-510849 from −7% to −33% were observed in patients with an increasing severity of HI. Potency of BMS-510849 to inhibit DPP-4 is twofold less than saxagliptin and therefore safety related to usage of saxagliptin is a prime concern in patients with HI. In addition, an open-label study on saxagliptin with a dose of 10-mg daily in patients with RI revealed that severe RI raised AUC values of saxagliptin and BMS-510849 2.1- and 4.5-fold, respectively, and hence a dose adjustment to 2.5 mg is recommended (EEIG, 2012).

Vildagliptin is metabolized extensively by hydrolysis, glucuronidation, and oxidation before being eliminated (He et al., 2007b, 2009). An in vitro
A study revealed that vildagliptin undergoes hydrolysis in liver while 23% was found to be excreted unchanged from the kidney (Neumiller, 2011). An open-label study in subjects with varying HI given a single oral dose of 100-mg vildagliptin showed no difference in AUC or $C_{\text{max}}$ of vildagliptin (He et al., 2007a). Since 23% of vildagliptin is excreted unchanged from kidney, EU guidelines suggest a dosage adjustment in patients with moderate to severe RI (EMA, 2013). Linagliptin is a novel xanthane-based DPP-4 inhibitor. An open-label study involving 51 subjects revealed that elimination and exposure of linagliptin is unaltered in RI patients (Graefe-Mody et al., 2011), and it can therefore be prescribed to patients with any degree of RI with no dosage adjustment prerequisite. Prescription characteristics of GLP-1R agonists and DPP-4 inhibitors for patients with renal and hepatic insufficiency are provided in Table 4.

### 2.1.5 Cardiovascular Effect

Incretin therapy offers important cardiovascular protective effects in addition to glycemic control. Attributed to the difference in pharmacological actions of GLP-1R agonists mentioned before, they exert greater effects on triglyceride and cholesterol as well as systolic blood pressure (BP) reduction than DPP-4 inhibitors (Graefe-Mody et al., 2011; Moretto et al., 2008; Pratley et al., 2010). A modest reduction in systolic BP of $\sim1$–7 mmHg has been reported for GLP-1R agonists but not DPP-4 inhibitors (Bergenstal et al., 2010; Morales, 2011). Exenatide twice daily was found to be more effective than sitagliptin in lowering triglyceride level (DeFronzo et al., 2008). Similarly, liraglutide (1.2 and 1.8 mg) was found to be more effective in decreasing triglyceride and cholesterol level than once daily 100-mg sitagliptin (Pratley et al., 2010). GLP-1R agonists and DPP-4 inhibitors have also been shown to have cardioprotective effect in ischemia reperfusion injury. Mechanisms such as the efficacy of GLP-1 to ameliorate insulin resistance in cardiomyocytes and increase glucose transport protein have been proposed (Nikolaidis et al., 2004a; Noyan-Ashraf et al., 2013; Villanueva-Peñacerrillo et al., 2001). In the EXAMI study, although high doses of exenatide did not affect left-ventricular function or area at risk, a trend of smaller infarct size was observed (Bernink et al., 2013). In human subjects with elevated myocardial infarction undergoing primary percutaneous coronary intervention, exenatide administration in a randomized, placebo-controlled trial resulted in a reduction in infarct size and increased myocardial salvage (Lønborg et al., 2012). When infused with recombinant GLP-1 for 72 h following angioplasty, patients with acute myocardial infarction showed improvements in
### Table 4  Prescription characteristics of GLP-1R agonists and DPP-4 inhibitors for patients with renal and hepatic insufficiency.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>References</th>
<th>Mild (CrCl ( \geq ) 50–&lt;80 mL/min)</th>
<th>Moderate (CrCl ( \geq ) 30–&lt;50 mL/min)</th>
<th>Severe/ESRD (CrCl &lt;30 mL/min)</th>
<th>Hepatic insufficiency</th>
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<td>Exenatide</td>
<td>EU (SPC)</td>
<td>✓</td>
<td>✓ With caution</td>
<td>×</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>US (PI.)</td>
<td>✓</td>
<td>✓ With caution</td>
<td>×</td>
<td>✓ Clinical experience lacking</td>
</tr>
<tr>
<td>Lixisenatide</td>
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<td>✓ With caution</td>
<td>×</td>
<td>✓ Limited clinical experience</td>
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<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>EU (SPC)</td>
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<td>× Limited clinical experience</td>
<td>× Limited clinical experience</td>
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<tr>
<td></td>
<td>US (PI.)</td>
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<td>✓ With caution</td>
<td>✓ With caution due to limited clinical experience</td>
</tr>
<tr>
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<td>× Limited clinical experience</td>
<td>×</td>
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<tr>
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</table>

