The glucagon-like peptides: a double-edged therapeutic sword?

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Glucagon-like peptide-1(7–36)-amide (GLP-1) is an endogenous peptide that is secreted from the gut in response to the presence of food. Recent studies have established that GLP-1 and its longer-acting analog exendin-4 have multiple synergistic effects on glucose-dependent, insulin secretion pathways of the pancreatic β-cell and on plasticity in neuronal cells. Recent interest has focused on the development of these peptides as a novel therapeutic strategy for non-insulin-dependent (type 2) diabetes mellitus and associated neuropathy. This is with a view to developing lead compounds, based on neurotrophic action, for central and peripheral degenerative disorders such as stroke and Alzheimer's disease in addition to the peripheral neuropathy associated with type 2 diabetes mellitus. Here, we address recent advances in the biological action of GLP-1 and its related analogs.

Non-insulin-dependent (type 2) diabetes mellitus is a progressive disease that is prevalent in the elderly. Unlike type 1 diabetes, people with type 2 diabetes might make healthy, even high, levels of insulin, but there is a decrease in insulin action at insulin-sensitive tissues. Thus, the control of glucose levels in the blood is impaired. This resistance to insulin is often caused by obesity (reviewed by [1]). Approximately 90–95% of people with diabetes have type 2 diabetes [2]. There is also evidence to indicate that type 2 diabetes, or at least impaired glucose tolerance, is associated with impaired cognition, independent of age. Therefore, the normal, age-related decline in cognitive function might be exacerbated by the development of impaired glucose tolerance and insulin resistance. The age-related decline in pancreatic β-cell function results in ~19% of those over 65 in the US being diagnosed with type 2 diabetes. Although type 2 diabetes is associated typically with older people, it has become much more prevalent among children and young adults, in line with the alarming rise of obesity in the general population. Present treatments are less than satisfactory.

Diabetes has become the major cause of peripheral neuropathy, afflicting some 20–30% of type 2 diabetics, for which there is currently no treatment other than strict control of blood glucose levels. A major focus of endocrinology research over the past 5 years has been the development of a new therapeutic strategy for type 2 diabetes and neuropathy, based on the insulinotropic actions of endogenous peptides. Specifically, glucagon-like peptide-1(7–36)-amide (GLP-1) and glucose-dependent insulinotropic peptide (GIP) (reviewed in [3]) are released from entero-endocrine cells of the gastrointestinal mucosa following the ingestion of nutrients. They regulate nutrient metabolism via effects on insulin release from pancreatic islets (insulinotropic release), gastric motility and acid secretion, islet cell proliferation and nutrient disposal [4,5]. Infusions (either intravenous or subcutaneous) of GLP-1, at pharmacological concentrations, lower blood glucose in type 2 diabetic and non-diabetic subjects [6,5].

An important regulator of the biological activity of GLP-1 and GIP is N-terminal degradation by the common, endogenous, aminopeptidase enzyme, dipeptidyl peptidase IV (DPP-IV). This enzyme cleaves GLP-1 at the alanine residue at position 2 [7], which not only inactivates GLP-1, but might turn it into an antagonist at the GLP-1 receptor (Box 1) [8]. Several studies confirm that DPP-IV-mediated inactivation of these peptides is a crucial control mechanism that regulates the biological activity of both GIP and GLP-1 in rodents [9] and humans [7,10]. Indeed, it is this rapid inactivation that poses important challenges for therapeutic efforts directed at enhancing GIP and GLP-1 activity *in vivo*. Thus, a continuous infusion of peptide is required to maintain steady-state levels of active GLP-1 in plasma. The finding that the glucose-lowering effects of GLP-1 are preserved in type 2 diabetic individuals, irrespective of patient age and the duration of diabetes [11], and that it can preserve and augment β-cell mass [12,13] has provided the impetus for developing GLP-1-based pharmaceuticals with more suitable pharmacokinetics than those of native GLP-1.

Insulinotropic action

GLP-1 exerts its insulinotropic activity via interaction with a specific receptor, the GLP-1 receptor, on the cell membrane of pancreatic β-cells. The GLP-1 receptor has been cloned [14] and is a G-protein-coupled receptor of the subfamily that includes receptors for the related peptides, secretin, vasoactive intestinal peptide, pituitary adenylyl cyclase activating peptide and GIP. The GLP-1 receptor is coupled positively to the adenylyl cyclase system [15]. Ligand activation of the GLP-1 receptor stimulates adenylyl cyclase (Fig. 1), leading to an increase in intracellular cAMP in pancreatic β-cells [16], rat

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http://tips.trends.com 0165-6147/03/$ - see front matter. Published by Elsevier Science Ltd. doi:10.1016/S0165-6147(03)00160-3

Box 1. Amino acid sequences and binding profiles of GLP-1, exendin-4 and selected analogs

The amino acid sequences of glucagon-like peptide-1 (7–36)-amide (GLP-1), exendin-4 and selected analogs are shown in Fig. I, with IC_{50} values that are derived from competitive binding studies in Chinese hamster ovary (CHO) cells transfected with the human GLP-1 receptor. Binding of [^{125}I]GLP-1 to intact CHO/GLP-1 receptor cells was competed with various concentrations of the polypeptides shown. The IC_{50} values represent the concentration required to displace 50% of the bound [^{125}I]GLP-1 (United States Provisional Patent Application No. 60/309 0706: Long-acting insulinotropic peptides and uses thereof) [53,54,65].

The active, circulating form of GLP-1 in humans is GLP-1 (7–36)-amide [65]. This is a short-lived peptide (half-life 1–2 min) partly because of N-terminal cleavage of residues His7 and Ala8 (shown in red) by the protease dipeptidyl peptidase IV (DPP-IV). Exendin-4, a naturally occurring GLP-1 analog, is a log factor more potent than GLP-1 as an insulinotropic agent because of more avid binding at the GLP-1 receptor [53]. This 39-amino-acid peptide, which has 53% amino acid homology with GLP-1, is not a substrate for DPP-IV because of a glycine at position 2. Potent, long-acting, insulinotropic GLP-1 analogs are derived following amino acid substitutions at the N-terminal end of the peptide, which render them resistant to DPP-IV. Thus, the N terminus largely determines the duration of action, whereas the C terminus is crucial for receptor binding affinity [53,54,65].

