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REVIEW

Emerging cardiovascular actions of the incretin hormone glucagon-like peptide-1: potential therapeutic benefits beyond glycaemic control?

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Glucagon-like peptide-1 (GLP-1) is an incretin hormone secreted by the small intestine in response to nutrient ingestion. It has wide-ranging effects on glucose metabolism, including stimulation of insulin release, inhibition of glucagon secretion, reduction of gastric emptying and augmentation of satiety. Importantly, the insulinotropic actions of GLP-1 are uniquely dependent on ambient glucose concentrations, and it is this particular characteristic which has led to its recent emergence as a treatment for type 2 diabetes. Although the major physiological function of GLP-1 appears to be in relation to glycaemic control, there is growing evidence to suggest that it may also play an important role in the cardiovascular system. GLP-1 receptors (GLP-1Rs) are expressed in the heart and vasculature of both rodents and humans, and recent studies have demonstrated that GLP-1R agonists have wide-ranging cardiovascular actions, such as modulation of heart rate, blood pressure, vascular tone and myocardial contractility. Importantly, it appears that these agents may also have beneficial effects in the setting of cardiovascular disease (CVD). For example, GLP-1 has been found to exert cardioprotective actions in experimental models of dilated cardiomyopathy, hypertensive heart failure and myocardial infarction (MI). Preliminary clinical studies also indicate that GLP-1 infusion may improve cardiac contractile function in chronic heart failure patients with and without diabetes, and in MI patients after successful angioplasty. This review will discuss the current understanding of GLP-1 biology, examine its emerging cardiovascular actions in both health and disease and explore the potential use of GLP-1 as a novel treatment for CVD.
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Abbreviations: CVD, cardiovascular disease; DPP-4, dipeptidyl peptidase-4; ERK, extracellular signal-regulated kinase; GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide-1; GLP-1R, glucagon-like peptide-1 receptor; LV, left ventricular; MAPK, mitogen-activated protein kinase; MI, myocardial infarction; PACAP, pituitary adenylate cyclase-activating polypeptide; PI3K, phosphoinositide 3-kinase; VIP, vasoactive intestinal peptide

Introduction

The global prevalence of diabetes mellitus is increasing at an exponential rate, and it is estimated that the number of affected individuals will rise to 300 million within the next 20 years (King *et al.*, 1998). Patients with diabetes are characterized by an increased risk of developing both microvascular complications, such as retinopathy, nephropathy and neuropathy, and atherosclerotic macrovascular disease, which may lead to the development of peripheral vascular disease, stroke and ischaemic/hypertensive heart failure (Clements and Bell, 1985). A significant proportion of diabetic patients may also develop diabetic cardiomyopathy in the absence of such aetiological factors, which is associated with a high incidence of congestive heart failure (Fang *et al.*, 2004; Asbun and Villarreal, 2006). Cardiovascular disease (CVD) is the leading cause of mortality in the UK, accounting for premature death in up to 40% of the population, and patients with diabetes are characterized by a significantly elevated risk compared to normoglycaemic individuals (Garcia *et al.*, 1974; Scandinavian Simvastatin Survival Study Group, 1994). Indeed, the Framingham Heart Study found that heart failure was twice as common in diabetic men, and five times as common in

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diabetic women aged 45–74 compared to the normal population, and that this association was even stronger in younger patients (Kannel and McGee, 1979).

Therapeutic agents such as statins, angiotensin-converting enzyme inhibitors and β-blockers have been demonstrated to cause a significant reduction in the incidence of CVD, and to exert morbidity and mortality benefits (The SOLVD Investigators, 1992; Scandinavian Simvastatin Survival Study Group, 1994; Colucci *et al*., 2007). However, there remains a substantial incidence of CVD in optimally treated patients, especially those with underlying pathologies, such as diabetes. Thus, there is an ongoing search for more effective therapeutic alternatives. One such candidate may be glucagon-like peptide-1 (GLP-1), a peptide hormone which forms the basis of a recently approved therapy for controlling hyperglycaemia in type 2 diabetes (Kendall *et al*., 2005). Interestingly, recent evidence suggests that in addition to its established glucose-lowering actions, GLP-1 may also exert several beneficial actions on the cardiovascular system. This review will discuss the emerging cardiovascular actions of GLP-1 and its potential as a treatment for CVD in both diabetic and non-diabetic patients.