Mild to moderate. Not studied for severe

Clinical experience lacking

Boehringer Ingelheim Pharmaceuticals, I.T.P.
PL., A.P.B.
PL., B.-M.S.O.
PL., M.S.D.C.J.
PL., N.N.V.
SPC, A.P.B.
SPC, N.N.V.
SPC., A.P.B.
SPC., B.-M.S.O.
SPC., M.S.D.C.J.
SPC., N.G.
regional and global left-ventricular functions (Nikolaidis et al., 2004b). Similarly, in patients having coronary artery-disease with preserved left ventricular function, a placebo-controlled trial revealed that increase in GLP-1 levels due to DPP-4 inhibition have led to the amelioration of postischemic stunning along with improvements in regional and global left-ventricular function (Khan et al., 2010). In a combination trial with sitagliptin and G-CSF based stem cell mobilization named SITAGRAMI-Trial, promising results were demonstrated (Theiss et al., 2010). Therapeutic effect of GLP-1 in failing heart have also been investigated, and attenuation of insulin resistance by GLP-1 being the prime mechanism behind (Ingelsson et al., 2005). Nikolaidis and coworkers in 2004 demonstrated that after infusion of recombinant GLP-1 in canine models of pacing-induced dilated cardiomyopathy, systolic/diastolic function and other hemodynamic parameters were improved (Nikolaidis et al., 2004a). Since then several studies with GLP-1 agonists and DPP-4 inhibitors have supported the results in various animal models (Bhashyam et al., 2010; Liu et al., 2010; Vyas et al., 2011). Many earlier studies in heart failure patients infused with GLP-1 have shown improved cardiac profiles (Thrainsdottir et al., 2004). One such study by Sokos and coworkers demonstrated the infusion of GLP-1 improved left-ventricular ejection fraction, myocardial ventilation oxygen consumption in heart failure patients with or without T2DM (Sokos et al., 2006). However, some other studies have also reported that only minor cardiovascular effect in heart failure patients without T2DM was found (Halbirk et al., 2010). Nathason and others have reported positive chronotropic effect and favorable improvements in cardiac index and hemodynamic in male T2DM patients with chronic heart failure infused with exenatide, yet the direct effect of exenatide in such patients remained unclear (Nathanson et al., 2012). To clearly elucidate the effects of GLP-1 in chronic heart failure patients, long term and detailed studies are required (Munaf et al., 2012). More recently, several reports were published regarding the vasoprotective actions of GLP-1R agonists and DPP-4 inhibitors against endothelial dysfunction and atherosclerosis (Ding and Zhang, 2012; Nathanson et al., 2009). With regard to the cardiovascular effects of GLP-1R agonists and DPP-4 inhibitors, it is important to delineate if the effects observed are direct effects of the drug or indirect secondary effects (Sheikh, 2013).

2.1.6 Other Actions

Apart from its main pharmacological actions described in detail earlier, other actions of GLP-1R agonists include reduction in lipogenesis of white
adipose tissue, increase in thermogenesis of brown adipose tissue, neuroprotective actions, and antiinflammatory functions (Campbell and Drucker, 2013; McClean et al., 2007).

2.2 Safety and Tolerability Concerns

Clinical studies involving GLP-1R agonists and DPP-4 inhibitors demonstrated that both groups of drugs are safe. Hypoglycemia, an obvious risk factor when using glucose-lowering agents, is monitored closely in all trials involving incretin therapy. Generally, it has been seen that DPP-4 inhibitors have slightly better tolerability than GLP-1R agonists with respect to adverse effects like nausea, GI upset, and vomiting (Amori et al., 2007; Scheen, 2012). The primary safety concerns in incretin therapy are given further in the chapter and are addressed in clinical trials before approval.

2.2.1 Pancreatitis

Pancreatitis initially emerged as a potential side effect of exenatide therapy as case reports and subsequently through numerous reports by FDA (EMA, 2012e). Amylin Corporation responded by suggesting that this is a consequence of the association of obesity and T2DM with pancreatitis rather than the effect of the drug itself (Whitcomb, 2004). Liraglutide was also reported to be associated with pancreatitis (Buse et al., 2009) and recently there have been more than 80 documented cases reported by the FDA on pancreatitis in patients treated with sitagliptin (US-FDA, 2009). Merck also holds a stand similar to Amylin Corporation (S, 2009). It is indeed true that patients with T2DM exhibit a twofold to threefold increased risk of being affected by acute pancreatitis (Noel et al., 2009). Two insurance claims databases were utilized for an analysis and the results indicated that usage of most antidiabetic agents carry potential pancreatitis risk in an equal measure (Dore et al., 2009; Garg et al., 2010). It was shown by one particular analysis that incidence rates of pancreatitis per 1000 patients per year for exenatide twice daily, sitagliptin and metformin were 5.7, 5.6, and 5.6, respectively. Regardless of the aforementioned study, manufacturers of the incretin therapies are required to perform further epidemiological clinical studies pertaining to the incidence of acute pancreatitis as requested by the FDA (Parks and Rosebraugh, 2010). It has also been recommended by the FDA that patients at a particular high risk of pancreatitis should avoid exenatide (EMA, 2012e) while usage of liraglutide (EMA, 2012f) should be closely monitored. Safety information on DPP-4 inhibitor prescription to patients on pancreatitis risk is not available (EMA, 2012a,c). Patient education on early spotting of the signs and
symptoms of pancreatitis is a possible measure to help reduce the associated complications (Morales, 2011).

2.2.2 Cancer Risk

GLP-1 based incretin therapies have been implicated to increase the potential risk of pancreatic cancer and thyroid C-cell carcinoma. As mentioned in Section 2.1, incretin therapy has a potential risk of pancreatitis, which is known to have a predisposition toward pancreatic cancer (Lowenfels et al., 1993). Chronic pancreatitis has been shown to increase the risk of pancreatic cancer 26-fold (Lowenfels et al., 1993). Both FDA and German regulatory databases indicate signs for development of pancreatic cancer for exenatide and FDA database for sitagliptin (Elashoff et al., 2011; Spranger et al., 2011). There were 258 pancreatic cancers reported for exenatide, 63 for liraglutide, 81 for sitagliptin, 18 for saxagliptin, and 1 for linagliptin. Similar to pancreatitis, the incidence of pancreatic cancer is higher in T2DM patients (Wideroff et al., 1997), therefore, the incidence of pancreatic cancer due to other factors such as age and T2DM have to be differentiated. Chronic inflammation and enhanced intraductal pressure due to stenosis of the pancreatic duct occurring secondary to the development of chronic pancreatitis could lead to the development of pancreatic carcinoma (Bhanot and Möller, 2009; Strobel et al., 2010; Vincent et al., 2011). It is unclear if acute pancreatitis has similar consequences while most incidences associated with incretin therapy are acute pancreatitis (Cure et al., 2008). More reliable long-term clinical studies covering a minimum of 5–6 years are required to correlate incretin drugs and the development of malignancy in the pancreas in the future (Nauck and Friedrich, 2013).

