Glucagon and Glucagon-Like Peptide Receptors as Drug Targets

J.L. Estall and D.J. Drucker*

Departments of Laboratory Medicine and Pathobiology, and Medicine, University of Toronto, The Banting and Best Diabetes Centre, Toronto General Hospital, University of Toronto, Toronto, Canada

Abstract: Glucagon and the glucagon-like peptides are derived from a common proglucagon precursor, and regulate energy homeostasis through interaction with a family of distinct G protein coupled receptors. Three proglucagon-derived peptides, glucagon, GLP-1, and GLP-2, play important roles in energy intake, absorption, and disposal, as elucidated through studies utilizing peptide antagonists and receptor knockout mice. The essential role of glucagon in the control of hepatic glucose production, taken together with data from studies employing glucagon antagonists, glucagon receptor antisense oligonucleotides, and glucagon receptor knockout mice, suggest that reducing glucagon action may be a useful strategy for the treatment of type 2 diabetes. GLP-1 secreted from gut endocrine cells controls glucose homeostasis through glucose-dependent enhancement of β−cell function and reduction of glucagon secretion and gastric emptying. GLP-1 administration is also associated with reduction of food intake, prevention of weight gain, and expansion of β−cell mass through stimulation of β−cell proliferation, and prevention of apoptosis. GLP-1R agonists, as well as enzyme inhibitors that prevent GLP-1 degradation, are in late stage clinical trials for the treatment of type 2 diabetes. Exenatide (Exendin-4) has been approved for the treatment of type 2 diabetes in the United States in April 2005. GLP-2 promotes energy absorption, inhibits gastric acid secretion and gut motility, and preserves mucosal epithelial integrity through enhancement of crypt cell proliferation and reduction of epithelial apoptosis. A GLP-2R agonist is being evaluated in clinical trials for the treatment of inflammatory bowel disease and short bowel syndrome. Taken together, the separate receptors for glucagon, GLP-1, and GLP-2 represent important targets for developing novel therapeutic agents for the treatment of disorders of energy homeostasis.

Key Words: Proglucagon, GLP-1, GLP-2, glucagon, g protein-coupled receptors, diabetes, intestinal disease, PGDP.

INTRODUCTION

The proglucagon gene encodes a large prohormone precursor, proglucagon, predominantly expressed in the endocrine pancreas, gut endocrine cells, and the central nervous system. Tissue-specific post-translational processing of proglucagon by prohormone convertases yields multiple biologically active proglucagon-derived peptides (PGDPs) that have attracted increasing interest due to their regulatory actions on nutrient absorption and energy homeostasis. The principal peptide products include glucagon in the pancreatic α-cells, and glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2), glicentin, and oxyntomodulin in the intestinal L-cells (Fig. **1**).

The primary function of glucagon is to maintain appropriate levels of glucose in the fasting state, and to raise plasma glucose levels in response to hypoglycemia (for review, see [1]). The other PGDPs have also been shown to regulate energy homeostasis in a nutrient-dependent manner *via* a multitude of biological actions including the regulation of insulin and glucagon secretion, gastric emptying, acid secretion, food intake, and intestinal epithelial integrity (for review, see [2, 3]).

Given the diverse actions of these hormones, and the demonstration that modulation of GLP-1 or GLP-2 action may have therapeutic benefits in experimental models of diabetes and intestinal diseases, respectively, there is now substantial interest in the development of specific peptide and non-peptide agonists and antagonists that target the glucagon-like peptide receptors. The aim of this review is to explore the therapeutic potential of agents that modulate glucagon-like peptide receptor signaling for the treatment of human disease.

THE GLUCAGON RECEPTOR FAMILY

The receptors for glucagon, GLP-1 and GLP-2 share extensive homology and are members of the Family B (II) Glucagon-Secretin G Protein-Coupled Receptor (GPCR) superfamily, which also includes receptors for secretin, glucose-dependent insulinotropic polypeptide (GIP), vasoactive intestinal peptide (VIP), pituitary adenylate cyclase-activating polypeptide (PACAP) and growth-hormone-releasing hormone (GHRH) [4]. The majority of the receptor agonists in this family are naturally occurring peptide hormones. Structural characteristics shared by this receptor family include: a relatively long, extracellular N-terminal domain; highly conserved cysteine residues in the extracellular domains which likely form disulfide bridges; an amino acid signal peptide directing membrane localization; and several N-linked glycosylation sites [5, 6].

Several studies have identified interactions between peptide ligands and the N-terminus, the extracellular loops, and the transmembrane helices of the Family B GPCRs (for review, see [6]). Studies using site-directed mutagenesis and the creation of chimeric receptors have demonstrated that the N-termini of receptors within this family, specifically the

*Address correspondence to this author at the Toronto General Hospital, Banting and Best Diabetes Centre, 200 Elizabeth Street MBRC4R-402, Toronto, Canada M5G 2C4; Tel: 416-340-4125; Fax: 416-978-4108; E-mail: d.drucker@utoronto.ca

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glucagon, GLP-1, VIP, and secretin receptors, recognize and bind the C-terminus of their cognate peptide ligands, while specific residues in the extracellular loops and the borders of the transmembrane helices bind the N-terminus of the ligand [7-12]. This indicates that ligand binding to this family of receptors likely involves an intricate and precise interaction between specific receptor/ligand residues and the requirement of intact receptor secondary structure, potentially explaining the high specificity and affinity of ligand binding.

All of the family members activate adenylyl cyclase through regulation of $G_{\alpha s}$; however some Family B receptors also couple to other G-proteins. Many of the receptors in this family exhibit some degree of agonist-independent (constitutive) activity [5]. While glucagon and the glucagon-like peptides are encoded by a single gene (proglucagon) (Fig. **1**), the genes for each of their receptors evolved separately (For review, see [4]). Although the receptors share considerable sequence identity, differential cellular localization and signaling properties allows their cognate peptide hormone agonists to regulate a diverse array of biological functions.

Glucagon

Glucagon is a 29 amino acid peptide synthesized mainly in pancreatic α-cells following cleavage of proglucagon by prohormone convertase 2 [13]. Glucagon is the principal counter-regulatory hormone that opposes insulin action leading to coordinate bihormonal control of glucose homeostasis. Through a variety of mechanisms, including stimulation of hepatic glycogenolysis and gluconeogenesis, glucagon acts to increase the level of available glucose by mobilizing available energy stores. Glucagon also inhibits glycogen synthesis and glycolysis in the liver. Glucagon excess, in the setting of insulin resistance and/or deficiency, contributes to the development of hyperglycemia in human subjects with diabetes mellitus (for review, see [1]).

Glucagon elicits it metabolic effects following binding to a highly specific G protein-coupled receptor. The glucagon receptor is widely expressed in many tissues, including liver, brain, pancreas, heart, kidney, and the smooth muscle of the gastrointestinal tract and peripheral vasculature (for a more in-depth review, see [14]). Consistent with this diverse receptor localization, glucagon has been used to treat conditions such as refractory bradycardia and cardiogenic shock due to its inotropic and chronotropic properties in the heart [15]. Glucagon acts as a vasodilator at supraphysiological concentrations [16] and exogenous glucagon administration can increase glomerular filtration rate, regulate ion transport, and electrolyte excretion in the kidney [17, 18]. Glucagon is also used as a spasmolytic agent to modulate motility in the gastrointestinal tract during radiological examinations [19]. Although glucagon may stimulate lipolysis in animals [20-23], evidence for the lipolytic effect of glucagon in humans is controversial [24, 25]. Irrespective of the numerous physiological targets of glucagon action, the therapeutic potential of modulating glucagon receptor activity has focused mainly on its role in glucose homeostasis. Although glucagon receptor activation clearly produces intriguing effects on blood pressure control and food intake, the therapeutic potential in modulating glucagon receptor action more likely resides in the blockade of glucagon receptor signaling for the treatment of diabetes.