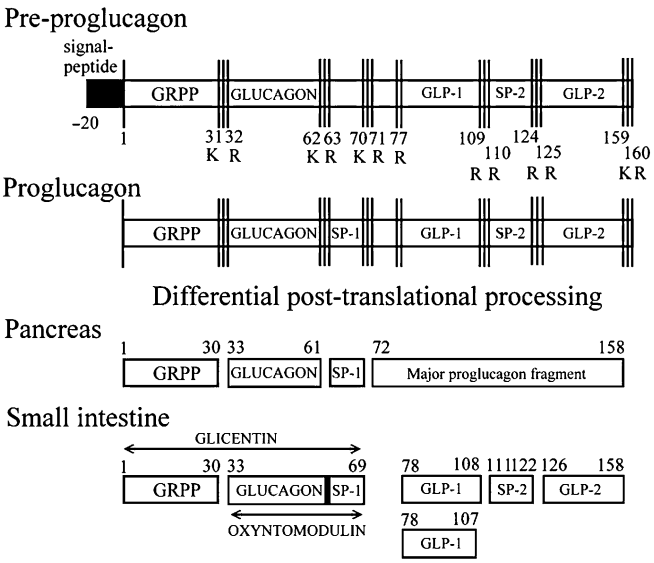

Figure 1 Structure and post-translational processing of the pre-proglucagon gene product. Pre-proglucagon is transcribed and processed to form glicentin, oxyntomodulin, GLP-1(7–36) amide, GLP-1(7–37) and GLP-2 in intestinal L-cells. GRPP, glicentin-related polypeptide; SP1, spacer peptide 1; SP2, spacer peptide 2.

The biology of GLP-1

Synthesis and secretion of GLP-1 in humans

The scientific study of GLP-1(7–36) amide originated almost 30 years ago with the cloning of a 'glucagon-related peptide' from the pre-proglucagon gene in anglerfish cDNA (Lund *et al*., 1981). Subsequent sequence analysis performed in numerous species indicated that there is complete conservation of the GLP-1 amino acid sequence in mammals (Bell *et al*., 1983; Heinrich *et al*., 1984). GLP-1(7–36) amide is a 30 amino acid polypeptide transcribed on the proglucagon gene, which is located on the long arm of chromosome 2 and is expressed in both pancreatic α-cells and intestinal L-cells (White and Saunders, 1986). However, the expression product is differentially processed between these cell types due to tissue-specific expression of pro-hormone convertase enzymes (Dhanvantari *et al*., 1996). The majority of active GLP-1 occurs in the form of two equipotent isotypes: a minor form, known as GLP-1(7–37) (transcribed from the proglucagon 78–108), which accounts for approximately 20% of active GLP-1, and a major form, GLP-1(7–36) amide (transcribed from proglucagon 78–107; herein referred to as GLP-1), which accounts for most of the other 80% of active GLP-1 (Orskov *et al*., 1994). Production of these isoforms results from post-translational processing of the proglucagon gene product that occurs in the L-cells (Figure 1). The greatest density of L-cells occurs in the distal ileum and colon, where they act as the major endogenous source of GLP-1 (Kervran *et al*., 1987; Eissele *et al*., 1992). L-cells are open-type epithelial endocrine cells which are juxtaposed with the intestinal lumen, where they appear to respond to local nutrient signals (Gribble, 2008). They are also in close contact with nervous and vascular tissues of the intestine, and a number of neurotransmitters and endocrine hormones are believed to play a role in the regulation of GLP-1 secretion.