The nine amino acids from the C terminus of exendin-4 were added sequentially to the C terminus of native GLP-1 and DPP-IV-protected GLP-1 analogs (GLP-1 ET and GG–GG_3, respectively). Binding of GLP-1 ET to the GLP-1 receptor was improved moderately relative to GLP-1, as illustrated by the twofold reduction in the IC_{50} value. Conversely, binding of GG, a DPP-IV-protected GLP-1 analog, to the GLP-1 receptor increased the IC_{50} value fivefold, thus yielding a longer acting analog with poorer binding affinity for the receptor. However, addition of the C-terminal sequence from exendin-4 to GG (GG_3) improved the IC_{50} value to nearly that of GLP-1. Addition of truncated C-terminal sequences to GG, exendin 31–33 (GG_1) and exendin 31–36 (GG_2), resulted in an increase in binding affinity relative to GG, although not to that of GG_3. Thus, the extreme C terminus of exendin-4 is likely to convey important receptor binding characteristics.

Exendin-4 has the highest affinity of all the compounds for the GLP-1 receptor. Sequential deletion of the C terminus [exendin (1–36) through exendin (1–26)] results in a progressive increase in the IC_{50} value, with exendin (1–36) and exendin (1–35) having similar binding profiles to exendin-4. The shorter peptides exendin (1–33), exendin (1–30) and exendin (1–28) behaved more like GLP-1, with exendin (1–28) representing the minimal sequence required for binding. The GLP-1 receptor antagonist exendin (9–39) is structurally identical to exendin-4 except it lacks the two residues at the extreme N terminus.

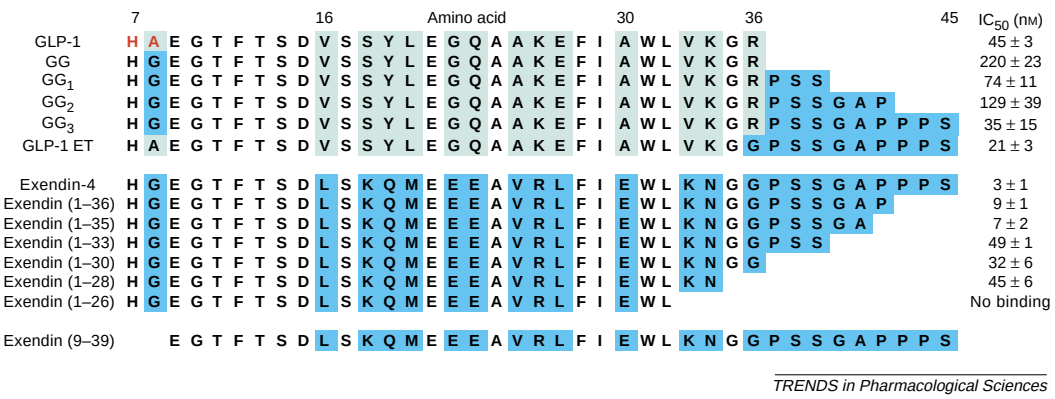

Fig. I. The amino acid sequences of glucagon-like peptide-1 (7–36)-amide (GLP-1), exendin-4 and selected analogs. Blue represents amino acid substitutions in the exendin-4 sequence relative to the GLP-1 sequence (green).

hypothalamic membrane preparations [17], PC12 cells [18] and primary hippocampal neurons in culture [19]. Subsequent activation of protein kinase A (PKA) leads to a plethora of biochemical events, including altered ion channel activity and intracellular Ca^{2+} handling, and enhanced exocytosis of insulin-containing granules [20]. PKA-independent signaling pathways have also been proposed in β-cells and neurons (Fig. 1).

The insulinotropic effects of GLP-1 are strictly glucose dependent; it has no effect on insulin secretion at glucose concentrations below ~4.5 mM [21]. In addition, GLP-1 strongly potentiates the insulinotropic actions of glucose itself. It enhances all steps of insulin biosynthesis and transcription of the insulin gene [22], which provides a continuous, augmented supply of insulin for secretion. Indeed, the expression of genes that are essential for β-cell function, such as glucokinase and Glut 2 [23], are upregulated following GLP-1 treatment. GLP-1 also has trophic effects on β-cells. It both stimulates β-cell proliferation [24] and enhances the differentiation of new β-cells from progenitor cells in the epithelium of the pancreatic duct [25]. Subsequently, Perfetti and colleagues [13] demonstrated GLP-1-mediated endocrine proliferation in aging, glucose-intolerant rats, with a resulting improvement in glucose tolerance. This indicates that GLP-1 might be capable of stimulating the growth of new β-cells in individuals who have an insufficient number of functioning cells, such as occurs in type 2 diabetic patients.

Chemical names

LY294002: 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one

PD98059: 2′-amino-3′-methoxyflavone

Inhibition of glucagon secretion

In addition to its effects on β-cells, GLP-1 inhibits glucagon secretion [26] in a glucose-dependent manner [27]. Glucagon opposes the effects of insulin on maintaining levels of blood glucose, which contributes significantly to the development of hyperglycemia in diabetic subjects.