The glucose-liberating actions of glucagon are thought to be regulated mainly by activation of adenylyl cyclase, increased levels of intracellular cAMP, and activation of protein kinase A (PKA). Glucagon enhances the activity of key gluconeogenic enzymes such as phosphoenolpyruvate carboxykinase (PEPCK) and the catalytic subunit glucose-6-phosphatase (G-6-Pase) *via* induction of cyclic-AMP response element binding protein (CREB) and peroxisome proliferator activated receptor gamma coactivator-1 (PGC-1) [26, 27]. Although the number of glucagon receptors in diabetic patients may be reduced, the ability of glucagon to stimulate cAMP production remains unchanged [28]. The hyperglucagonemia associated with diabetes most likely results in increased glucagon receptor signaling and subsequent elevation of plasma glucose in the absence of sufficient counter-regulatory insulin signaling. Conversely, al-

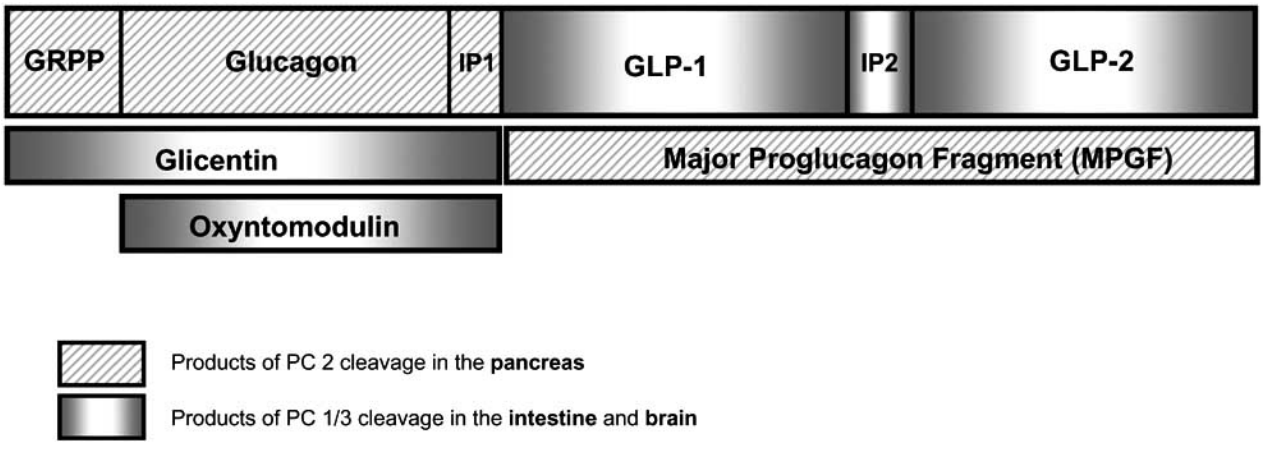

Fig. (1). Proteolytic Cleavage of the Proglucagon Hormone. Proglucagon encodes multiple biologically active peptides that are produced following enzymatic cleavage by prohormone convertases (PC). PC2 cleavage yields glicentin-related pancreatic polypeptide (GRPP), glucagon, intervening peptide 1 (IP1), and the major proglucagon fragment (MPGF) in the pancreatic α-cells and the central nervous system, while PC1/3 enzymatic activity produces glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2), glicentin, oxyntomodulin, and intervening peptide 2 (IP2) in the intestine and brain.

though most diabetic subjects with hypoglycemia maintain a preserved therapeutic response to exogenous glucagon administration, endogenous glucagon secretion frequently becomes defective in patients with diabetes following repeated episodes of hypoglycemia [29-31].

Oxyntomodulin

Oxyntomodulin is a 37 amino acid proglucagon-derived peptide that contains the sequence of glucagon and a carboxyterminal 8 amino acid extension, including the sequence of intervening peptide-1 (Fig. **1**). Although oxyntomodulin regulates gastric acid secretion, a separate oxyntomodulin receptor has not been identified. Administration of oxyntomodulin, either *via* intracerebroventricular or peripheral injection, reduces food intake and produces weight loss in rodent studies [32-35]. Although oxyntomodulin is capable of interacting as an agonist at both the glucagon and GLP-1 receptors [35], the anorectic actions of oxyntomodulin are blocked by the GLP-1 receptor antagonist exendin (9-39) [32]. Similarly, oxyntomodulin significantly reduces food intake in $GCGR^{-/-}$ but not $GLP\text{-}1R^{-/-}$ mice, hence the glucagon receptor is not required for transduction of oxyntomodulin actions on food intake [35]. Intriguingly, short term infusion studies demonstrate that oxyntomodulin also reduces appetite and energy intake in normal human subjects [36], and oxyntomodulin produces weight loss following sustained administration in human subjects[1].

Therapeutic Potential of Targeting the Glucagon Receptor

Treatment of Diabetes

The ratio of insulin to glucagon is normally tightly regulated. In healthy human subjects, hyperglycemia following nutrient absorption stimulates insulin release and decreases glucagon levels, while hypoglycemia inhibits insulin and stimulates glucagon secretion. In patients with type 2 diabetes, insulin secretion is often delayed and reduced, together with defects in insulin action, while glucagon levels remain unchanged or elevated [1, 37]. In type 1 diabetes, where post-prandial insulin release is virtually absent, hyperglucagonemia may be attributed largely to the lack of insulin's negative feedback on glucagon release from islet α−cells [38]. Persistently elevated levels of glucagon or increased glucagon/insulin ratios are often observed in diabetic humans and in animal models of diabetes [39, 40], and most likely play a substantial role in the development of hyperglycemia, the metabolic signature of both type 1 and type 2 diabetes [38, 41, 42].

Although the unopposed actions of glucagon in diabetic subjects have been described for years, until recently, there has been little experimental focus on determining the potential role of glucagon antagonists in the treatment of experimental diabetes. A glucagon analogue, [l-N alpha-trinitrophenylhistidine, 12-homoarginine]-glucagon (THG), was demonstrated to have antagonistic properties at the hepatic glucagon receptor *in vitro*. Bolus injections and long-term infusion of the peptide *in vivo* (diabetic rats) decreased blood glucose levels by up to 65% in the absence of exogenous insulin treatment [43]. These observations suggested that glucagon receptor antagonists might be safe and effective therapeutic agents for the treatment of diabetes. Nevertheless, in the two decades following the publication of these findings, only a handful of additional studies described characterization of peptide antagonists for the glucagon receptor [44-50]. Although many of these glucagon antagonists reduced the capacity of native glucagon to lower hepatic cAMP generation *in vitro*, their usefulness in rodent models of diabetes appeared limited. Concurrently, efforts were made to decrease circulating levels of glucagon with highly specific glucagon-neutralizing antibodies. Infusion of these antibodies into rodent models of diabetes effectively reduced free glucagon levels and decreased blood glucose [51-53]. These studies provided proof of concept that peptide-based glucagon antagonists and/or neutralizing antibodies directed against glucagon may have therapeutic value for the treatment of diabetes.

More recently, focus has shifted to the development of non-peptide glucagon receptor antagonists (for review, see [54]). The first non-peptide competitive human glucagon receptor antagonist, 2-(benzimidazol-2-ylthio)-1-(3, 4-dihydroxyphenyl)-1-ethan one, NNC 92-1687 (2), was shown to specifically bind to the glucagon receptor, and inhibit glucagon-stimulated cAMP accumulation in cells expressing the glucagon receptor [55]. Several competitive and non-competitive glucagon receptor antagonists have been developed including L-168, 049 (Merck) [56]; the triarylimidazoles (Merck) [57]; the alkylidene hydrazides - Compound 27 (Agouron/Novo Nordisk) [58], Compound 28 [4-hydroxy-3-cyanobenzoic acid (4-isopropylbenzyloxy-3, 5-dimethoxymethylene) hydrazide] (Anadys/Novo Norkisk) [59], and NNC 25-2504 (Novo Nordisk) [60]; Bay 27-9955 (Bayer) [61]; 5-Hydroxyalkyl-4-phenylpyridines (Bayer) [62]; and the urea-based compounds (Novo Nordisk) including the biaryl amides (Abbott) [63] (For chemical structures, see Fig. **2**). Skyrin, a fungal bisanthroquinone, was found to specifically and non-competitively inhibit glucagon receptor signaling in primary hepatocytes (i.e. independent of binding to the glucagon receptor) [64]. All of these small molecule glucagon receptor antagonists were able to significantly inhibit glucagon-induced cAMP accumulation *in vitro*, and a few antagonists have also shown efficacy in lowering blood glucose in rodent models [43, 58, 59, 63]. Bay 27-9955, has been administered to human subjects and attenuated the development of glucagon-induced hyperglycemia in a short-term study over several hours [61]. The advantage of these synthetic chemical entities includes their oral bioavailability, cheaper cost of manufacturing, together with the reduced likelihood of inducing an immune response compared to peptide-based antagonists.

Mice lacking a functional glucagon receptor have been generated ($GCGR^{-/-}$) and exhibit a number of interesting phenotypes. These mice have elevated levels of circulating glucagon with no changes in insulin levels, but are otherwise viable and healthy, and exhibit mild fasting hypoglycemia and improved glucose tolerance [65, 66]. $GCGR^{-/-}$ mice also have an increase in pancreatic weight, due in part to significant α-cell hyperplasia. $GCGR^{-/-}$ mice display reduced adi-

[1]Wynne K, Park AJ, Small CG, Patterson M, Ellis SM, Murphy KG, *et al*. Subcutaneous oxyntomodulin reduces body weight in overweight and obese subject: a double-blind, randomized, controlled trial. Diabetes 2005; 54(8): 2390-5.