Thyroid cancer is a rare disease (Aschebrook-Kilfoy et al., 2011). In all cases of thyroid cancer, ~2% female and <4% male patients are diagnosed with medullary thyroid cancer with an incidence that increases with age (Aschebrook-Kilfoy et al., 2011). GLP-1R stimulation in rodents is known to increase cAMP levels in thyroid C cells initiating the release of calcitonin (CCT) and subsequently on long-term exposure induces C-cell proliferation and formation of C-cell adenomas and carcinomas (medullary thyroid) (Bjerre Knudsen et al., 2010). Once-daily injection of liraglutide was found to induce C-cell abnormalities in mice and C-cell carcinomas in male rats (Bjerre Knudsen et al., 2010; Elbrønd et al., 2002). Treating rodent C-cell derived cell-lines with GLP-1, exenatide, and liraglutide could induce cAMP and CCT release while the same effect was absent in human cells even at a high concentration of 1 μM (Gier et al., 2011). Clinical trials with
incretin therapy tested for changes in CCT concentrations also give similar results (Agersø et al., 2002; Elbrønd et al., 2002). Lower expression levels of GLP-1R in human thyroid C-cells compared to those in rodents are likely the reason for the observed differences. However, recently immunohistochemical stainings for GLP-1R have shown its expression in human C- and follicular cells (Hegeduš et al., 2011) although another study using a radio-ligand assay failed to confirm these data (Waser et al., 2010). Higher reported cases of thyroid cancer on both liraglutide (57 events) and exenatide (74 events) were shown in adverse event reports by FDA (Elashoff et al., 2011) but not in DPP-4 inhibitors (Butler et al., 2013; Spranger et al., 2011). Long-term clinical studies are therefore required while one precautionary measure to be taken is not to treat subjects at high risk for developing medullary thyroid carcinoma with liraglutide (according the label), and be cautious when prescribing incretin drugs to such subjects.

2.2.3 Hypoglycemia
Past clinical data have shown that incretin therapies alone when not combined with other antidiabetic agents, carry a low risk of hypoglycemia. This is attributed to the fact that they exert their effects on glucose-dependent insulin release while limiting the postprandial glucagon secretion by α-cells (Näslund et al., 1999). Risk of hypoglycemia is further reduced as this glucagon suppression is absent when glucose levels are below 65 mg/dL (Nauck et al., 2002). Mild and moderate hypoglycemia have been reported in clinical studies of monotherapy of exenatide (4–9%) (Moretto et al., 2008; Nelson et al., 2007) and liraglutide (0–12%) but the levels of hypoglycemia are negligible when compared with sulphonylurea therapy such as glimepiride (24%) (Garber et al., 2009). As mentioned earlier, an even milder incidence of hypoglycemia has been reported with DPP-4 inhibitors such as sitagliptin (0–4%). For patients who have had hypoglycemia episodes earlier and for patients in whom hypoglycemia incidence is undesirable (pilots as an example), American diabetes association (ADA) recommends incretin therapy (Campbell, 2011).

2.2.4 Nausea
Nausea is one of the most common adverse events when using GLP-1R agonists, particularly for exenatide and liraglutide (Moretto et al., 2008; Nelson et al., 2007). In a head-to-head incretin study, it was reported that twice-daily exenatide and liraglutide once daily had 28 and 26% patients experiencing transient nausea (Buse et al., 2009) as opposed to nausea
incidence of merely 1–2% and 2–4% in the case of sitagliptin and saxagliptin (Aschner et al., 2006; Morales, 2011; Raz et al., 2006). Dose escalation strategies are often used to alleviate this problem, for example, patients are prescribed 5-μg exenatide twice daily for the first month and eventually escalated to 10 μg (EMA, 2012e). Regarding liraglutide, doses are from 0.6-mg daily for a week and eventually escalated to 1.2 or 1.8 mg (EMA, 2012f). Patient education on nausea generally peaks at 2 months of treatment can also improve compliance.

2.2.5 Hypersensitivity Reactions

Clinical studies of liraglutide (EMA, 2012f), twice-daily exenatide (EMA, 2012e), sitaglitptin (EMA, 2012c), linagliptin (EMA, 2012a), and saxagliptin (EMA, 2012b) have reported hypersensitivity reaction as one of the adverse events related to the treatment. A small minority of serious hypersensitivity reactions have been reported with the usage of exenatide (EMA, 2012e). Immunogenic reaction in a small minority of patients (0.8%) experiencing urticaria has been reported with the usage of liraglutide. For the usage of more traditional antidiabetic agents, only 0.4% of patients experienced hypersensitivity (Campbell, 2011; EMA, 2012f). DPP-4 inhibitor sitagliptin also induces hypersensitivity reactions (EMA, 2012c) including angioedema, anaphylaxis, and exfoliative dermatitis in a minority of patients normally within 3 months of treatment.