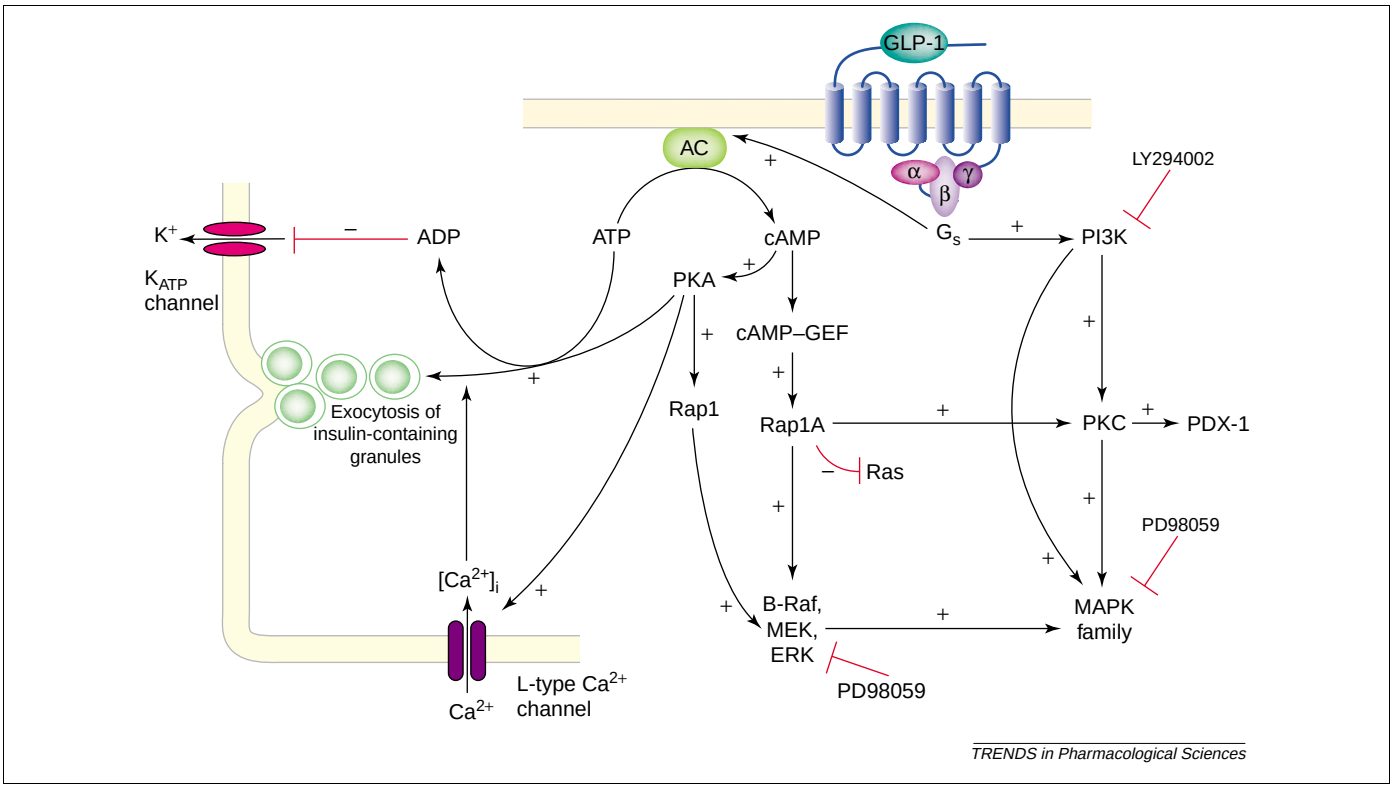

Fig. 1. Proposed signaling mechanism for glucagon-like peptide-1 (7–36)-amide (GLP-1) receptor agonists. GLP-1 action is mediated by binding to a specific, seven-trans-membrane G-protein-coupled receptor (GLP-1 receptor) [14] that is coupled positively to the adenylyl cyclase (AC) system [15]. Ligand activation of the Gα subunit of the GLP-1 receptor stimulates AC, which leads to an increase in intracellular cAMP and activation of protein kinase A (PKA). GLP-1 acts directly through the cAMP–PKA pathway to enhance and sensitize β-cells to glucose-stimulated insulin secretion. Glucose metabolism in the β-cell causes an increase in the concentration of ATP and raises the cytoplasmic ATP:ADP ratio, which leads to depolarization of the plasma membrane following closure of ATP-sensitive K^+ channels. This permits opening of voltage-dependent L-type Ca^{2+} channels and increases cytosolic Ca^{2+}, which triggers fusion of insulin-containing secretory vesicles to the plasma membrane. Exocytosis of insulin follows rapidly. Activation of GLP-1 receptors by GLP-1 leads to an increase in $[Ca^{2+}]_i$ as a result of activation of the L-channel following phosphorylation of PKA and/or mobilization of intercellular Ca^{2+} stores, an effect that might (or might not) be PKA dependent (reviewed by [58]). Much is known about the signaling pathways that occur following the binding of GLP-1 to the GLP-1 receptor in pancreatic β-cells but, as yet, little has been confirmed in other cell types [18,36]. The Gβγ dimer activates phosphatidylinositol 3-kinase (PI3K), which subsequently activates mitogen-activated protein kinases (MAPKs) (by a PKC-dependent or independent mechanism). This pathway is associated strongly with the GLP-1-induced proliferative signal in β-cells and the trophic effect in neuronal cells in culture; inhibition of PI3K (with LY294002) or MAPK (with PD98059) results in limited GLP-1-stimulated neurite outgrowth [18]. GLP-1-mediated activation of PI3K and downstream effectors [such as the transcription factor pancreatic and duodenal homeobox gene-1 (PDX-1)] are thought to regulate expression of the gene encoding insulin, β-cell growth, and differentiation of the β-cell phenotype in islet, ductal and exocrine cells [23,25]. cAMP activates a GTPase of the Ras superfamily, Rap1, following PKA-dependent phosphorylation. Certainly, cAMP activates multiple intracellular signaling cascades independently of its activation of PKA. An alternative, PKA-independent pathway was recently proposed in β-cells [59], which involves two types of cAMP–GEFs. GEFs are activated by binding to cAMP and activation of Rap1A [60]. Rap1A inhibits Ras but activates PKC and B-Raf, the latter two events both leading to activation of MAPKs. Abbreviations: ADP, adenosine diphosphate; cAMP–GEFs, cAMP–guanine-nucleotide-exchange factors; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; ERK, extracellular signal-regulated kinase; MEK, mitogen-activated protein kinase kinase (see Chemical names).