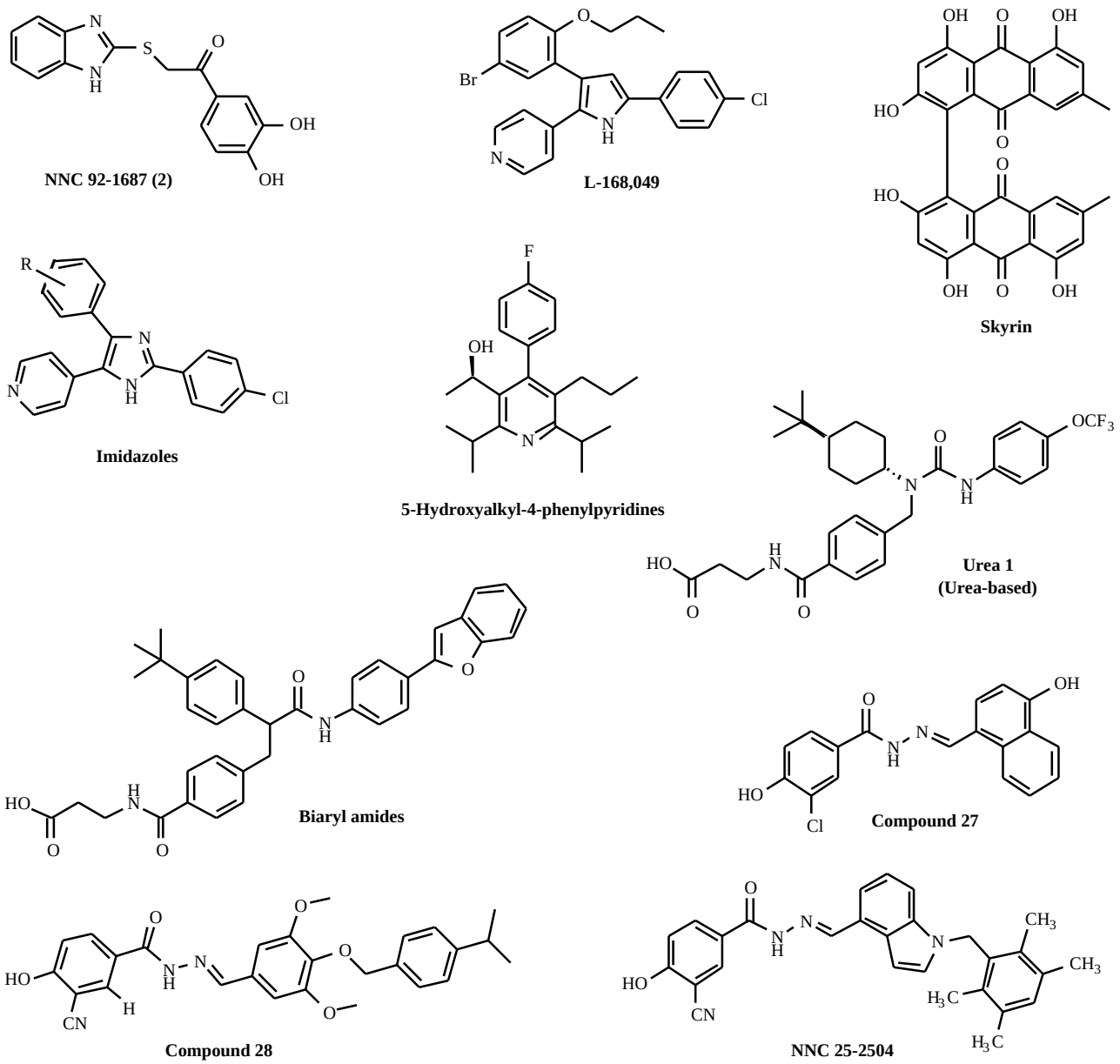

Fig. (2). Chemical Structures of Small Molecule Glucagon Receptor Antagonists. The published chemical structures/backbones of molecules currently being investigated as glucagon receptor antagonists. Compound **27**, compound **28**, and NNC 25-2504 are all alkylidene hydrazides. Each compound inhibits glucagon induced cAMP production by acting in a competitive or non-competitive manner on the glucagon receptor. All structures have been reproduced with permission from the publishers.

posity and leptin levels, but have a normal body weight, food intake, and energy expenditure [66]. Mice engineered to express the human glucagon receptor, were recently used to study the binding and efficacy of two glucagon-receptor antagonists, one peptide-based and one small molecule, *in vivo*. It was determined that the majority of specific glucagon binding was localized to the liver, and that each of the antagonists displaced 70-80% of the radiolabeled glucagon from the hepatic human glucagon receptor *in vivo* and effectively lowered glucagon-induced hyperglycemia [67]. While the exact binding domain of the glucagon receptor antagonists within the receptor has not yet been identified, this study suggests that there may be direct competition between these molecules for the ligand-binding pocket.

A complementary approach to determining the potential efficacy of reducing glucagon action for the treatment of type 2 diabetes involves the use of anti-sense oligonucleotides (ASO) to reduce glucagon receptor expression in the liver of diabetic mice. Db/db mice treated with glucagon receptor ASO exhibited lowered blood glucose, triglyceride, and free fatty acid levels and improved glucose tolerance with no apparent hypoglycemia or changes in pancreatic islet architecture [68]. In a similar study, glucagon receptor ASO

decreased cAMP-dependent gene transcription in the liver and not only preserved insulin secretion, but increased serum levels of both insulin and GLP-1 [69]. ASO treatment resulted in significant α-cell hypertrophy and/or hyperplasia [69] and increased levels of glucagon; however, the hyperglucagonemia appeared to be reversible. The data obtained with glucagon receptor ASO treatment is consistent with the phenotype observed in $GCGR^{-/-}$ mice, providing further evidence that this treatment specifically targets glucagon receptor expression.

There is strong evidence suggesting that hyperglucagonemia plays a major role in the hyperglycemia associated with both type 1 and type 2 diabetes. The growing amount of data demonstrating improved glucose tolerance and decreased glycemia following administration of agents that block glucagon receptor action accentuates the importance of glucagon for the control of glucose homeostasis. Both GLP-1 and amylin exert potent inhibitory effects on glucagon secretion in human subjects, even in patients with type 1 diabetes [70, 71], hence GLP-1R agonists or amylin analogues may exert glucose-lowering effects in diabetic subjects in part *via* reduction of glucagon action. The discovery of molecules that permit safe and effective lowering of glucagon receptor signaling may prove therapeutically useful for the treatment of diabetes in human subjects.

Glucagon can be further processed in the liver by an endopeptidase to a truncated form consisting of only the C-terminal 11 amino acids [72]. Glucagon(19-29), or miniglucagon, inhibited glucagon-induced increases in intracellular calcium and was a powerful inhibitor of insulin release in cultured pancreatic β-cells [73] (Fig. **3**). Interestingly, miniglucagon had no effect on glucagon-induced cAMP accumulation in this model. While the effects of miniglucagon on calcium signaling in heart [74-76] and liver [77] cells have been investigated, little data is available on the potential use of miniglucagon as an antagonist of glucagon action in rodent models of diabetes. Nevertheless, miniglucagon blocks the insulinotropic effect of co-administered glucagon and the administration of antisera directed against miniglucagon enhanced glucose-stimulated insulin release from the rat pancreas [78, 79]. Hence modulating miniglucagon action may also be theoretically useful for enhancing β−cell function in the setting of type 2 diabetes.

THE GLUCAGON-LIKE PEPTIDES

While glucagon secretion from islet α−cells is stimulated by hypoglycemia and suppressed following food intake, the two glucagon-like peptides, GLP-1 and GLP-2, are released from intestinal enteroendocrine L-cells following nutrient ingestion [3, 80, 81], primarily by meals rich in carbohydrates and lipids [82-85]. Secretion of these intestinal PGDPs is regulated by glucose-dependent insulinotropic polypeptide [86], somatostatin [87], gastrin-releasing peptide [88], and neural stimuli [89], in a species-specific manner [90]. For a more detailed review on the regulation of glucagon-like peptide secretion, see [90-92]. Although the biologically active forms of GLP-1 and GLP-2 share almost 40% amino acid identity, they possess very distinct and separate biological actions due to their high specificity of binding to their respective receptors. While both peptides regulate energy homeostasis, GLP-2 acts mainly in the gastrointestinal tract to promote nutrient absorption and maintain bowel integrity, while GLP-1 inhibits gastric emptying and glucagon secretion and stimulates insulin synthesis and release in a glucose-dependent manner.