2.3 Conclusions

The growing complexity in the treatment of T2DM is evident in recent years with the arrival of incretin therapies as a new add-on pharmacological option. Both GLP-1R agonists and DPP-4 inhibitors are now very important in a second line treatment of T2DM. Although their glycemic control cannot match the efficacy of metformin or sulphonylureas, GLP-1R agonists seem to lower blood glucose and induce weight loss to a greater extent than DPP-4 inhibitors that are weight neutral. However, long-term clinical studies are required to compare the efficacy of these two groups of drugs. Compared to traditional therapies, the safety of incretin therapies is equal if not better in most cases, while specifically DPP-4 inhibitors have better tolerability profile compared to GLP-1R agonists. DPP-4 is administered orally when compared to the subcutaneous administration route of GLP-1R agonists, which is an undoubted advantage as it increases compliance in some patient groups and reduces the risk of hypoglycemia in patient groups at-risk. Personalization of incretin therapy to patient is important to achieve
glycemic control while simultaneously preventing adverse events. Patients with renal and hepatic insufficiency are examples for such. Novel DPP-4 inhibitors such as linagliptin can be prescribed to patients with any degree of RI while GLP-1R agonists are not suitable for this patient group but glycemic control efficacy is likely to be reduced. Further clinical trials and long-term postmarketing surveillance is required for further clarification to personalize incretin therapy most suited for different individuals.

3. GIP

Gastric inhibitory polypeptide (GIP), a 42-amino acid peptide released from the enteroendocrine K cells (Alana et al., 2007), was first discovered in 1969 from porcine intestinal extract (Unger and Eisentraut, 1969), and was later characterized as an inhibitor of gastric acid secretion (Brown et al., 1969). It was subsequently established that GIP is an incretin that can stimulate insulin release in a glucose-dependent manner, and hence, the hormone was also named “glucose-dependent insulinotropic polypeptide” (Andersen et al., 1978; Pederson and Brown, 1976; Pederson et al., 1975). It has also been shown to stimulate proinsulin gene transcription and translation (Wang et al., 1996). The receptor for GIP (GIPR) (Lynn et al., 2003) is present in pancreas, and in brain, testis, lung pituitary, heart, bone, adrenal cortex, small intestine, and adipose tissue (Baggio and Drucker, 2007). Like other hormones, GIP exerts multiple effects in our body including bone reabsorption (Bollag et al., 2001; Tsukiyama et al., 2006), locomotion activity (Ding et al., 2006), stimulation of intestinal GLP-1 release (Simpson et al., 2007), and proliferation of hippocampal progenitor cells (Nyberg et al., 2005).

3.1 Therapeutic Potential

As an incretin, GIP clearly possesses therapeutic potential, which is yet to be thoroughly exploited (Gault and Holscher, 2008; Tsukiyama et al., 2006). In glucose homeostasis, GLP-1 and GIP effects overlap, for example, they both regulate the secretion and biosynthesis of insulin in a glucose-dependent fashion, inhibition of gastric acid release and emptying (Gautier et al., 2005; Preitner et al., 2004). GIP also stimulate β-cells growth, proliferation, differentiation, and survival (Ehses et al., 2002; Trumper et al., 2001, 2002). A stable GIP analog was shown to stimulate functional differentiation of mouse
embryonic stem cells expressing islets-specific genes and hormones (Marenah et al., 2006). The secretion of GIP is primarily in direct correlation to blood nutrient levels as seen by the fasting levels of GIP, which were found to be 10 pmol/L while after 1 h of ingestion of carbohydrate and lipid-rich meals, it was found to peak up to 70–150 pmol/L (Vilsboll et al., 2001). However, the half-life of the peptide is known to be very short as the peptide is also cleaved by the DPP-4 at first two amino acids, resulting in a truncated GIP (3–42), which has no insulinotropic activity (Deacon et al., 2006). It was also observed that there is a direct correlation between the GIP levels and obesity (Flatt et al., 1983; Salera et al., 1982). High-fat diet (HFD) has been shown to enhance the GIP gene expression and hyperplasia in K cells and intestinal GIP levels (Bailey et al., 1986; Flatt et al., 1983). This, therefore, is the reason why GIP antagonists are acknowledged to have a potential as an antiobesity therapeutic (Irwin and Flatt, 2009).

3.1.1 Type-2 Diabetes

We have already discussed the use of GLP-1 in the treatment of T2DM, the importance of GIP as a complementary drug for the treatment of T2DM along with the conventional drugs like Metformin remains exciting (Nauck et al., 1993). Some reports suggest that reduction of hyperglycemia in T2DM subjects is established by GIP-stimulated insulin secretion (Gaman et al., 2015; Hojberg et al., 2009; Puddu et al., 2015; Seino et al., 2015). Similar to GLP-1, utilization of GIP-R agonist and DPP-4 inhibitors are the two major therapeutic strategies and both approaches have had some success in improving the symptoms of T2DM. There are many primary studies exhibiting early insulin response by infusion with GIP in T2DM (Kjems et al., 2003; Ward et al., 1984), although conflicting data have also suggested the development of β-cell resistance in patients with T2DM (Deacon et al., 2000; Meier et al., 2004).