Therefore, injectable glucagon can be used as a treatment for diabetics at risk of hypoglycemia. However, repeated hypoglycemia often results in the development of a defective counter-regulatory glucagon response. In the hyperglycemic condition, GLP-1 infusion suppresses glucagon secretion. Conversely, glucagon secretion is stimulated in low glucose conditions. Thus, the glucose-lowering effect of GLP-1 might be a consequence of the inhibition of glucagon secretion, indicating that treatment with GLP-1 is unlikely to interfere with the glucagon-mediated defense against hypoglycemia.

Gastrointestinal effects

GLP-1 inhibits gastrointestinal secretion and motility, most notably gastric emptying [26]. The speed of this response (within a couple of minutes) is indicative of either a neural or endocrine signaling mechanism from the upper gastrointestinal area. Recent evidence implicates GLP-1 as one of the main hormones of the 'ileal brake', the primary, inhibitory feedback mechanism that controls the transit of a meal through the upper gastrointestinal tract to optimize nutrient digestion and absorption.

During physiological malabsorption, GLP-1 secretion is stimulated and gastric and pancreatic secretion is inhibited [28]. Infusion of GLP-1 during the ingestion of a meal dose-dependently diminishes the insulin responses, rather than enhances them [29]. This is likely to be related to reduced gastric emptying and subsequent decrease in absorption of insulinotropic nutrients. The GLP-1-mediated potentiation of nutrient-stimulated insulin secretion might be achieved, in part, through the control of chyme levels in the digestive tract by retarding propulsion and digestion of gastric contents [28,29].

CNS effects of GLP-1

A role for GLP-1 in the CNS has been established by considerable evidence that the GLP-1 receptor is expressed in the brains of rodents [30,31] and humans [32]. Its early identification on hypothalamic nuclei [31,33] supported a role of GLP-1 in the central regulation of food

intake, although GLP-1 receptor expression has now been demonstrated in the thalamus, brainstem, lateral septum, subfornical organ and the area postrema. In addition, specific GLP-1 binding sites for GLP-1 are also evident in neurons in the caudate–putamen, cerebral cortex, hippocampus and cerebellum, albeit at lower densities [34,35,30]. The stimulus for activation of neuronal GLP-1 receptors in the CNS is unclear (reviewed by [36]), and it remains to be established if GLP-1 is produced by neural cells. GLP-1 in the bloodstream can enter the brain [37], but what is its function?

Intestinally derived peptides, such as GLP-1, are classified as both hormones and growth factors – peptides that can regulate diverse cellular processes, including mitosis, growth and differentiation. The recent demonstration that GLP-1 can induce the differentiation of neuronal cells in culture [18] in a way that is similar to nerve growth factor, reflects the GLP-1-mediated neogenesis that occurs in pancreatic β-cells [24]. Much is known about the cellular signaling pathways triggered by GLP-1 binding to its receptor in pancreatic β-cells but, as yet, little has been confirmed in neuronal cells (Fig. 1) [36].

Effects on appetite and food intake

Recent studies indicate that GLP-1 dose-dependently inhibits feeding behavior in rodents [38,39,33], which can be reversed with the GLP-1 receptor antagonist, exendin (9–39) [40]. Such satiety-related effects also appear to occur in humans, because peripheral administration of GLP-1 significantly enhances satiety and reduces appetite in both healthy [41] and diabetic subjects [42], although such effects are transient [5]. Whether GLP-1 receptor-mediated signaling pathways are essential for the physiological control of appetite and body weight remains unclear. Certainly, inhibition of gastric emptying might account for a component of the satiety experienced after GLP-1 administration. Likewise, nutrients in the ileum are thought to have a satiating effect and curtail food intake [43]. Because hypothalamic nuclei that control feeding behavior express GLP-1 receptors [31,33] it has been suggested that peripheral GLP-1 might exert indirect effects on satiety centers in the CNS through neuronal relay mechanisms. However, GLP-1 receptor knockout mice appear to be resistant to the development of obesity [38,44]. This strongly suggests that GLP-1 receptor signaling is either nonessential for the long-term control of body weight (at least in this particular knockout mouse), or that other processes compensate.

Neuroprotective effects of GLP-1

The importance of cAMP in GLP-1 signaling has been demonstrated robustly. Signals that stimulate cAMP production can protect neurons against death in various paradigms. Activation of the GLP-1 receptor modulates cell survival mechanisms in diverse cell types. GLP-1 treatment reduces the number of apoptotic cells in the pancreas of Zucker diabetic fatty (ZDF) rats [45] and db/db mice [46] (rodent models of diabetes that undergo spontaneous, apoptotic destruction of pancreatic β-cells), with an associated increase in islet size and β-cell mass. In addition, GLP-1 can protect against hydrogen-peroxide-induced apoptotic cell death in an insulin-secreting cell line [47], which provides further evidence that GLP-1 receptor agonists might have greater therapeutic potential than just their insulinotropic properties. Furthermore, in rat hippocampal neurons in culture, which express functional GLP-1 receptors, GLP-1 and exendin-4 completely protect against glutamate-induced cell death [19] in a manner that is similar to other neurotrophic factors [48]. This provided the first indication of a neuroprotective role for GLP-1 receptor agonists and demonstrates that the anti-apoptotic action mediated by GLP-1 might not be restricted to insulin-secreting cells.

Although much is known about the signaling pathways involved in apoptotic cell death, the precise mechanism how GLP-1 challenges pro-apoptotic stimuli in diverse cell types is unclear. Phosphatidylinositol 3-kinase (PI3K), mitogen-activated protein kinase (MAPK) and cAMP are important signaling molecules involved in GLP-1-mediated cell proliferation and differentiation (Fig. 1). Recent literature indicates a role for PI3K-dependent, MAPK-independent signaling in the anti-apoptotic activity of GLP-1 (Fig. 2) [47].