Glucagon-Like Peptide-1 (GLP-1)

GLP-1 exists as two equipotent bioactive molecular forms, GLP-1 (7-37) and GLP-1 (7-36)amide (Fig. **3**). GLP-1 secretion facilitates lowering of blood glucose and improved glucose tolerance by increasing the insulin:glucagon ratio. GLP-1 also inhibits post-prandial gastrointestinal motility and secretion, and suppresses food intake *via* the central nervous system (CNS). For a more detailed account of the biological actions of GLP-1, refer to [92-94]. More recently, considerable experimental evidence indicates that activation of GLP-1R signaling can also protect β−cells from programmed cell death (apoptosis) and stimulate β−cell proliferation in cultured rodent and human islets and in rodent models of diabetes [95-99].

GLP-1 binds to the β−cell GLP-1 receptor (GLP-1R) with high affinity and specificity. The GLP-1R is also found in the lung, stomach, intestine, kidney, heart, and the central and peripheral nervous systems [100-102]. Whether the GLP-1 receptor is also expressed in adipose tissue [103, 104], muscle [105, 106], liver [107, 108], and pancreatic α-cells [109, 110] in all mammals remains unclear. The existence of GLP-1-activated signaling pathways in these tissues has stimulated interest in the search for a second GLP-1R receptor.

The GLP-1R is coupled to multiple downstream signaling pathways. Activation of the GLP-1R in transfected cells and cultured islets leads to increases in intracellular cAMP, and the activation of PKA, cAMP/guanine-nucleotide exchange factor (Epac), and PI-3K [111, 112]. GLP-1R signaling inhibits ATP-sensitive and voltage-dependent K^+ channels [113], thus reducing cell depolarization, and increasing cytosolic calcium [114], resulting in the stimulation of cellular exocytosis machinery [115]. GLP-1R activation stimulates insulin release and induces glucose competence in cultured islet cells, up-regulates insulin gene expression, and enhances insulin biosynthesis. GLP-1Rs are also widely distributed in multiple regions of the brain where receptor activation stimulates expression of c-fos [116, 117], in association with inhibition of food intake and regulation of hypothalamic-pituitary function. The cytoprotective actions of GLP-1 are largely mediated through cAMP/PKA-dependent activation of CREB and PI-3K-dependent activation of Akt/PKB [96, 99, 118, 119]. GLP-1R signaling leads to decreased cleavage of pro-apoptotic molecules, including caspase-3 and poly-ADP-ribose polymerase (PARP), and increased expression of cytoprotective molecules such as Bcl-2, Bcl-xL, and the inhibitor of apoptosis protein-2 (IAP-2) (For review, see [120]). Mice with a targeted deletion of the GLP-1R exhibit mild fasting hyperglycemia and glucose intolerance in association with decreased circulating insulin levels following glucose challenge [121], but are otherwise viable and healthy. Furthermore, consistent with a role for endogenous GLP-1R signaling in the control of β-cell mass, $GLP\text{-}1R^{-/-}$ mice exhibit reduced numbers of large islets [122]

Fig. (3). The amino acid structure of the human proglucagon-derived peptides and their degradation products. Glicentin, oxyntomodulin, glucagon, and miniglucagon are all derived by differential cleavage of the N-terminal domain of proglucagon, and thus share high sequence identity. However, only glucagon has been shown to have high affinity and potency at the glucagon receptor. Miniglucagon may act as a glucagon receptor antagonist *in vivo*. Ubiquitously expressed DPP-IV rapidly cleaves off the two N-terminal amino acids from both GLP-1 and GLP-2, yielding the biologically inactive GLP-1 (9-37/9-36^{amide}) and GLP-2 (3-33). These byproducts may serve as functional receptor antagonists *in vivo*. Substitution of a glycine residue for the alanine at position two renders [Gly2]GLP-2 (1-33) insensitive to DPP-IV cleavage and dramatically increases its biological half-life.

and enhanced β−cell apoptosis following administration of streptozotocin [96].

Therapeutic Potential of GLP-1

Treatment of Diabetes Mellitus

Activation of GLP-1 receptor signaling in the pancreas leads to increased production and secretion of insulin in response to hyperglycemia, with concurrent protection of β-cell mass, improving glucose tolerance and maintaining islet integrity. GLP-1R signaling also improves the glucose-sensing ability of the β-cell [123], possibly through up-regulation of glucose transporter and glucokinase expression [124]. All of these biological actions, but specifically its ability to potently stimulate glucose-dependent insulin release makes GLP-1-based therapies an attractive approach for the treatment of type 2 diabetes.

Patients with type 2 diabetes exhibit elevated levels of fasting blood glucose, insulin resistance, reduced glucose-stimulated insulin release, and progressive β-cell failure. Administration of native GLP-1 or GLP-1R agonists has consistently improved glucose tolerance and glycemic control in studies of human subjects with type 2 diabetes (For review, see [125, 126]). Although the GLP-1 secretory response in patients with type 2 diabetes may be diminished compared to healthy human subjects [127], low-dose infusion of GLP-1 significantly increases insulin secretion and improves β−cell responsiveness to glucose [123]. Type 2 diabetics treated continuously for six weeks with a subcutaneous infusion of GLP-1 exhibited enhanced insulin secretion, reduced fasting and post-prandial blood glucose, improved insulin sensitivity and decreased levels of hemoglobin A_{1c} (HbA_{1c}) [128]. Administration of GLP-1 to patients with type 2 diabetes has shown promising results even following the failure of other insulinotropic agents to lower glucose [129]. Moreover, GLP-1 has also been shown to increase the effectiveness of commonly used diabetes medications from various drug families, including metformin [130], pioglitazone [131], and glibenclamide [132], when administered concurrently. As the effects of GLP-1 on insulin secretion and biosynthesis are glucose-dependent, there is far less likelihood of developing hypoglycemia in response to GLP-1 administration relative to the mechanism of action of other insulinotropic drugs [133].

While the principal effect of GLP-1 administration is the improvement of blood glucose in diabetic subjects, pro-

longed treatment with GLP-1R agonists may also have additional benefits in diabetic individuals. Moreover, there is tremendous interest in determining whether long-term activation of GLP-1R signaling in human subjects will inhibit β−cell apoptosis and stimulate β−cell proliferation thereby preventing the progressive deterioration in β−cell function that is frequently associated with the natural history of type 2 diabetes.

The majority of interest in the therapeutic potential of GLP-1 is focused on type 2 diabetes; however, there is some interest in GLP-1 action in the setting of type 1 diabetes. Although GLP-1 failed to augment insulin-mediated hepatic glucose uptake [134], infusion of native GLP-1, or the GLP-1R agonist exendin-4 (see GLP-1R agonists), normalized postprandial glucose excursions and improved glycemic control in patients with type 1 diabetes [135, 136]. Normalization of blood glucose in subjects with type 1 diabetes was most likely achieved through reduction of the rate of gastric emptying and inhibition of glucagon secretion. Given the cytoprotective and proliferative capabilities of GLP-1 demonstrated in pre-clinical studies, there is considerable interest in the potential use of GLP-1 to stimulate β-cell proliferation and/or neogenesis in patients with type 1 diabetes. Furthermore, the recent demonstration that islet neogenesis may be achieved using a combination of peptides such as GLP-1 and gastrin, suggests new lines of future experimentation for the treatment of type 1 diabetes.

GLP-1, Food Intake and the Control of Body Weight

Activation of the GLP-1R signaling axis in the central nervous system regulates feeding behavior. GLP-1, administered *via* intracerebroventricular (ICV) injection inhibits food intake in rodents [137, 138] and may induce a conditioned taste aversion and an aversive stress response [139, 140]. GLP-1, and long-acting GLP-1R peptide agonists cross the blood-brain barrier [141, 142], hence hormone signals released from the gastrointestinal tract following a meal may communicate with CNS centers regulating subsequent feeding activity. Furthermore, administration of much larger GLP-1R agonists (Albugon) that do not readily cross the blood brain barrier results in reduced food intake and activation of c-fos expression in the murine CNS following intraperitoneal administration [143]. In human studies, type 2 diabetics receiving GLP-1 therapy exhibited reduced food intake and subsequent weight loss [128, 144]. Hence GLP-1R agonists have the ability to decrease body weight in diabetic patients following chronic administration *in vivo*.