The primary stimulus for GLP-1 secretion is enteral nutrient ingestion. Indeed, consumption of an equicaloric test meal of either carbohydrate, fat or protein has been shown to stimulate GLP-1 secretion in human subjects (Elliott *et al*., 1993). Furthermore, the same authors reported that an oral glucose load (75 g) leads to increased plasma GLP-1 concentrations (Elliott *et al*., 1993). Secretion of GLP-1 largely depends upon the specific nutrient composition of the meal, and it has been reported that a particular caloric threshold or nutrient delivery rate must be reached in order to trigger significant secretion (Schirra *et al*., 1996). For an extensive overview of nutrient, neural and endocrine factors involved in GLP-1 secretion, the reader is directed towards an excellent recent review paper (Dubé and Brubaker, 2004).

Clearance and metabolism of GLP-1

GLP-1 is thought to be eliminated from the circulation by up to three separate mechanisms: renal clearance, hepatic clearance and degradation in the circulation. Renal clearance of GLP-1 is well documented in studies which have measured GLP-1 levels in plasma or renal filtrates from uremic patients (Orskov *et al*., 1992), anaesthetized pigs (Deacon *et al*., 1996) and rats undergoing nephrectomy or uretal ligation (Ruiz-Grande *et al*., 1993). Significant hepatic extraction of GLP-1 has also been observed in pigs following exogenous systemic infusion (Deacon *et al*., 1996).

Following its release into the circulation, GLP-1 undergoes rapid enzymatic degradation by its primary endogenous inactivator, dipeptidyl peptidase-4 (DPP-4) (Deacon, 2004). This physiologically ubiquitous enzyme rapidly degrades GLP-1(7–36) amide to GLP-1(9–36) amide by the removal of an N-terminal dipeptide. The resultant metabolite has a 1000-fold lower affinity for the GLP-1 receptor (GLP-1R) and is characterized by a complete lack of insulinotropic activity (Knudsen

and Pridal, 1996; Deacon *et al.*, 2002; Green *et al.*, 2004c). Thus, the glucose-lowering activity of GLP-1 is relatively short lived, with a circulating half-life of ~2 min. This rapid inactivation of GLP-1 was clearly demonstrated by a study in human subjects which reported that 30 min after subcutaneous GLP-1 injection, inactive GLP-1(9–36) amide accounted for 78% of total immunoreactive GLP-1 (Deacon *et al.*, 1995). Interestingly, it appears that GLP-1 may undergo further enzymatic break-down subsequent to the action of DPP-4. For example, multiple degradation fragments have been observed following incubation of GLP-1 with both purified human neutral endopeptidase (neprilysin) (Hupe-Sodmann *et al.*, 1995) and a neprilysin activity-containing pancreatic β-cell line (Hupe-Sodmann *et al.*, 1997), suggesting that this enzyme may also be involved in the metabolism of GLP-1. However, a recent study indicated that up to 50% of native GLP-1 entering the circulation may be directly degraded by neprilysin (Plamboeck *et al.*, 2005), indicating that a significant proportion of the GLP-1 breakdown may occur independently of DPP-4, although this remains controversial.

The GLP-1R

First sequenced in rat pancreatic tissue, the GLP-1R is a G-protein coupled receptor that consists of 463 amino acids (Thorens, 1992). The GLP-1R is ubiquitously expressed and has been detected in tissues such as pancreatic islets (Orskov and Poulsen, 1991), heart (Wei and Mojsov, 1995), aorta (Green *et al.*, 2008), lung (Kanse *et al.*, 1988), gastric glands (Uttenthal and Blazquez, 1990) and parts of the central and peripheral nervous system (Shimizu *et al.*, 1987; Kanse *et al.*, 1988; Wei and Mojsov, 1995).