The amyloid-β peptide (Aβ), particularly Aβ1–42, cellular oxidative stress and membrane-lipid peroxidation are believed to play important roles in the dysfunction and death of neurons in Alzheimer's disease (AD). Hippocampal neurons in culture have been exposed to toxic levels of Aβ and iron in the presence and absence of GLP-1 and exendin-4 [49]. Both peptides dose-dependently protect neurons against the insults, which indicates possible anti-apoptotic and antioxidant actions. Furthermore, GLP-1 and exendin-4 reduce the depletion of choline acetyltransferase immunoreactivity, a marker of acetylcholine-containing neurons in the basal forebrain, in a well-established model of neurodegeneration in rats [19]. Collectively, these data indicate that GLP-1 agonists are likely to play a role in protecting neurons against several types of brain injury, including excitotoxic and oxidative damage.

The mechanisms that underlie the dysfunction and death of acetylcholine-containing neurons in degenerative diseases such as AD are not understood fully. However, the altered processing of β-amyloid precursor protein in the disease process and subsequent increase in the levels of cytotoxic Aβ is likely to contribute to impaired acetylcholine-mediated signaling and neuronal degeneration [50]. Recent evidence indicates that GLP-1 dose-dependently reduces endogenous Aβ concentrations in the brains of normal, control mice [49]. Acetylcholine receptor agonists and anticholinesterases are effective clinically in alleviating the symptoms of AD. They also decrease Aβ production [51], but it is not known whether the Aβ-lowering action of these agents contributes to their clinical efficacy. Nevertheless, the therapeutic potential of agents such as nerve growth factor as a treatment for AD will be undermined by the decline in acetylcholine-containing neurons and concomitant reduction in the expression trkA receptors in the disease process (trkA receptors often colocalize with cells that are vulnerable to the AD process and lost relatively early during the disease). Alternative treatments that utilize pathways unaffected by the

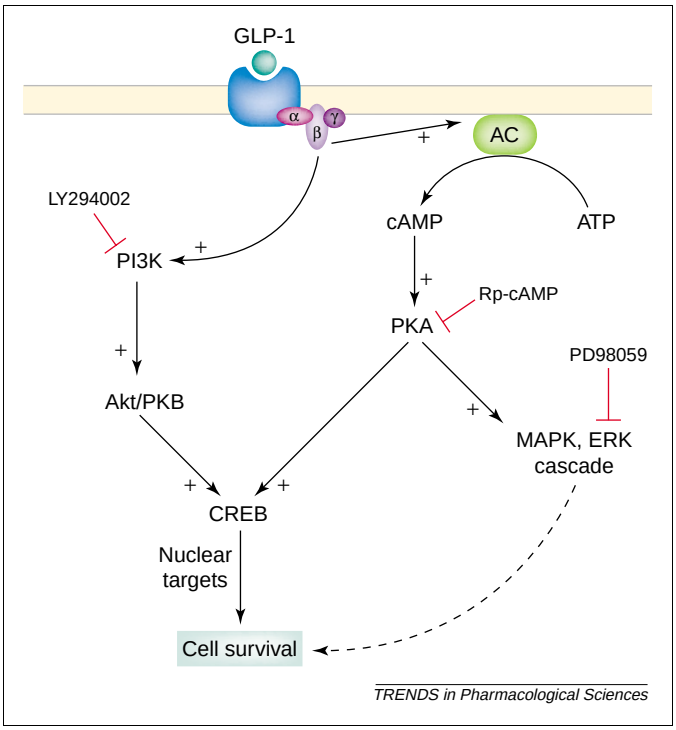

Fig. 2. Survival-promoting effects of glucagon-like peptide-1 (7–36)-amide (GLP-1). A proposed schematic of the signaling events involved in the survival-promoting effects elicited by GLP-1 receptor agonists is shown. Many growth factors, which are known characteristically for their pro-proliferative properties and/or their effects on cell differentiation, interfere with the sequence of events that leads to apoptosis. GLP-1 receptor activation reduces the number of apoptotic cells in the pancreas of Zucker diabetic rats [45] and protects against apoptotic insult in hippocampal neurons [44] and mouse insulinoma cells [47] in culture. GLP-1-mediated protection from apoptosis can be abolished by Rp-cAMP [a cAMP-dependent inhibitor of protein kinase A (PKA)], which indicates that cAMP is a positive mediator in the prevention of apoptosis of insulin-secreting cell lines [47]. Phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) are two important signaling molecules that mediate cell proliferation, differentiation and apoptosis. The PI3K inhibitor LY294002 inhibits the anti-apoptotic activity of GLP-1, which indicates that this effect of GLP-1 is, at least in part, regulated by a PI3K-dependent signaling mechanism. GLP-1 activates MAPK/ERK [61,18], although MAPK/ERK signaling appears not to be involved in the protection of insulinoma cells from apoptosis (because the MAPK inhibitor PD98059 does not inhibit the protective properties of GLP-1). This corroborates the anti-apoptotic action of the related, structurally similar peptide hormone GLP-2, which also protects cells from apoptosis via a MAPK-independent pathway [62]. There is insufficient evidence to rule out an involvement for all MAPK pathways in the anti-apoptotic action of GLP-1, and studies to address this question in diverse cell types are required. Activation of the GLP-1 receptor inhibits hydrogen peroxide-induced apoptosis in rat insulinoma cells, probably through the cAMP response element-binding protein (CREB)-stimulated expression of cellular genes following both PI3K-mediated phosphorylation at Ser133 and activation of the growth factor-dependent Ser/Thr kinase Akt/PKB (Akt/PKB-induced CREB activity can be suppressed by the PI3K inhibitor LY294002) [47]. Phosphorylation of CREB at Ser133 is implicated in the resistance of cells to various insults, and several well-established neuroprotective agents are believed to exert their action via pathways that converge on CREB [63]. The PI3K and cAMP pathways might be compensatory because inhibition of both pathways simultaneously produces a greater pro-apoptotic effect than inhibition of either pathway alone [47]. The limited data that exist indicates that GLP-1-mediated promotion of cell survival might be achieved, at least in part, by stimulating gene expression via the CREB nuclear-transduction pathway. Whether phosphorylation of Ser133 is a key event in the anti-apoptotic action of GLP-1 receptor agonists remains to be elucidated. Certainly, other transcription factors are essential in anti-apoptotic mechanisms (reviewed in [64]), but their involvement in GLP-1-mediated cell survival is not yet established. Abbreviations: AC, adenylyl cyclase; ERK, extracellular signal-regulated kinase.