To address the effectiveness of GLP-1 in the setting of obesity, GLP-1 was administrated to normal and obese human subjects in short-term infusion studies where it enhanced satiety and decreased energy intake [145, 146]. Although it is possible that the anorectic actions of GLP-1 are mediated *via* GLP-1R receptor activation in the central nervous system, the effects of GLP-1 on satiety and decreased appetite may be due to in part to its ability to decrease gastric motility [147]. Whether chronic GLP-1R agonist administration will promote weight loss in non-diabetic obese human subjects in the absence of type 2 diabetes merits further investigation.

GLP-1 and the Treatment of Central Nervous System Disorders

Due to the anti-apoptotic and proliferative actions of GLP-1R signaling and the diverse expression of the GLP-1R throughout the brain, GLP-1 may also play a role in neuroprotection and neuronal regeneration in the central nervous system. Consistent with this hypothesis, GLP-1 treatment of PC12 cells, a commonly used model of neuronal cell differentiation, promoted neurite outgrowth and nerve growth factor (NGF)-induced differentiation [148]. Furthermore, GLP-1 prevented apoptosis in PC12 cells and cultured rat hippocampal neurons after NGF withdrawal or glutamate treatment, respectively [148, 149]. GLP-1 reduced the levels of amyloid beta-peptide in the brain, and reduced the levels of amyloid precursor protein (APP) and inhibited amyloid beta-peptide and iron-induced cell death in cultured hippocampal neurons [149]. These studies have generated interest in the potential of GLP-1-based therapies for the treatment of neurodegenerative diseases, such as Alzheimer's disease, or diabetic peripheral neuropathy (for review, see [150]).

GLP-1 may also play a role in the regulation of cognitive function. Activation of GLP-1R signaling in the CNS enhanced associative and spatial learning in mice whereas GLP-1R knockout mice exhibit learning deficits that are reversed upon recovery of GLP-1R expression using gene therapy [151]. Moreover, over-expression of GLP-1R in the hippocampus enhances learning and memory in rats and GLP-1R agonist administration reduced kainite-induced neuronal apoptosis and seizure activity in normal mice and in GLP-1R knockout mice after GLP-1R gene transfer in hippocampal cells [151]. Hence GLP-1 may facilitate protection and modeling of areas of the central nervous system responsible for learning and memory.

GLP-1 and the Cardiovascular System

GLP-1 has acute effects on the cardiovascular system that appear different in rodents vs. human subjects. Treatment of rats with GLP-1 produced rapid significant dose-dependent increases in both diastolic and systolic blood pressure and heart rate that could be blocked by a specific GLP-1R antagonist [152-154]. Conversely, GLP-1R knockout mice exhibit reduced resting heart rate, elevated left ventricular (LV) end diastolic pressure, and increased LV thickness at two months of age. Although basal cardiac function appeared similar to wild-type mice, 5-month old GLP-1R$^{-/-}$ mice exhibited impaired LV contractility and diastolic function following insulin-induced hypoglycemia [155]. Although GLP-1R activation in rat cardiomyocytes stimulated accumulation of intracellular cAMP, increased GLP-1 receptor signaling paradoxically decreased contraction amplitude and did not affect cellular calcium levels [156]. Therefore the contractile and chronotropic effects of systemic GLP-1 infusion may be indirectly mediated through activation of neural or hormonal mechanisms. GLP-1 or exendin-4 can also activate central sympathetic neurons and adrenal medullary chromaffin cells that produce catecholamines, suggesting that GLP-1R signaling networks may modulate cardiovascular function *via* central nervous system-dependent pathways [157].

Further evidence for multiple signal transduction pathways regulating central GLP-1R-dependent cardiovascular events derives from studies in rats demonstrating a role for nicotinic, muscarinic, and vasopressin receptor signaling in GLP-1R-dependent regulation of blood pressure [158]. More recently, the therapeutic potential of GLP-1 administration has been examined in specific models of cardiovascular dysfunction. GLP-1 was administered to dogs with heart failure induced following 28 days of rapid electrical pacing. GLP-1 increased ventricular contractility, stroke volume and cardiac output, and decreased left ventricular end-diastolic pressure, heart rate, and systemic vascular resistance. GLP-1 also increased myocardial insulin sensitivity and myocardial glucose uptake [159].

Paradoxically, despite the acute inotropic effects of GLP-1 on cardiovascular function, a two week GLP-1 infusion in salt-sensitive rats attenuated hypertension, reduced proteinuria and albuminuria, improved endothelial function, and decreased cardiac and renal damage [160, 161]. It was hypothesized that these effects were due mainly to the diuretic and natriuretic properties of GLP-1R signaling in the kidney. Similarly, a 3-hour infusion of GLP-1 in healthy human subjects and insulin-resistant obese men enhanced sodium excretion, reduced H^+ secretion, and reduced glomerular hyperfiltration [162].

Complementary studies examined the effects of GLP-1 administration in patients with acute myocardial infarction (AMI) and severe systolic dysfunction after successful angioplasty. GLP-1-treated patients had significantly improved LV function, reduced levels of plasma glucose and free fatty acids and improved global wall motion score indexes and regional wall motion score indexes [163]. This data suggests that GLP-1 therapy merits further investigation in the treatment of specific cardiovascular diseases, and additional studies are needed to determine the precise mechanisms for the beneficial aspects of GLP-1 action in these patients.

The Biological Half-Life of the Glucagon-Like Peptides - Role of DPP-IV

One of the major drawbacks of using native GLP-1, or GLP-2, as a therapeutic agent for the treatment of human disease is their rapid enzymatic degradation and clearance, leading to extremely short biological half lives *in vivo*. The alanine residue in position two makes both glucagon-like peptides targets for cleavage by a ubiquitously expressed protease, dipeptidyl peptidase-IV (DPP-IV) (Fig. **3**). Native GLP-1 has a half-life ($t_{1/2}$) of 1 – 2 minutes in human subjects, due mainly to enzymatic degradation and extensive renal clearance, while GLP-2 has a slightly longer half-life of 7 minutes. Once cleaved, the biologically inactive forms of GLP-1 (9-37/36amide) and GLP-2 (3-33) are also rapidly excreted *via* the kidney. These peptide metabolites may serve as receptor antagonists, thus decreasing the activity of agonists [164], or may also have additional biological functions. It has recently been demonstrated that GLP-1 (9-36amide) reduces blood glucose in pigs [165], however this metabolite has been shown to have no effect on control of blood glucose in humans [166]. Thus, research is primarily focused on the biological and physiological actions of the intact peptides and their potential usefulness as therapeutic agents.

Although both native GLP-1 and GLP-2 have been shown to be effective following chronic administration to patients with diabetes and short bowel syndrome, respectively [128, 167], continuous infusion or multiple injections of the native peptide were needed to achieve sufficient levels of bioactive peptide throughout the treatment period. Therefore, intense efforts have been made to prolong drug action through the development of long-acting GLP-1 and GLP-2 analogues with improved pharmacokinetic profiles that are resistant to DPP-IV cleavage or through drugs that target and inhibit the DPP-IV enzyme directly.

GLP-1R Agonists

In 1993, a naturally occurring peptide isolated from the venom of the *Heloderma suspectum* lizard was characterized as a specific GLP-1R agonist with enhanced biological activity compared to native GLP-1 [168-171]. Exendin-4 exhibits approximately 53% amino acid identity relative to native GLP-1 (Fig. **4**); a glycine residue in position two renders the peptide resistant to DPP-IV cleavage, and thus confers a significantly longer half-life of ~ 26 minutes in humans following IV exendin-4 administration [172]. Exendin-4 shares a similar structure to GLP-1 and it has been shown that the additional 9 amino acids at the C-terminus increase the affinity of the GLP-1R for exendin-4 [173]. While multiple GLP-1R extracellular domains are required for binding of native GLP-1, exendin-4 binding appears to primarily involve only the N-terminus [174]. Thus, the enhanced actions of exendin-4 are mostly likely the combined result of reduced DPP-IV cleavage and enhanced binding to the GLP-1R. The requirement of the N-terminus of native GLP-1 for specific binding to the GLP-1R may explain why modifications made to the native GLP-1 amino acid sequence to render them insensitive to DPP-IV significantly decrease the receptor affinity for the new ligand. It is interesting to note that although many of these DPP-IV resistant GLP-1 analogues exhibit decreased binding and cAMP accumulation, they appear to have similar or enhanced insulinotropic and glucose-lowering effects, *in vitro* and *in vivo*, compared to native GLP-1 [175-177].