3.1.1.1 GIPR Agonists

It is well known that GIP, along with several other peptide hormones, is degraded by DPP-4. GIP analogs with modifications at the N-terminus are generally not targeted by the DPP-4 (Gault et al., 2002a, 2003b; O’Harte et al., 1999, 2000, 2002). All the reported Tyr1 modification at the N-terminus displayed boosted resistance to DPP-4 to increase their bioavailability (Gault et al., 2003a,b; Hinke et al., 2002). The effects of these modifications have been studied and reviewed extensively elsewhere (Gault et al., 2003b; Meier and Nauck, 2004).
3.1.1.1 GIP Analog (D-GIP₁⁻³₀) The GIP is a 42 amino acid long peptide and the N-terminal of the peptide is recognized by the DPP-4 and hence cleaves the mature peptide rapidly in the system. It was found that GIP₁⁻³₀ had equipotent insulinotropic effect. This GIP analog contains substitution of a d-alanine (Ala) at the 2nd position (D-GIP₁⁻³₀) that makes it DPP-4 resistant without affecting its biological activity (Hinke et al., 2002; Kuhn-Wache et al., 2000). The analog has been shown to exhibit full insulinotropic activity in in vitro studies (Hinke et al., 2001). Intraperitoneal (i.p.) glucose tolerance tests (IPGTT) and subcutaneous (s.c.) injection of D-GIP₁⁻³₀ (8 nmol/kg) demonstrated moderately improved glucose profile in VDF rats (Lynn et al., 2001). The effect was not observed when tested in the GIPR knockout mice, while it was observed in the WT (Widenmaier et al., 2010). A similar study done on the mouse islet cultures treated with D-GIP₁⁻³₀ provided evidence for insulin release from WT but not from GIPR knockout islets. Nevertheless, the effect was observed only in 11 mM but not in 3-mM glucose, which is consistent with the threshold of glucose required for GIP-stimulated insulin secretion (McIntosh et al., 2009; Widenmaier et al., 2010). In staurosporine-treated INS-1 cells, it was found that both D-GIP₁⁻³₀ and GIP₁⁻⁴₂ (i.e., 1–100 nM) were able to suppress cell death to a parallel extent, nonetheless in case of D-GIP₁⁻³₀, somewhat reduced mean efficacy was observed (Widenmaier et al., 2010).

3.1.1.1.2 N-AcGIP(LysPAL₁₆) and N-AcGIP(LysPAL₃₇) These analogs were designed essentially to enhance its stability against DPP-4 by N-terminal acetylation and by adding a palmitate C-16 at the amino group of Lys₁₆ or Lys₃₇ (Kurtzhals et al., 1995; O’Harte et al., 2002). As tested in in vitro system, the N-AcGIP(LysPAL₁₆) agonist was shown to have similar or slightly better insulin release and cAMP stimulatory function as the native peptide (O’Harte et al., 2002). The plasma glucose level was observed to go down along with an increase in insulin release in the ob/ob mice model (Irwin et al., 2005) (Bailey et al., 1982). N-AcGIP(LysPAL₃₇) was found to be more potent than the N-AcGIP (LysPAL₁₆), as it has displayed significantly enhanced insulin releasing effect (Irwin et al., 2005). The reason of this remains unclear, although it was hypothesized that a fatty acid chain associated to Lys closer to the C-terminus of the peptide may have less of a negative effect on the bioactive region (Gault et al., 2002b; Hinke et al., 2001; Irwin et al., 2005; Manhart et al., 2003). When matched to native GIP peptide’s effect on the ob/ob mice (Jones et al., 1987; Meier et al., 2004; Nauck et al., 1993), the agonist exhibits effect even in the lowest
dose (6.25 nmol/kg) to produce substantial reduction in blood glucose and insulinotropic function (Irwin et al., 2005). The prolonged duration of action of the analog is made evident by the reduced hyperglycemia and sustained 13% decrease in plasma glucose 24-h after injection (Irwin et al., 2005), indicating the potential of this analog as a future therapeutic agent in T2DM (Irwin et al., 2005).

3.1.1.2 DPP-4 Inhibitors

As shown in Table 3, the pros and cons of using these drugs in the treatment of T2DM have already been discussed in detail in the previous sections (Fig. 3).

3.1.1.3 GIP Antagonist

Upon knocking out the GIP-R mediated signaling actions of GIP, chemically or genetically, several studies have reported only minor impairments in the secretion of insulin as well as glucose homeostasis in rodents (Irwin et al., 2004; Miyawaki et al., 1999). Conversely, upon examining the effect of long-term disruption of GIP-R mediated signaling in obesity-diabetes models, specifically HFD-fed animals, a different scenario has emerged (Gault et al., 2005). A large number of studies therefore have carried out and revealed the importance of GIP-R antagonism for the purpose of treating obesity-diabetes (Althage et al., 2008; Flatt, 2008; Gault et al., 2005, 2007; Hansotia et al., 2007; Irwin et al., 2007; McClean et al., 2007, 2008; Miyawaki et al., 2002; Parker et al., 2007). Another approach by K-cell specific knockdown of GIP has indicated reduced obesity and associated insulin resistance in HFD fed mice (Althage et al., 2008). In extremely obese patients, there are evidences indicating cure of diabetes by surgically evading GIP-secreting K cells present in the upper region of small intestine (Clements et al., 2004; Flatt, 2007). Also, glucose intolerance could be improved by actively immunizing ob/ob mice against GIP (Irwin et al., 2009). In summary, it can be concluded that blocking the GIP-R pathway results in reduction of fat deposition, body weight gain as well as enhancement of insulin resistance and glucose tolerance (Hansotia et al., 2007; Miyawaki et al., 2002). The most significant observation therefore is that antagonizing the GIP pathway drastically and rapidly restore insulin sensitivity (Figs. 4 and 5).