degenerative condition are preferable. Currently, there is no evidence to demonstrate reduced expression of the GLP-1 receptor in the brains of patients with AD. In addition, GLP-1 fulfills several strategic criteria appropriate to the development of therapeutic targets for the prevention and treatment of AD; namely, either reduced production of Aβ or enhanced clearance from the brain, and suppression of neurodegenerative cascades. That GLP-1 receptor activation reduces cell death in several cell types, including neurons [18,19,49], transfected fibroblasts [52] and islet β-cells [45,46], indicates that direct coupling to anti-apoptotic and trophic signaling pathways might represent a generalized feature of GLP-1 receptor activation. In this regard, GLP-1 might represent an alternative, potentially valuable, novel approach for the treatment of neurodegeneration [36].

Optimizing GLP-1 as a therapeutic agent

In this review we have highlighted considerable evidence that indicates that activation of the GLP-1 receptor is an important determinant for cell survival, particularly following organ and tissue damage. This makes GLP-1 an attractive therapeutic agent. However, such potential is limited by the susceptibility of GLP-1 to proteolytic degradation. Basic and clinical investigations have focused on strategies to circumvent DPP-IV-mediated inactivation of GLP-1. These include modifying GLP-1 to make it resistant to DPP-IV (Box 1) and inhibiting DPP-IV. Several reports document the enhanced biological activity of DPP-IV-resistant molecules in both normal and diabetic rodents. Exendin-4, a naturally occurring GLP-1 receptor agonist that occurs in the saliva of the Gila monster lizard, is resistant to DPP-IV-mediated degradation (Box 1). This contributes to the enhanced stability of exendin-4 *in vivo* and its ability to maintain insulinotropic, GLP-1-like actions, as well as positive effects on β-cell mass and nutrient disposal. In diabetic mice, once-daily intraperitoneal administration for 13 weeks normalized blood glucose and reduced hemoglobin A_{1c} (glycosylated hemoglobin) to near normal values [53]. Subsequently, it was demonstrated that treatment with exendin-4 for 8 weeks substantially improved glycemia, hyperinsulinemia and body weight in ZDF rats [54]. Based on this and other experimental data, exendin-4 is now in Phase III clinical studies for type 2 diabetes (Amylin Pharmaceuticals, San Diego, USA).

The addition of an acyl chain to native GLP-1 creates a peptide (NN2211) that binds to albumin. This limits sensitivity to DPP-IV and delays absorption from the injection site. This GLP-1 analog appears to delay and reduce the development of diabetes in ZDF rats [55,56], and it is currently in clinical development (NovoNordisk, Denmark). In humans, NN2211 has an excellent pharmacokinetic profile, with a half-life of 12 h [57]. Therefore, once-daily administration of adequate concentrations of NN2211 might lower plasma glucose for a full 24 h.

Concluding remarks

That GLP-1 can stimulate the formation of new β-cells in rodents (partly by enhancing β-cell proliferation and partly by enhancing the differentiation of duct progenitor cells into mature β cells) has fueled interest in a neurological role of GLP-1. Based on the actions of GLP-1 on islet cell differentiation, a neurotrophic role for GLP-1 has been demonstrated in the nervous system. As a result, developing GLP-1 analogs to optimize the

neurotrophic actions is an emerging area of therapeutic drug development. Although numerous mechanistic questions remain, GLP-1 receptor agonists might represent a potentially valuable, novel, therapeutic intervention, and provide an alternative to the existing treatment of diabetes, central and peripheral degenerative disorders, such as stroke, AD, and the peripheral neuropathy associated with type 2 diabetes mellitus.

Acknowledgements

This work was supported by the Intramural Research Program National Institute on Aging.