A synthetic version of exendin-4, Exenatide (AC2993), has been studied in human patients with Type 2 diabetes. Exenatide administered subcutaneously for 5 days reduced fasting and post-prandial blood glucose levels, in association with increased levels of plasma insulin, decreased circulating glucagon, and reduced gastric emptying in patients with type 2 diabetes [178] (For review, see [171]). The main adverse events included transient headache, nausea, and vomiting. Exenatide has also been shown to be efficacious when used in conjunction with other diabetes therapies, leading to lowering of HbA_{1c} levels in diabetic patients not currently achieving optimal glycemic control with diet and/or oral anti-diabetic medications [133]. Exenatide has completed Phase 3 clinical testing and long acting release formulations of Exenatide (Exenatide LAR), which may allow once a week dosing, are under clinical development.

Exenatide was approved for the treatment of type 2 diabetes in the United States in April 2005 on the basis of 3 separate Phase 3 clinical trials.[2-4] Patients with diabetes not optimally controlled on either metformin, sulphonylurea therapy, or a combination of metformin and sulphonylurea

received twice daily injections of Exenatide, 5 or 10 ug twice daily. The therapy was generally well tolerated, although nausea was the principal adverse event reported in about 40% of the Exenatide-treated subjects. Exenatide reduced levels of HbA1c ~ 0.9-1% over 30 weeks, in association with modest weight loss (1-2 kg). The incidence of mild to moderate hypoglycemia was increased in patients receiving Exenatide plus a sulphonylurea agent but not in subjects treated with Exenatide plus metformin. Although anti-Exenatide antibodies were detected in up to 40% of patients following initiation of Exenatide therapy, there was no correlation between the presence or absence of antibodies and the therapeutic response to Exenatide.[2-4]

Addition of amino acid side-chains on the GLP-1 peptide also decreases the extent of DPP-IV cleavage [179]. Similar to data represented for other GLP-1 analogs resistant to enzymatic cleavage, these GLP-1R agonists have reduced affinities for the receptor and a corresponding diminished cAMP response. However, these DPP-IV resistant analogs produced potent insulinotropic effects *in vitro* and when modified at the N-terminus, these agonists significantly improved glucose tolerance and insulin secretion in diabetic mice [177, 180].

Liraglutide (NN2211) is a human GLP-1 analogue that possesses a fatty-acyl moiety on the GLP-1 peptide backbone (Fig. **4**), increasing the half-life in humans to approximately 12 hours by conferring DPP-IV resistance and promoting non-covalent binding to serum albumin, thus protecting the peptide from degradation and reducing renal clearance. Liraglutide was well tolerated when administered to humans in short-term studies, and a one week treatment course in diabetic patients improved β−cell function and decreased blood glucose and levels of plasma glucagon [181-183]. Liraglutide administered once daily for 8 weeks to obese diabetic patients resulted in improved glycemic control with no changes in overall body weight or energy expenditure, although a trend toward reduction of fat mass was noted [184]. Administration of Liraglutide for 12 weeks reduced HbA_{1c} and the ratio of proinsulin:insulin, improved glycemic control, and reduced body weight in diabetic patients [185]. Adverse events following Liraglutide treatment were reported to be mild and transient.

The theoretical attractiveness of prolonging the circulating $t_{1/2}$ of GLP-1 has stimulated the development of analogues that utilize binding to albumin, which has a biological half-life of 11-14 days in human subjects, for prolonging the duration of action of GLP-1. Prototype drugs that utilize albumin technology include CJC-1131 (Conjuchem) and Albugon (Human Genome Sciences). To create CJC-1131, a maleimidoproprionic acid (MPA) group was attached to the carboxy terminus of a degradation-resistant GLP-1 analogue (Fig. **4**) [186]. This modification increased the circulating half-life to 10 – 12 days in humans. CJC-1131 binds to the human GLP-1 receptor and significantly reduced levels of blood glucose, increased insulin transcripts, and increased islet size when administered for 4 weeks to db/db mice [187]. Albugon is a recombinant protein encoding GLP-1 fused in the same open reading frame as human serum albumin. This GLP-1-albumin fusion protein improved glucose tolerance, reduced food intake, inhibited gastric emptying, and activated c-fos expression in the murine central nervous system [143]. Although albumin-based drugs show efficacy in animal models, there is limited information about their effectiveness in human clinical studies.

Glucagon-Like Peptide-2 (GLP-2)

GLP-2 is co-secreted together with GLP-1 from intestinal L-cells in a 1:1 ratio. While GLP-1 elicits its main effects on the endocrine pancreas, the majority of GLP-2 action is observed directly in the gastrointestinal mucosa. Bioactive GLP-2 (1-33) inhibits gastric emptying and gastric acid secretion [188-190], while enhancing nutrient absorption and epithelial barrier function [191-193]. A role for GLP-2 in the regulation of intestinal mucosal growth was discovered following studies in rodents. Exogenously administered native GLP-2 stimulated significant small bowel growth in mice, which was largely due to lengthening of the intestinal villi [194]. The growth-promoting effects of GLP-2 on the bowel were due in part to increased crypt cell proliferation and inhibition of enterocyte apoptosis (For review, see [195, 196]). GLP-2 is also produced in the brainstem and transported to different regions of the central nervous system. Although the precise actions of GLP-2 in the central nervous system remain unclear, there is some evidence that pharmacological amounts of GLP-2 can inhibit food intake in rodents following intracerebroventricular administration [197, 198], but peripheral infusion or intermittent injection of GLP-2 in humans has not been associated with a reduction in food intake [167, 199, 200].

The GLP-2 receptor (GLP-2R) was cloned from rat and human hypothalamic and intestinal cDNA libraries [201]. In contrast to the widespread tissue distribution of the GLP-1R, GLP-2R expression is highly restricted, predominantly to the gastrointestinal tract. GLP-2 receptor transcripts have been detected in the stomach, small and large bowel, the brain, and the lung [197, 202, 203]. Immunocytochemistry has localized the GLP-2R to human enteroendocrine cells [202], myofibroblasts[5] and specific regions of the murine central nervous system [198], while *in situ* hybridization has localized the receptor in the CNS [203] and murine enteric neurons [204]. The GLP-2R recognizes GLP-2, but not related members of the glucagon peptide superfamily, in a highly specific manner [201, 205]. Agonist binding results in dose-dependent activation of adenylyl cyclase, increases in intracellular cAMP, and activation of PKA in cells expressing a heterologous rat or human GLP-2R, as well as in primary cell cultures from the CNS and the intestinal mucosa [201, 203, 206, 207]. Furthermore, GLP-2 has been shown to

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Exenatide – AC2993 (Amylin)

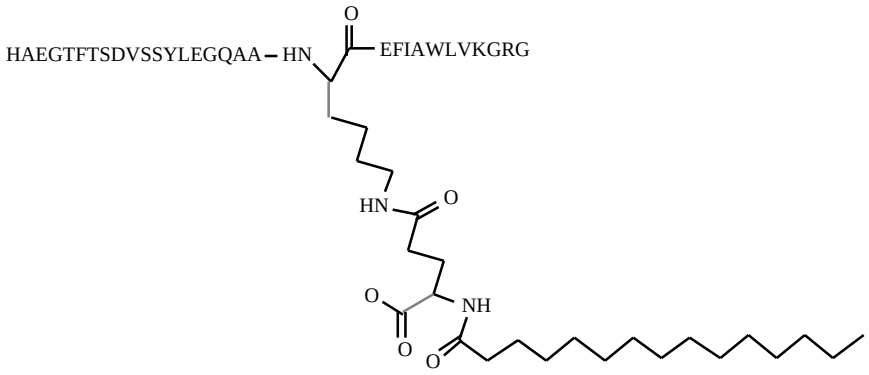
H***A***EGTFTSDVSSYLEGQAAKEFIAWLVKGRG GLP-1(7-37)

H EGTFTSD+S +E +A + FI WL G

H***G***EGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS Exenatide (Exendin-4)

DLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS Exendin(9-39)

Liraglutide – NN2211 (Novo Nordisk)



$Arg^{34}Lys^{26}$-(N-ε-(γ-Glu(N-α-hexadecanoyl)))-GLP-1(7–37)

CJC-1131 – (Conjuchem)