In 1993, Thorens and Waeber subsequently isolated and cloned the human GLP-1R from a pancreatic cDNA library. Further studies revealed that the human GLP-1R has 90% sequence homology to the rat GLP-1R, and that its gene is localized to chromosome 6p21 (Stoffel *et al.*, 1993). Interestingly, this membrane-spanning receptor has been found to share sequence similarities with several other receptors, including those for parathyroid hormone, calcitonin, growth hormone-releasing hormone, pituitary adenylate cyclase-activating polypeptide (PACAP), glucose-dependent insulinotropic polypeptide (GIP), secretin, vasoactive intestinal peptide (VIP) and glucagon (Kieffer and Habener, 1999). The GLP-1R, (illustrated in Figure 2) comprises eight hydrophobic domains, seven of which span the membrane, with a further extracellular N-terminal domain. GLP-1 and similar agonists have been demonstrated to bind to the receptor in an orthosteric manner. However, recent studies employing both a small molecule GLP-1R agonist and molecular modelling of the three-dimensional structure of the receptor, have also identified a non-allosteric activation site (Knudsen *et al.*, 2007; Lin and Wang, 2009).

It is well established that activation of the GLP-1R stimulates the production of cyclic AMP via the action of adenylate cyclase. Cyclic AMP signalling may then be further amplified and diversified via the activation of several downstream factors, such as protein kinase A and cyclic AMP-regulated guanine nucleotide exchange factors (Holz, 2004). Agonism of the GLP-1R is also known to be associated with phosphorylation of the cyclic AMP response binding element, elevation of intracellular calcium, inhibition of voltage-dependent potassium channels, and activation of a number of kinases, including extracellular signal-regulated kinase (ERK)1/2, protein kinase C, phosphoinositide 3-kinase (PI3K), mitogen-activated protein kinase (MAPK) and protein kinase B (Buteau *et al.*, 2001; Arnette *et al.*, 2003; Kang *et al.*, 2007).

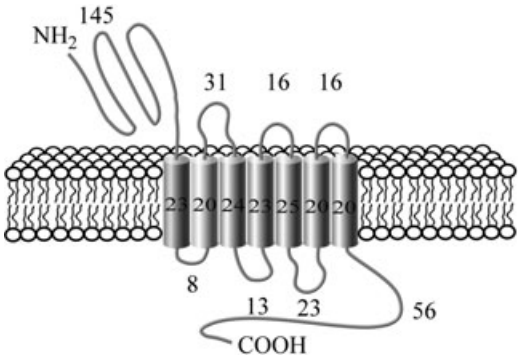

Figure 2 Illustration of the structure of the glucagon-like peptide-1 receptor (GLP-1R) including the number of amino acid residues in each segment. The GLP-1R is a seven-domain, membrane-spanning G-protein coupled receptor comprised of 463 amino acids, which is expressed in tissues such as heart, aorta, pancreas, kidney, lung and the central and peripheral nervous systems.

The GLP-1R has an extremely high affinity for GLP-1 which it selectively binds at nanomolar concentrations, while other peptides of the glucagon superfamily, such as glucagon, PACAP, GIP and VIP, bind either poorly or not at all. Interestingly, exendin-4(1–39), a peptide which was originally isolated from the venom of the Arizona desert lizard, *Heloderma suspectum*, which shares 50% structural homology with GLP-1, has also been found to be a potent GLP-1R agonist, binding with comparable affinity to GLP-1 (Goke *et al.*, 1993; Thorens *et al.*, 1993). Exendin-4(1–39) mimics almost every documented physiological action of GLP-1; however, it is becoming apparent that it does exert some effects which are not strictly related to GLP-1. For example, infusion of GLP-1, but not exendin-4, into the rat portal vein has been found to activate vagal afferent nerves (Nishizawa *et al.*, 2000). Conversely, studies in 3T3-L1 adipocytes reported exendin-4, but not GLP-1, to improve insulin sensitivity via a PI3K-dependent mechanism (Idris *et al.*, 2002). It is unclear why these differences between the actions of GLP-1 and exendin-4 exist, although it has been speculated that they may occur via an unidentified, functionally distinct receptor (Nishizawa *et al.*, 2000; Burcelin *et al.*, 2001; Idris *et al.*, 2002). Importantly, exendin-4 possesses the unique characteristic of being resistant to enzymatic degradation by the endogenous inactivator of GLP-1, DPP-4, which has led to its recent exploitation as a treatment for the hyperglycaemia associated with type 2 diabetes (Kendall *et al.*, 2005).