References

1 Cavaghan, M.K. *et al.* (2000) Interactions between insulin resistance and insulin secretion in the development of glucose intolerance. *J. Clin. Invest.* 106, 329–333
2 United Kingdom Prospective Diabetes study, (1995) *Diabetes* 44, 1249–1258
3 Kieffer, T.J. (2003) GIP or not GIP? That is the question. *Trends Pharmacol. Sci.* 24, 110–112
4 Doyle, M.E. and Egan, J.M. (2001) Glucagon-like peptide-1. *Recent Prog. Horm. Res.* 56, 377–399
5 Drucker, D.J. (2001) Minireview: the glucagon-like peptides. *Endocrinology* 142, 521–527
6 Todd, J.F. *et al.* (1997) Glucagon-like peptide-1 (GLP-1): a trial of treatment in non-insulin-dependent diabetes mellitus. *Eur. J. Clin. Invest.* 27, 533–536
7 Deacon, C.F. *et al.* (1995) Degradation of glucagon-like peptide-1 by human plasma *in vitro* yields an N-terminally truncated peptide that is a major endogenous metabolite *in vivo*. *J. Clin. Endocrinol. Metab.* 80, 952–957
8 Knudsen, L.B. and Pridal, L. (1996) Glucagon-like peptide-1-(9-36) amide is a major metabolite of glucagon-like peptide-1-(7-36) amide after in vivo administration to dogs, and it acts as an antagonist on the pancreatic receptor. *Eur. J. Pharmacol.* 318, 429–435
9 Kieffer, T.J. *et al.* (1995) Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 *in vitro* and *in vivo* by dipeptidyl peptidase IV. *Endocrinology* 136, 3585–3596
10 Deacon, C.F. *et al.* (1995) Both subcutaneously and intravenously administered glucagon-like peptide I are rapidly degraded from the NH2-terminus in type II diabetic patients and in healthy subjects. *Diabetes* 44, 1126–1131
11 Egan, J.M. *et al.* (2002) Glucagon-like peptide-1 augments insulin-mediated glucose uptake in the obese state. *J. Clin. Endocrinol. Metab.* 87, 3768–3773
12 Wang, Y. *et al.* (1997) Glucagon-like peptide-1 can reverse the age-related decline in glucose tolerance in rats. *J. Clin. Invest.* 99, 2883–2889
13 Perfetti, R. *et al.* (2000) Glucagon-like peptide-1 induces cell proliferation and pancreatic-duodenum homeobox-1 expression and increases endocrine cell mass in the pancreas of old, glucose-intolerant rats. *Endocrinology* 141, 4600–4605
14 Thorens, B. (1992) Expression cloning of the pancreatic beta cell receptor for the gluco-incretin hormone glucagon-like peptide 1. *Proc. Natl. Acad. Sci. U. S. A.* 89, 8641–8645
15 Drucker, D.J. *et al.* (1987) Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. *Proc. Natl. Acad. Sci. U. S. A.* 84, 3434–3438
16 Holz, G.G. *et al.* (1999) cAMP-dependent mobilization of intracellular Ca2 + stores by activation of ryanodine receptors in pancreatic beta-cells. A Ca2 + signaling system stimulated by the insulinotropic hormone glucagon-like peptide-1-(7-37). *J. Biol. Chem.* 274, 14147–14156
17 Kanse, S.M. *et al.* (1988) Identification and characterization of glucagon-like peptide-1 7-36 amide-binding sites in the rat brain and lung. *FEBS Lett.* 241, 209–212
18 Perry, T. *et al.* (2002) A novel neurotrophic property of glucagon-like peptide 1: a promoter of nerve growth factor-mediated differentiation in PC12 cells. *J. Pharmacol. Exp. Ther.* 300, 958–966
19 Perry, T. *et al.* (2002) Protection and reversal of excitotoxic damage by glucagon-like peptide-1 and exendin-4. *J. Pharmacol. Exp. Ther.* 302, 881–888
20 Gromada, J. *et al.* (1998) Cellular regulation of islet hormone secretion by the incretin hormone glucagon-like peptide 1. *Pflugers Arch.* 435, 583–594
21 Weir, G.C. *et al.* (1989) Glucagon-like peptide I (7-37) actions on endocrine pancreas. *Diabetes* 38, 338–342
22 Fehmann, H.C. and Habener, J.F. (1992) Insulinotropic hormone glucagon-like peptide-I(7-37) stimulation of proinsulin gene expression and proinsulin biosynthesis in insulinoma beta TC-1 cells. *Endocrinology* 130, 159–166
23 Buteau, J. *et al.* (1999) Glucagon-like peptide-1 promotes DNA synthesis, activates phosphatidylinositol 3-kinase and increases transcription factor pancreatic and duodenal homeobox gene 1 (PDX-1) DNA binding activity in beta (INS-1)-cells. *Diabetologia* 42, 856–864
24 Stoffers, D.A. *et al.* (2000) Insulinotropic glucagon-like peptide 1 agonists stimulate expression of homeodomain protein IDX-1 and increase islet size in mouse pancreas. *Diabetes* 49, 741–748
25 Zhou, J. *et al.* (1999) Glucagon-like peptide 1 and exendin-4 convert pancreatic AR42J cells into glucagon- and insulin-producing cells. *Diabetes* 48, 2358–2366
26 Orskov, C. *et al.* (1988) Effect of truncated glucagon-like peptide-1 [proglucagon-(78-107) amide] on endocrine secretion from pig pancreas, antrum, and nonantral stomach. *Endocrinology* 123, 2009–2013
27 Nauck, M.A. *et al.* (2002) Effects of glucagon-like peptide 1 on counterregulatory hormone responses, cognitive functions, and insulin secretion during hyperinsulinemic, stepped hypoglycemic clamp experiments in healthy volunteers. *J. Clin. Endocrinol. Metab.* 87, 1239–1246
28 Layer, P. *et al.* (1995) Ileal release of glucagon-like peptide-1 (GLP-1). Association with inhibition of gastric acid secretion in humans. *Dig. Dis. Sci.* 40, 1074–1082
29 Nauck, M.A. *et al.* (1997) Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. *Am. J. Physiol.* 273, E981–E988
30 Goke, R. *et al.* (1995) Distribution of GLP-1 binding sites in the rat brain: evidence that exendin-4 is a ligand of brain GLP-1 binding sites. *Eur. J. Neurosci.* 7, 2294–2300
31 Shughrue, P.J. *et al.* (1996) Glucagon-like peptide-1 receptor (GLP1-R) mRNA in the rat hypothalamus. *Endocrinology* 137, 5159–5162
32 Satoh, F. *et al.* (2000) Characterization of human and rat glucagon-like peptide-1 receptors in the neurointermediate lobe: lack of coupling to either stimulation or inhibition of adenylyl cyclase. *Endocrinology* 141, 1301–1309
33 Turton, M.D. *et al.* (1996) A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* 379, 69–72
34 Calvo, J.C. *et al.* (1995) Structural characterization by affinity cross-linking of glucagon-like peptide-1(7-36)amide receptor in rat brain. *J. Neurochem.* 64, 299–306
35 Campos, R.V. *et al.* (1994) Divergent tissue-specific and developmental expression of receptors for glucagon and glucagon-like peptide-1 in the mouse. *Endocrinology* 134, 2156–2164
36 Perry, T. and Greig, N.H. (2002) Glucagon-like peptides: a new genre in therapeutic targets for intervention in Alzheimer's disease. *J. Alzheimers Dis.* 4, 487–496
37 Orskov, C. *et al.* (1996) Glucagon-like peptide I receptors in the subfornical organ and the area postrema are accessible to circulating glucagon-like peptide I. *Diabetes* 45, 832–835
38 Scrocchi, L.A. *et al.* (1996) Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene. *Nat. Med.* 2, 1254–1258
39 Tang-Christensen, M. *et al.* (1996) Central administration of GLP-1-(7-36) amide inhibits food and water intake in rats. *Am. J. Physiol.* 271, R848–R856
40 Meeran, K. *et al.* (1999) Repeated intracerebroventricular administration of glucagon-like peptide-1-(7-36) amide or exendin-(9-39) alters body weight in the rat. *Endocrinology* 140, 244–250
41 Flint, A. *et al.* (1998) Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. *J. Clin. Invest.* 101, 515–520
42 Toft-Nielsen, M.B. *et al.* (1999) Continuous subcutaneous infusion of glucagon-like peptide 1 lowers plasma glucose and reduces appetite in type 2 diabetic patients. *Diabetes Care* 22, 1137–1143