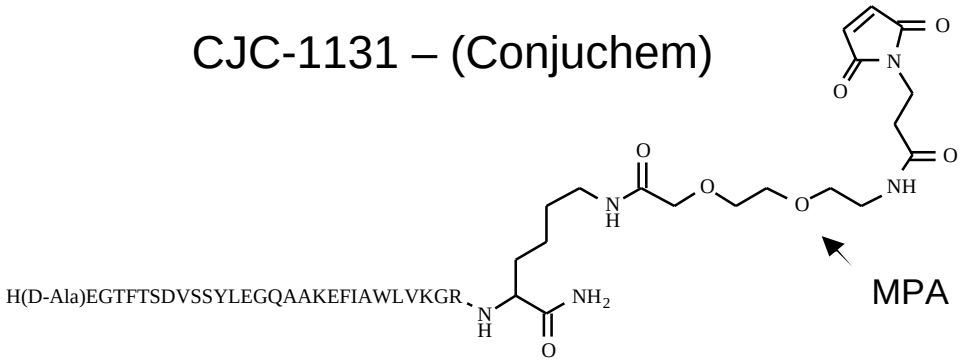


Fig. (4). Long-acting, degradation-resistant GLP-1R agonists. A synthetic version of the lizard derived peptide exendin-4 (exenatide) is a highly potent GLP-1R agonist which is highly homologous to GLP-1, but resistant to DPP-IV cleavage due to the glycine residue in position two. Identical (letters) and similar (+) amino acids are shown to illustrate homologous regions. N-terminally truncated Exendin (9-39) is a potent GLP-1R antagonist *in vitro* and *in vivo*. The biological half-life of GLP-1 is significantly extended by the addition of a fatty-acyl motif (liraglutide) [256] or maleimidoproprionic acid (MPA) [186, 187] which promote binding of the peptide to serum albumin. Chemical structures have been adapted and reproduced with permission from the publishers.

activate c-fos in cells transfected with the GLP-2R [206] and in the murine intestinal mucosa [204]. GLP-2R signaling has also been shown to activate intestinal constitutive nitric oxide synthase (NOS) activity and endothelial NOS protein abundance that appears to play a role in GLP-2-induced intestinal blood flow and glucose uptake [208].

The direct cytoprotective effects of GLP-2R signaling are mediated *via* a number of signaling mechanisms depending on the specific apoptotic stimulus and experimental model. GLP-2R activation inhibits cycloheximide–induced apoptosis in a PKA-independent manner [209], while PKA appears to regulate the anti-apoptotic properties of GLP-2R signaling when apoptosis is induced by either inhibition of PI-3K [210] in transfected fibroblasts or glutamate in hippocampal neurons [203]. There is conflicting evidence on whether GLP-2R activation can directly stimulate cellular proliferation. Treatment of cultured colonic intestinal cells or astrocytes with GLP-2 results in increased cell proliferation [211-

213], whereas cells transfected with the GLP-2 receptor fail to exhibit a significant mitogenic effect after treatment with moderate concentrations of GLP-2 [206]. This suggests that GLP-2R signaling may regulate cell proliferation indirectly, possibly *via* release of as yet unidentified growth factors. Furthermore, GLP-2 treatment may inhibit proliferation in cultured epithelial cells from the small intestine yet high concentrations of GLP-2 stimulate cell proliferation in cell lines derived from the large bowel [214], suggesting that GLP-2R activation of downstream mitogenic effectors may be cell type- or tissue-specific. Importantly, the effects of GLP-2 in different cell types are often observed in the absence of documented expression of the known GLP-2R, leaving open the possibility that GLP-2 may exert some of these effects through as yet undefined receptors and signaling pathways.

Therapeutic Potential of GLP-2

Intestinal Disease and Injury

Following the identification of GLP-2 as the PGDP most likely responsible for the marked small bowel hyperplasia observed in humans or rodents with proglucagon-producing tumours [194, 215], interest has focused on the potential use of GLP-2 as a therapeutic agent to stimulate bowel growth and/or repair mucosal damage in the setting of gastrointestinal injury. In addition to proliferative and cytoprotective actions, GLP-2 also enhances nutrient absorption, and inhibits gastric acid secretion and emptying, further extending the potential benefits of using this peptide hormone to treat human patients with compromised gastrointestinal function.

GLP-2 (1-33) is rapidly inactivated by DPP-IV cleavage and GLP-2 (3-33) has recently been shown to act as a weak agonist and a competitive antagonist at the GLP-2R *in vitro* and *in vivo* [216]. In an effort to extend the circulating half-life of GLP-2 *in vivo*, a peptide analogue was developed with a glycine residue substituted for the alanine in position two, thus preventing DPP-IV-mediated cleavage. This analogue, designated ALX-0600, or Teduglutide specifically activates the GLP-2R *in vitro* [206] and is more potent relative to native GLP-2 *in vivo* [217].

h[Gly2]GLP-2 and native GLP-2 have been used to examine the therapeutic benefit of enhanced GLP-2 action in diverse models of intestinal injury. Infusion of GLP-2 in pigs or rats prevented the mucosal atrophy associated with total parental nutrition (TPN) [218-221] and h[Gly2]GLP-2 administration significantly decreased intestinal permeability in a rodent model of acute necrotizing pancreatitis [222]. Furthermore GLP-2 stimulated mucosal growth and reduced immunosuppression following acute burn injury in rats [223]; enhanced gastrointestinal absorptive function and induced mucosal hyperplasia in rat models of ischemia-reperfusion and following massive intestinal resection [224, 225]; and decreased the severity of bowel injury in the setting of experimental enteritis or colitis in mice [226-230]. GLP-2 also acutely reduced mucosal permeability and diminished both immediate and late-phase hypersensitivity reactions by enhancing barrier function in mice [193, 231]. Although there is limited experience with GLP-2 administration in humans, twice daily injections of GLP-2 in patients with short bowel disease resulted in increased nutrient absorption, increased body weight, and delayed gastric emptying [167]. Due to its significant protective and regenerative properties in the bowel, NPS Pharmaceuticals Inc. is currently investigating the efficacy of h[Gly2]GLP-2, currently named Teduglutide, in humans with intestinal diseases characterized by insufficient repair of the intestinal mucosa and/or compromised nutrient absorption.

Chemotherapy and Cancer

Significant bowel injury is often associated with intensive chemotherapy for the treatment of cancer. Due to its cytoprotective properties, GLP-2 administration to patients undergoing chemotherapy may lessen the damaging effects of chemotherapy on the intestinal epithelium. In support of this hypothesis, administration of GLP-2 prior to treatment of mice with either irinotecan hydrochloride or 5-fluorouracil reduced mortality, decreased bacteremia, and enhanced intestinal crypt cell survival [232]. Similarly, a DPP-IV inhibitor was recently shown, in combination with metformin, to reduce 5-fluorouracil-induced intestinal atrophy, potentially through its effect on increasing the levels of GLP-2 *in vivo* [233].

As GLP-2 is a potent growth factor for the bowel mucosa, sustained GLP-2 administration may exhibit potential for promotion of intestinal tumor growth. In studies with tumor-bearing rats, GLP-2 administration did not promote existing tumor growth [219]. Similarly, administration of h[Gly2]-GLP-2 to tumor-bearing mice did not effect tumor growth or the effect of chemotherapy on reduction of tumor size [232]. In contrast, administration of GLP-2 increased the number of small intestinal polyps in mice pre-injected with the carcinogen 1, 2-dimethylhydrazine (DMH), suggesting that GLP-2, in the setting of co-administered carcinogens may promote the growth of chemically-induced murine tumors [234].

GLP-2 and Bone

A significant increase in bone density was observed in human patients with short-bowel syndrome following short-term administration of GLP-2 [235], suggesting that GLP-2 may also regulate bone growth and/or remodeling. Although the precise localization of GLP-2 receptor expression in isolated bone tissue or in bone-derived cell populations remains unclear, GLP-2 caused a dose-dependent decrease in bone-resorption and stimulated bone formation when administered to postmenopausal women [236, 237]. These findings suggest that GLP-2 may also be effective, either directly or indirectly, in the prevention and treatment of osteoporosis.

GLP-2 Signaling in the Central Nervous System and Regulation of Feeding

ICV administration of GLP-2 in rats inhibited food intake [197]; however, a subsequent study showed only a modest yet significant inhibition of short-term food intake following intracerebroventricular administration of pharmacological amounts of GLP-2 in mice [198]. Intriguingly, whereas the actions of GLP-2 on food intake in rats were blocked by the GLP-1R antagonist exendin(9-39), similar experiments using exendin(9-39) in mice resulted in augmentation of the anorectic actions of GLP-2 [198]. These discrepancies may potentially be explained by species-specific differences in GLP-2 action in the brain. Furthermore, studies in humans

have not demonstrated a reduction in food intake following peripheral GLP-2 administration [167, 199, 200]. These data suggest that GLP-1R and GLP-2R signaling may impinge upon common downstream targets in the CNS in a species-specific manner.

Consistent with the anti-apoptotic properties of GLP-1 in the CNS, GLP-2 also inhibits apoptosis in cultured hippocampal neurons [203]. As GLP-2 receptor expression has been localized to multiple regions of the central nervous system involved in learning and memory and in enteric neurons, GLP-2R signaling may play a role in neuronal function and/or survival.