Physiological actions of GLP-1

A wide range of biological actions for GLP-1 (summarized in Figure 3) have been reported in several experimental systems from *in vitro* cell lines to human subjects (Green *et al.*, 2004b; Drucker, 2006). Many of these effects relate to the efficient

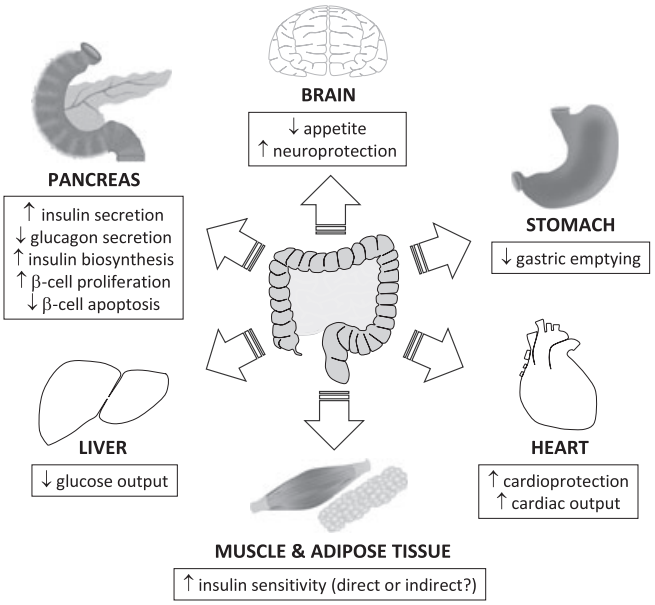

Figure 3 Physiological effects of glucagon-like peptide-1 receptor agonists. Glucagon-like peptide-1 administration exerts diverse biological actions on a number of human target organs, such as the pancreas, heart, brain, liver, stomach, muscle and adipose tissue.

storage, utilization and disposal of glucose and other nutrients. GLP-1 is one of two physiological hormones that meet the criteria of an 'incretin', that is, released from the intestine in response to nutrients with the ability to stimulate insulin secretion at physiologically relevant concentrations (Creutzfeldt, 1979). GLP-1 exerts a potent insulin-releasing effect on pancreatic β-cells (Hargrove *et al.*, 1995), while inhibiting the release of glucagon from α-cells (Creutzfeldt *et al.*, 1996). The most remarkable feature of the insulin-releasing action of GLP-1 is that it occurs in a glucose-dependent manner (Hargrove *et al.*, 1995), and it is this particular characteristic which prompted widespread interest in its potential as an anti-diabetic treatment (Green and Flatt, 2007). Indeed, it is now well established that GLP-1-induced insulin secretion leads to significant postprandial glucose lowering in both diabetic animal models (Doyle *et al.*, 2001; Xiao *et al.*, 2001; Green *et al.*, 2003; 2004a) and patients with type 2 diabetes (Gutniak *et al.*, 1994; Juntti-Berggren *et al.*, 1996; Nauck *et al.*, 1996).

Therapeutic applications of GLP-1

Drug therapies targeting the GLP-1R offer several new possibilities for the treatment of type 2 diabetes. There is persuasive clinical evidence that prolonged therapy with GLP-1 analogues/mimetics exerts beneficial actions on cardiovascular risk factors such as hyperglycaemia, dyslipidaemia and body weight in diabetic subjects (Juntti-Berggren *et al.*, 1996; Madsbad *et al.*, 2004; DeFronzo *et al.*, 2005; Suzuki *et al.*, 2007; Zinman *et al.*, 2007). These effects are likely to occur subsequent to the multiple glucose-lowering actions of GLP-1, such as stimulation of satiety and energy expenditure, reduction of gastric emptying and development of conditioned taste aversion (Turton *et al.*, 1996; Willms *et al.*, 1996; Thiele *et al.*, 1997; Hwa *et al.*, 1998). Other reported anti-diabetic actions of GLP-1 include the improvement of insulin sensitivity (related to both direct and indirect effects on muscle and adipose tissues, although these have yet to be demonstrated in humans) (Sandhu *et al.*, 1999; Gedulin *et al.*, 2005; Green *et al.*, 2006), reduction in hepatic glucose output (Larsson *et al.*, 1997) and possible protective and regenerative actions on the pancreatic β-cell (Buteau *et al.*, 2003; Drucker, 2003; Liu *et al.*, 2004; Gedulin *et al.*, 2005).