43 Read, N. *et al.* (1994) The role of the gut in regulating food intake in man. *Nutr. Rev.* 52, 1–10
44 Scrocchi, L.A. and Drucker, D.J. (1998) Effects of aging and a high fat diet on body weight and glucose tolerance in glucagon-like peptide-1 receptor −/− mice. *Endocrinology* 139, 3127–3132
45 Farilla, L. *et al.* (2002) Glucagon-like peptide-1 promotes islet cell growth and inhibits apoptosis in Zucker diabetic rats. *Endocrinology* 143, 4397–4408
46 Wang, Q. and Brubaker, P.L. (2002) Glucagon-like peptide-1 treatment delays the onset of diabetes in 8 week-old db/db mice. *Diabetologia* 45, 1263–1273
47 Hui, H. *et al.* (2003) Glucagon-like peptide-1 inhibits apoptosis of insulin-secreting cells via a cyclic 5′-adenosine monophosphate-dependent protein kinase A- and a phosphatidylinositol 3-kinase-dependent pathway. *Endocrinology* 144, 1444–1455
48 Cheng, B. *et al.* (1994) Neurotrophin-4/5 protects hippocampal and cortical neurons against energy deprivation- and excitatory amino acid-induced injury. *Brain Res.* 650, 331–335
49 Perry, T. *et al.* (2003) Glucagon-like peptide-1 decreases amyloid-β peptide (Aβ) production and protects neurons against death induced by Aβ and iron. *J. Neurosci. Res.* 72, 603–612
50 Sambamurti, K. *et al.* (2002) Advances in the cellular and molecular biology of the beta-amyloid protein in Alzheimer's disease. *Neuromol. Med.* 1, 1–31
51 Lahiri, D.K. *et al.* (2000) Cholinesterase inhibitors, beta-amyloid precursor protein and amyloid beta-peptides in Alzheimer's disease. *Acta Neurol. Scand. Suppl.* 176, 60–67
52 Li, Y. *et al.* (2003) Glucagon-like peptide-1 receptor signaling modulates beta cell apoptosis. *J. Biol. Chem.* 278, 471–478
53 Greig, N.H. *et al.* (1999) Once daily injection of exendin-4 to diabetic mice achieves long-term beneficial effects on blood glucose concentrations. *Diabetologia* 42, 45–50
54 Szayna, M. *et al.* (2000) Exendin-4 decelerates food intake, weight gain, and fat deposition in Zucker rats. *Endocrinology* 141, 1936–1941
55 Pick, A. *et al.* (1998) Role of apoptosis in failure of beta-cell mass compensation for insulin resistance and beta-cell defects in the male Zucker diabetic fatty rat. *Diabetes* 47, 358–364
56 Larsen, P.J. *et al.* (2001) Systemic administration of the long-acting GLP-1 derivative NN2211 induces lasting and reversible weight loss in both normal and obese rats. *Diabetes* 50, 2530–2539
57 Juhl, C.B. *et al.* (2002) Bedtime administration of NN2211, a long-acting GLP-1 derivative, substantially reduces fasting and postprandial glycemia in type 2 diabetes. *Diabetes* 51, 424–429
58 Doyle, M.E. and Egan, J.M. (2003) Pharmacological agents that directly modulate insulin secretion. *Pharmacol. Rev.* 55, 105–131
59 Leech, C.A. *et al.* (2000) Expression of cAMP-regulated guanine nucleotide exchange factors in pancreatic beta-cells. *Biochem. Biophys. Res. Commun.* 278, 44–47
60 Kawasaki, H. *et al.* (1998) A family of cAMP-binding proteins that directly activate Rap1. *Science* 282, 2275–2279
61 Montrose-Rafizadeh, C. *et al.* (1999) Pancreatic glucagon-like peptide-1 receptor couples positively to multiple G proteins and activates mitogen-activated protein kinase pathways in Chinese hamster ovary cells. *Endocrinology* 140, 1132–1134
62 Yusta, B. *et al.* (2000) The glucagon-like peptide-2 receptor mediates direct inhibition of cellular apoptosis via a cAMP-dependent protein kinase-independent pathway. *J. Biol. Chem.* 275, 35345–35352
63 Walton, M.R. and Dragunow, I. (2000) Is CREB a key to neuronal survival? *Trends Neurosci.* 23, 48–53
64 Mattson, M.P. *et al.* (2002) Neuroprotective and neurorestorative signal transduction mechanisms in brain aging: modification by genes, diet and behavior. *Neurobiol. Aging* 23, 695–705
65 Orskov, C. *et al.* (1994) Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide I in humans. *Diabetes* 43, 535–539

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