3.1.1.3.1 (Pro3) GIP

(Pro3) GIP is a GIP analog with proline substitution at the 3rd position of the GIP mature peptide (O’Harte et al., 2007). In metabolic testing of all GIPR antagonists, (Pro3) GIP has been
Figure 3  Insight of the active site of the DPP-4 with compound 1 retrieved from PDB ID 4D5A from RCSB PDB.
(Pro3) GIP has been shown to cause impaired glucose tolerance, as it specifically inhibits or blocks the insulinotropic effect of GIP (Gault et al., 2002c, 2003c). Daily (Pro3) GIP of 25-nmol/kg body weight, given to ob/ob mice, has been reported to reduce levels of hyperglycemia, improve insulin resistance as well as restore glucose tolerance (Gault et al., 2005). Consistently, (Pro3) GIP, upon daily administration of 25-nmol/kg body weight to ob/ob young mice prevent onset of abnormality including obesity-diabetes (Irwin et al., 2007). Knockout of GIPR in ob/ob mice and in HFD-fed mice have provided similar findings (Hansotia et al., 2007; Miyawaki et al., 2002). However, these beneficial effects could not be detected when the treatment was performed after blocking the GLP-1R by exendin(9–39)amide (Parker et al., 2007), suggesting that the beneficial metabolic effects of (Pro3) GIP in the case of obesity-diabetes are dependent on insulin (Flatt, 2008; Gault et al., 2007; McClean et al., 2007). Changes in circulating glucagon levels after (Pro3) GIP treatment is also consistent with the insulin-dependency of its actions (Flatt, 2008; Gault et al., 2007; Parker et al., 2007; Zhou et al., 2005).
Figure 5  Summary of the cellular actions of GIP, glucagon, and GLP-1.
It has been reported that the region exhibiting high-affinity receptor binding is the region contained within the fragment GIP\(_{6-30}\)amide, as the IC\(_{50}\)s of this analog and the native peptides were 2.39 ± 1.1 and 3.08 ± 0.57 nM (Table 5) (Gelling et al., 1997). Although the analog is capable of interacting and binding to the GIP receptor, it does not stimulate adenylyl cyclase, indicating antagonistic activity. In in vitro studies, GIP\(_{6-30}\)amide at a concentration of 100 nM was able to inhibit 58 ± 2.5% cAMP induced by 1-nM GIP (Gelling et al., 1997). The potential of in vivo GIP\(_{6-30}\)amide as functional antagonist of GIP, however, remains to be examined. In addition, a number of truncated GIP peptides were studied including GIP\(_{10-30}\), GIP\(_{7-36}\), and GIP\(_{7-30}\), while none of these peptides are functional with the exception of GIP\(_{7-30}\)amide that is a weak antagonist capable of inhibiting the cyclic AMP generation in receptor transfected cells as well as β-TC3 cells (Tseng et al., 1996).

### Table 5

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>Affinity to GIPR as compared to GIP</th>
<th>Stimulation potential for GIPR</th>
</tr>
</thead>
<tbody>
<tr>
<td>GIP(_{6-30})amide</td>
<td>Same</td>
<td>None</td>
</tr>
<tr>
<td>GIP(_{7-36})</td>
<td>74-fold lower</td>
<td>Inhibits GIP (1-nM range)</td>
</tr>
<tr>
<td>GIP(_{10-30})</td>
<td>235-fold lower</td>
<td>Inhibits GIP (1-nM range)</td>
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#### 3.1.1.3.2 GIP\(_{6-30}\)amide

It has been reported that the region exhibiting high-affinity receptor binding is the region contained within the fragment GIP\(_{6-30}\)amide, as the IC\(_{50}\)s of this analog and the native peptides were 2.39 ± 1.1 and 3.08 ± 0.57 nM (Table 5) (Gelling et al., 1997). Although the analog is capable of interacting and binding to the GIP receptor, it does not stimulate adenylyl cyclase, indicating antagonistic activity. In in vitro studies, GIP\(_{6-30}\)amide at a concentration of 100 nM was able to inhibit 58 ± 2.5% cAMP induced by 1-nM GIP (Gelling et al., 1997). The potential of in vivo GIP\(_{6-30}\)amide as functional antagonist of GIP, however, remains to be examined. In addition, a number of truncated GIP peptides were studied including GIP\(_{10-30}\), GIP\(_{7-36}\), and GIP\(_{7-30}\), while none of these peptides are functional with the exception of GIP\(_{7-30}\)amide that is a weak antagonist capable of inhibiting the cyclic AMP generation in receptor transfected cells as well as β-TC3 cells (Tseng et al., 1996).

#### 3.1.1.3.3 GIP-Oxytomodulin Hybrid Peptide

Oxytomodulin (Oxm) is a 37 amino acid peptide that has the potential for treatment of diabetes and obesity via glucagon and GLP-1 receptor. Recently, a novel GIP-Oxm hybrid peptide DA2GIP-Oxm was designed to exert a positive and beneficial effect on the secretion of insulin from β-cells, glucose homeostasis, and body weight, mediated by the activation of GIP, GLP-1 receptors, and glucagon. It was hope that a combined therapeutic approach can help in overcoming the individual receptor defects induced by hyperglycemia (Aaboe et al., 2009) along with the possible worries regarding the solubility as well as efficiency of two or more peptides together upon its administration by a single injection. These “multiple-targeting” peptides represent a novel therapeutic treatment plan for obesity-diabetes (Alana et al., 2007). HFD mice, when treated with DA2GIP-Oxm daily for 15 days, exhibit reduction in body weight and concentrations of nonfasting plasma glucose, increase in the concentrations of plasma insulin, and an improved glucose-mediated secretion of insulin, confirming the long-lasting effect of DA2GIP-Oxm on the insulinotropic
activity along with its ability of overcoming the defects associated with pancreatic β-cells (Bhat et al., 2013). Further, positive effects observed in glucose homeostasis were independent of the changes in the insulin sensitivity, highlighting its role primarily in the enhancement of β-cell function. Also, DA2GIP-Oxm treated mice demonstrated improvement in the glucose lowering as well as insulinotropic activity when compared to the effect of DA2GIP molecule (Hinke et al., 2002). The observed effects were not because of the food intake differences, as they remained constant during the course of study. Further investigation related to the feeding behavior and/or patterns, expenditure of energy, and locomotor activity must be conducted. Also, the long-term effects of DA2GIP-Oxm treatment on the lipid parameters, insulin-mediated signaling cascade, and on other crucial circulating hormones or adipokines must further be investigated.