The major advantage of GLP-1 over conventional anti-diabetic therapies, such as sulphonylureas, is that its insulinotropic actions are dependent on ambient glucose concentrations, thus mitigating the risks of hypoglycaemia (Hargrove *et al.*, 1995; Green and Flatt, 2007). Consequently, a range of GLP-1 analogue and mimetic compounds are currently in development and are gradually making their way towards the clinic (Table 1). GLP-1 analogues are those peptides which closely resemble the GLP-1 amino acid sequence (e.g. liraglutide) (Flatt *et al.*, 2009), whereas mimetics are compounds with alternative structures that seek to mimic the actions of GLP-1 (e.g. exendin-4, small molecule agonist Boc5) (Chen *et al.*, 2007; Knudsen *et al.*, 2007). Indeed, inhibitors of DPP-4 (known as gliptins, e.g. sitagliptin, alogliptin), which prevent physiological inactivation of endogenous GLP-1 thereby boosting its activity, have been used for several years to control hyperglycaemia in type 2 diabetes (see recent reviews: Green *et al.*, 2007; Flatt *et al.*, 2009).

Early research sought to develop GLP-1 analogues (closely resemble the GLP-1 amino acid sequence) which were resistant to endogenous degradation by DPP-4, while retaining potent activation of the GLP-1R and thereby extending the *in vivo* half-life to around 4 h (Green *et al.*, 2004b). In the search to obtain a once-daily formulation, it was discovered that the half-life of GLP-1 peptides could be further protracted (>12 h) through methods such as acylation, PEGylation or the attachment of chemical linkers (Green and Flatt, 2007). At present, no GLP-1 analogues are clinically available, although regulatory approval of liraglutide (Victoza®, NovoNordisk), an acylated form of GLP-1, is expected in 2009. However, the GLP-1 mimetic compound, Byetta® (exenatide, Eli Lilly) was successfully launched in the USA in 2005 and subsequently in the UK in 2007.

Initial pre-clinical findings with Byetta® in patients with type 2 diabetes have now been supported by substantial clinical evidence indicating that it exerts several beneficial actions on glucose metabolism. These studies have reported that type 2 diabetic patients chronically treated with Byetta® demonstrate marked increases in first- and second-phase insulin secretion, suppression of postprandial glucagon secretion, significant reductions in postprandial hyperglycaemia and improved basal glycaemic control (Kolterman *et al.*, 2003; Fehse *et al.*, 2005; Nauck *et al.*, 2007; Zinman *et al.*, 2007). Furthermore, in clinical trials Byetta® was reported to cause significant reductions in both plasma HbA1c levels and body weight (Buse *et al.*, 2004; Nauck *et al.*, 2007).

Clinical approval of several further GLP-1 compounds is expected within the next few years (Flatt *et al.*, 2009) (summarized in Table 1). It is hoped that these will offer additional benefits to those of Byetta®, such as longer duration of action, more desirable methods of administration (compared to

Table 1 Examples of glucagon-like peptide-1 (GLP-1) analogues/mimetics which are approved or being investigated for clinical therapeutic application