DPP-IV Inhibitors

Native GLP-1 and GLP-2 are both inactivated by enzymatic cleavage, hence inhibition of the activity of DPP-IV would be predicted to increase the circulating level of both peptides *in vivo*. DPP-IV (CD26) was originally identified as a glycoprotein found on the surface of lymphocytes, and has been shown to regulate cytokine production and T cell proliferation (For review, see [238]). Although DPP-IV appears to play an important role in multiple aspects of immune function, DPP-IV knockout mice are viable and show no overt immunological phenotype in the basal state [239]. However, $CD26^{-/-}$ mice exhibit alterations in the proportion of T cell subsets, and reduced immunoglobulin and cytokine responses following immunization with pokeweed mitogen [240]. Consistent with an essential role for CD26/DPP-IV in the control of GLP-1 degradation, $CD26^{-/-}$ mice exhibit increased levels of intact GLP-1, circulating insulin, and enhanced glucose tolerance in response to an oral glucose challenge state [239].

A number of DPP-IV inhibitors have been developed that mimic the glycemic phenotype produced in the knockout mouse, and may have therapeutic potential for the treatment of human disease. DPP-IV inhibitors have been shown to enhance post-prandial insulin secretion, increase pancreatic insulin content, maintain islet integrity, and improve peripheral insulin sensitivity in animal models of diabetes [241-244]. Clinical data with DPP-IV inhibitors in human subjects with type 2 diabetes, although limited, suggest that DPP-IV inhibitors improve glycemic control, inhibit glucagon secretion, and decrease HbA_{1c} levels in 4-12 week studies. For a more extensive overview of current data involving the use of DPP-IV inhibitors to treat diabetes, see [126, 238, 245-247]. DPP-IV inhibition also prevents the degradation of GLP-2 *in vivo* and enhances the intestinotrophic effect of exogenously administered GLP-2 in rodents. Although the use of DPP-IV inhibitors for the treatment of diabetes seems promising, inhibition of DPP-IV may have potential unwanted side effects on immunological function. Highly specific DPP-IV inhibitors that target the catalytic site of the enzyme without abrogating CD26 signaling in lymphocytes have been developed; whether they will prove safe in long-term human studies merits careful ongoing assessment.

DESENSITIZATION OF THE GLUCAGON RECEPTOR FAMILY

Following identification of the GPCR superfamily of receptors, extensive research has been conducted to identify native ligands and elucidate the biological functions associated with receptor activation. Since GPCRs serve as receptors for a multitude of naturally occurring hormones, neurotransmitters, and signal transduction molecules, GPCRs have proven to be successful targets for the development of drugs that modulate receptor function. Hence investigation of the mechanisms that regulate GPCR receptor signaling and the implications of repeated receptor stimulation is of interest to scientists and has direct relevance for the clinical use of long-acting GPCR agonists. GPCR signaling is tightly regulated at the cellular level, and even low-level receptor stimulation can lead to rapid receptor desensitization and/or receptor down-regulation (For review, see [248]). Taken together, these processes can lead to attenuated receptor signaling and potentially diminish the effect of natural and synthetic GPCR ligands.

Although limited data is available regarding the regulation of Family B GPCRs, members of the glucagon receptor subfamily significantly desensitize upon receptor activation. Rapid agonist-dependent (homologous) and -independent (heterologous) glucagon receptor desensitization was observed in cultured hepatocytes and was found to be dependent on protein kinase C (PKC) activity and independent of intracellular cAMP concentrations [249, 250]. Prolonged agonist stimulation resulted in extensive receptor down-regulation at the cell surface accompanied by delayed resensitization of glucagon signaling [249]. However, the role of glucagon receptor desensitization *in vivo*, or in the setting of experimental or clinical diabetes, has not been extensively investigated.

A series of elegant studies characterized the mechanisms regulating GLP-1R desensitization in transfected fibroblasts and cultured islets. The GLP-1R underwent rapid agonist-induced desensitization, accompanied by significant receptor internalization, and heterologous desensitization induced by PKC activation [251, 252]. A number of serine doublets in the C-terminus of the GLP-1R were identified by site-directed mutagenesis as regulators of either homologous or heterologous receptor desensitization [253, 254]. Currently, there is no available data on the consequences of repeated or prolonged GLP-1R activation on receptor signaling or expression *in vivo*.

Similarly, the GLP-2R has been shown to undergo rapid and prolonged desensitization in response to acute agonist stimulation in transfected fibroblasts and intestinal epithelial cells. Moreover, agonist treatment stimulated significant receptor endocytosis *via* a dynamin-independent, lipid-raft-dependent mechanism and the reappearance of cell surface GLP-2Rs was delayed [255]. It has not yet been determined whether prolonged stimulation of the glucagon, GLP-1, or GLP-2 receptors, results in extensive down-regulation of receptor expression *via* protein degradation; however, this process has been characterized for other GPCRs. The data derived from studies of receptor action *in vitro* indicate that persistent stimulation of receptor signaling, either though constant infusion of peptide or the use of long-acting analogues, may theoretically result in the diminution of receptor signaling or possibly limit the effectiveness of these receptor agonists in the long-term treatment of human diseases. Nevertheless, there have been no reports suggesting the devel-

opment of clinically significant desensitization or loss of a therapeutic response in human studies with long-acting GLP-1 or GLP-2 analogues *in vivo*.

SUMMARY

Considerable progress has been made over the past decade in refining our concepts of PGDP action, employing a combination of cell based receptor studies, and physiological experiments using both normal and transgenic rodents and experimental models of diabetes, obesity, and gastrointestinal diseases. The therapeutic efficacy of glucagon receptor antagonists or ASOs to reduce glucagon action in preclinical studies suggests that reducing or blocking glucagon receptor signaling is a promising approach for the treatment of type 2 diabetes. Furthermore multiple GLP-1R agonists have demonstrated considerable efficacy in clinical trials of diabetic subjects, and DPP-IV inhibitors are now in late stage clinical testing for the treatment of type 2 diabetes. Moreover GLP-2 analogues also appear promising in pre-clinical models of intestinal disease and are being evaluated in human subjects with compromised gastrointestinal function. Although the long-term side effects of these different receptor agonists and enzyme inhibitors remain unknown, their safety profile to date has been promising. There remains considerable interest in developing new agents for enhancing PGDP action, either through stimulation of PGDP secretion, or through development of orally active small molecule receptor agonists. Taken together, the PGDP receptors represent a unique family of Family B GPCRs that regulate metabolic functions and represent attractive pharmaceutical targets for the treatment of human disease.

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ABBREVIATIONS

PGDP = Proglucagon-derived peptides

GLP-1 = Glucagon-like peptide-1

GLP-1R = Glucagon-like peptide-1 receptor

GLP-2 = Glucagon-like peptide-2

GLP-2R = Glucagon-like peptide-2 receptor

GPCR = G-protein-coupled receptor

GIP = Glucose-dependent insulinotropic polypeptide

VIP = Vasoactive intestinal peptide

PACAP = Pituitary adenylate cyclase-activating polypeptide

GHRH = Growth-hormone-releasing hormone

PEPCK = Phosphoenolpyruvate carboxykinase

G-6-Pase = Glucose-6-phosphatase

CREB = Cyclic-AMP response element binding protein

PGC-1 = Peroxisome proliferator activated receptor gamma coactivator-1

THG = [l-N alpha-trinitrophenylhistidine, 12-homoarginine]-glucagon

cAMP = Cyclic adenosine monophosphate

ASO = Anti-sense oligonucleotides

IP = Intervening peptide

MPGF = Major proglucagon fragment

PKA = Protein kinase A

Epac = cAMP/guanine-nucleotide exchange factor

PI-3K = Phosphatidylinositol 3-kinase

ATP = Adenosine triphoshate

PKB = Protein kinase B

PARP = Poly-ADP-ribose polymerase

IAP-2 = Inhibitor of apoptosis protein-2

HbA_{1c} = hemoglobin A_{1c}

ICV = Intracerebroventricular

NGF = Nerve growth factor

APP = Amyloid precursor protein

CNS = Central nervous system

LV = Left ventricular

AMI = Acute myocardial infarction

DPP-IV = Dipeptidyl pepsidase-IV

$t_{1/2}$ = Half-life

MPA = Maleimidoproprionic acid

TPN = Total parental nutrition

DMH = 1, 2-dimethylhydrazine

PKC = Protein kinase C

GRPP = Glicentin-related pancreatic polypeptide

PC = Prohormone convertases

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