### 3.2 Conclusions

The major setback of using GIP as a therapeutic agent is its very low plasma half-life. Therefore, in order to exploit the therapeutic potential of GIP, several GIP analogs with modifications at N-terminus have been developed to increase its bioavailability, by making it DPP-4 resistant. For instance, D–GIP$_{1-30}$ with d-alanine substitution at 2nd position is not only resistant to DPP-4 activity but it also exhibits complete insulinotropic activity. Similarly, GIP analogs, N–AcGIP(LysPAL16) and N–AcGIP(LysPAL37), show increased stability, owing to N-terminal acetylation and addition of palmitate at the Lys16 or Lys37 amino group. There remains a necessity to find a chemical compound as agonist or antagonist molecule for GIP with high plasma half-life that can be administered orally. Importantly, the expression level of GIP gene is enhanced under the influence of HFD and hence, GIP antagonism undeniably has great potential as antiobesity therapy. Although many have been introduced as antiobesity drugs, pursuit for a stable drug with minimum side effect remains. Certain set of studies illustrated the significance of inhibiting GIP–R mediated pathways for restoring insulin sensitivity upon utilizing GIP–R antagonists such as (Pro3) GIP and GIP$_{6-30}$amide. (Pro3) GIP specifically inhibits the insulinotropic effect of GIP followed by impaired glucose tolerance, while GIP$_{6-30}$amide fails to stimulate adenylyl cyclase activity upon interacting with GIP–R. More interestingly, DA2GIP–Oxm, a novel GIP–Oxm hybrid peptide developed recently, can be exploited as an efficient combined therapeutic approach, as it
can be a potential treatment for diabetes and obesity acting via glucagon and GLP-1 receptor. Overall, the administration of GIP analogs characterizes a novel therapeutic treatment strategy for obesity–diabetes. Apart from hybrid peptide, further intensive research needs to be done for designing a drug molecule to act as GIP agonist and antagonist, considering its great potential.

4. GLUCAGON

Early studies in the late 19th century on canine pancreas showed that the organ might play a key modulatory role in controlling blood glucose levels, the dysfunction of which leads to diabetes mellitus. The effect of insulin and its association with the pancreas was established in early 1920s but in 1923, Murlin and coworkers identified that there were other factors in the pancreas which were responsible for the increase in blood sugar levels (Murlin et al., 1923). Proglucagon which is posttranslationally processed by prohormone convertase-2 enzyme to generate glucagon and other products in the α-cells of the islets of Langerhans in the pancreas is also expressed in the brain and L-cells of the small intestine (Rouille et al., 1994). Glucagon is a 29-amino acid peptide that acts via its receptor (GCGR) which is present on the cell membrane of the target tissues (Jelinek et al., 1993). The binding of the peptide to the GCGR triggers the activation of adenylate cyclase and phospholipase C cascading leading to augmentation of intracellular cAMP and calcium levels. In the liver, glucagon activates gluconeogenesis and glycogenolysis, which leads to the increase in blood glucose level. The infusion of somatostatin, a key negative regulator of glucagon secretion, leads to the conformation of glucagon’s role in regulation of circulating glucose (Gerich et al., 1974; Liljenquist et al., 1977). Glucagon release can be due to changes of insulin levels in response to blood glucose levels as insulin inhibits glucagon release (Ahren, 2013), for instance, hyperinsulinemia in many cases has been noted to prevent glucagon secretion even in hypoglycemic conditions. The proximity of the β- and α-cells in the islets of Langerhans is one of the explanations for insulin’s influence (Banarer et al., 2002). Animals treated with β-cell specific drug alloxan that inhibits insulin production did not respond to hypoglycemia in production of somatostatin and suppression of glucagon (Greenbaum et al., 1991). Insulin also suppresses glucagon production by activating K<sub>ATP</sub> channels leading to hyperpolarization of the α-cell membrane (Franklin et al., 2005). Somatostatin, a peptide hormone secreted from the δ-cells of the islets in the pancreas, the brain, and
duodenum, has been shown to be essential to the glucose-dependent inhibition of glucagon release. It is also important to note that somatostatin also plays a critical role in insulin secretion thereby regulating glucose metabolism (Hauge-Evans et al., 2009). Ghrelin another peptide hormone has been shown to have a dose-dependent effect on glucagon release and at the same time deletion of ghrelin receptor leads to reduced glucagon levels and fasting glucose (Chuang et al., 2011). Finally, glucagon also has an autocrine stimulatory effect on the α-cells via a cAMP-mediated activation (Ma et al., 2005). Studies by Salehi and Vieira suggested a U-shaped response of glucagon secretion to glucose. Maximum inhibition of glucagon secretion was attained at around 7 mmol/L glucose while stimulation of glucagon release also occurred at 25–30 mmol/L glucose. It was suggested that this mechanism might be a possible explanation for hyperglucagonemia under hyperglycemic conditions (Salehi et al., 2006).

4.1 Glucagon-Based Therapeutics

It is of great interest if any drug developed is capable of controlling hyperglycemia, and this is likely one of the reasons why, unlike GLP-1, glucagon-based therapeutics have not been a research focus in the past. After the initial synthesis of glucagon peptide in 1984, there were many exciting modifications of the peptide. In 1987, des-His1-[Glu9] glucagon-NH2 along with other synthetic peptides were studied. Des-His1-[Glu9] glucagon-NH2 peptide showed 40% higher potency compared to endogenous glucagon in the competitive displacement of 125I-labeled glucagon from liver, however, the analog did not exhibit any cAMP effects, while a concentration of 100 nM was found sufficient to inhibit cAMP production thereby neutralizing glucagon’s effect (Mojsov and Merrifield, 1984; Unson et al., 1987). In 1994, a novel antagonist des-His1-[Nle9-Ala11-Ala16]-glucagon-NH2 with 10 times higher potency than the former one was developed (Unson et al., 1994). Regarding glucagon agonist, [l-N α-trinitrophenylhistidine, 12-homoarginine]-glucagon (THG) was developed to have a hypoglycemic effect in diabetic rats treated with streptozotocin (Johnson et al., 1982). In a recent paper, Wang and coworkers analyzed the use of monoclonal anti-GCGR antibodies in streptozotocin-induced T1DM (Wang et al., 2015). The metabolic phenotype was normalized due to the blockage of glucagon-receptor activation. It was suggested that this strategy might aid in efficiently modulating glucose levels in the insulin administered to patients with T1DM by GCGR neutralizing antibodies (Wang et al., 2015). For T2DM,
hyperglycemia is partially the result of insulin insensitivity and increased plasma glucagon levels. It is believed that reduction in GCGR gene expression by a GCGR antisense oligonucleotide approach may aid to achieve glucose homeostasis. A study in db/db mice using antisense oligonucleotide treatment demonstrated reduced response to glucagon challenge in hepatic cAMP as well as lowered plasma glucose, triglyceride, and free fatty acid levels (Liang et al., 2004). In addition, a number of small molecules were developed in the past to agonize or antagonize glucagon, for example, Compound 1 proposed by Qureshi and coworkers was found to antagonize the biological response of glucagon in human hepatocytes and in mice with humanized GCGR (Qureshi et al., 2004). Bayer antagonist, Bay 27–9955 was able to partially control glucose production and plasma glucose levels in human subjects in response to hyperglucagonemia (Petersen and Sullivan, 2001). A list of all the small molecule glucagon antagonists was compiled by Bagger’s team, while they have raised questions on the effectiveness of unimolecular therapeutic effect of glucagon antagonism (Bagger et al., 2011). The prospects of dual- and even triagonist approaches were recently explored in depth as therapies activating or deactivating single hormones were unable to show more than 5–10% effect on obesity or embolism (Amori et al., 2007).

4.2 Dual-Agonist and Triagonist Approach

It should be noted that all the three peptides OXN, GLP-1, and glucagon, share the same precursor molecule; OXN and GLP-1 being secreted from the L-cells of the small intestine and glucagon from α-cells of the pancreas. OXN has also been shown to be capable of activating both GLP-1R and GCGR. A study in 2009, designed two agonists, one a GLP-1/glucagon dual-agonist which mimics OXN (DualAG) and the other a GLP-1 agonist (GLP1AG) but both of them had superior stability as they were resistant to DPP-4. Both DualAG and GLP1AG had considerable effects on diet-induced obese mice by lowering blood glucose, body weight, and food intake. DualAG interestingly exhibited superior weight loss and lipid lowering effect without inducing hyperglycemia. This was also reflected by improvements in plasma insulin, leptin, and adiponectin levels. These results were further substantiated in GLP-1R−−/− and GCGR−−/− mice (Pocai et al., 2009). The possible mechanisms for actions of DualAG are its dual effects in activating the gluconeogenic pathway in the liver and simultaneous GLP1-agonism to improve postprandial glucose tolerance. It was also stated that
hormone sensitive lipase, a key regulator of lipid metabolism in the adipocyte which is normally downregulated in diet-induced obese mice, was augmented when treated with DualAG (Pocai et al., 2009). Day and coworkers subsequently designed and evaluated a set of agonists with GLP-1/glucagon dual agonistic properties, and their potencies were studied specifically to GCGR in the consideration of its hyperglycemia effect. It was shown that DualAG achieved greater and more sustained weight loss at lower concentration when compared to the GLP-1 agonist. The hyperglycemic effect rendered by glucagon agonism was not prevalent in the dual-agonist treatments, and this can be the result of GLP-1 agonism, which antagonizes glucagon-mediated effects (Day et al., 2009). GIP/GLP-1 dual-agonist also exhibited significant effects on weight loss, antihyperglycemic and insulinotropic efficacy when compared to the GLP-1 agonist (Finan et al., 2013).

In early 2015, a triagonist that can stimulate receptors for GIP, GLP-1, and glucagon was designed and evaluated in rodents. The triagonist was found superior than dual-agonists of GLP-1/glucagon and GLP-1/GIP in weight loss and glycemic control at significantly lower dosages. The triagonist treated group showed a 26.6% reduction in body weight in DIO mice after 20 days compared to 15.7% GLP-1/GIP dual-agonist group, and similar dose-dependent effects were also observed in DIO rats. These observations were further validated using knockout and various other disease animal models (Finan et al., 2015). The enhanced energy expenditure and superior hepatic lipid handling characteristics of the triagonist were explained via the induction of FGF21 (Finan et al., 2015; Habegger et al., 2013).

4.3 Conclusions

Glucagon is clearly an important metabolic hormone in our body while agonists and antagonists of the hormone had little success in the past for treatment of metabolic diseases. The dual- and particularly the triagonist approaches have provided novel insights into treatments countering both obesity and T2DM simultaneously, while understanding the molecular mechanisms behind their actions is crucial. The scientific community has widely accepted that structure and atomic interactions in the proteins play a key role in the biological outcomes that proteins and other biological macromolecules elicit. Further refinement is particularly necessary for the clinical success of the triagonist due to differences in the mouse and human sequences of the involved receptors and peptides. With more structural information we could start a conversation on further refinement of the
triagonist as we believe that this peptide can be a potential solution to treat dysfunctions that haunt the developed and developing world at epic proportions.

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