Name	*Company*	*Structure*	*Status*
Exenatide (Exendin, Byetta®)	Amylin/Lilly	Exendin (twice-daily subcutaneous injection)	Approved and launched
Liraglutide (NN2211, Victoza®)	NovoNordisk	GLP-1-fatty acid (once-daily subcutaneous injection)	Approval pending
Taspoglutide (BIM-51077)	Ipsen/Roche	Long-acting GLP-1 analogue	Recruiting for phase III
Exenatide-LAR (Exendin-LAR)	Amylin/Lilly	Exendin long-acting release (once-weekly subcutaneous injection)	Phase II
Naliglutide (Albiglutide)	GlaxoSmithKline	GLP-1-albumin complex	Phase II
MKC253	MannKind	GLP-1-technospheres for inhalation	Phase I completed

twice-daily injection) and possible additional functionalities (Flatt *et al.*, 2009). For example, it now appears that the half-life of certain GLP-1-based therapies may be further extended. In this regard, exenatide-LAR, which constitutes a microsphere-encapsulated suspension of exendin-4 administered via once weekly subcutaneous injection, has been developed, and a preliminary phase II trial has indicated that it is effective in patients with type 2 diabetes. Other research efforts are also being invested in the direction of GLP-1-based therapies which may be administered via alternative, more desirable routes, such as inhalation or oral administration (Chen *et al.*, 2007; Knudsen *et al.*, 2007).

Interestingly, it appears that the therapeutic potential of GLP-1 may extend to other degenerative conditions in addition to type 2 diabetes. For example, GLP-1 has been reported to confer neuroprotective effects in both Alzheimer's and Huntington's diseases (Perry and Greig, 2004; Holscher and Li, 2008; Martin *et al.*, 2009), and to exert several direct cardioprotective actions (Bose *et al.*, 2005b; Nikolaidis *et al.*, 2005a; Sokos *et al.*, 2006).

GLP-1 in the cardiovascular system

Although the major physiological function of GLP-1 appears to be in relation to glycaemic control, GLP-1Rs have also been found in a variety of extra-pancreatic tissues. Interestingly, GLP-1Rs have been reported to be widely expressed in the heart and vasculature of both rodents and humans, with specific localization in vascular smooth muscle, cardiomyocytes, endocardium and coronary endothelium/smooth muscle (Wei and Mojsov, 1995; Bullock *et al.*, 1996), suggesting that GLP-1 may play an important role in the cardiovascular system. Indeed, recent work from several different laboratories, including our own, have reported GLP-1R agonists to exert wide ranging cardiovascular effects, such as modulation of heart rate, blood pressure, vascular tone and myocardial contractility (Barragan *et al.*, 1994; Vila Petroff *et al.*, 2001; Yamamoto *et al.*, 2002; Green *et al.*, 2008). Importantly, beneficial actions of these agents on CVD have also been reported in both experimental models and in human patients, either in the presence or absence of diabetes (Nikolaidis *et al.*, 2004a; Nystrom *et al.*, 2004; Ozyazgan *et al.*, 2005; Sokos *et al.*, 2006).

Effects of GLP-1 on blood pressure and heart rate

In normal rodents, it is well established that acute and chronic treatment with both GLP-1 and exendin-4 significantly increases blood pressure and heart rate in a dose-dependent manner within the picomolar to nanomolar range (Barragan *et al.*, 1994; Bojanowska and Stempniak, 2000; Yamamoto *et al.*, 2002; Isbil-Buyukcoskun and Gulec, 2004; Gardiner *et al.*, 2006). However, the data from larger animal and human studies are less clear. Although acute infusion of GLP-1 in conscious calves has also been reported to significantly increase heart rate, it has no effect on blood pressure (Edwards *et al.*, 1997). Similarly, a 2 h GLP-1 infusion in pigs (Kavianipour *et al.*, 2003), and both short- and long-term administration of GLP-1 in humans have been found to have no detectable chronotropic or pressor effects (Thrainsdottir *et al.*, 2004; Sokos *et al.*, 2006; 2007). These findings may suggest a species-specific effect of GLP-1, although it should be noted that the larger animal and human studies generally employed lower concentrations of GLP-1, within the low picomolar range, compared to the rodent studies which mostly administered large supra-physiological doses given as bolus injections. Interestingly, two groups have independently demonstrated a biphasic effect of GLP-1 in response to low-dose bolus administration, characterized by an initial increase in blood pressure followed by a prolonged hypotension which persists for over 30 min (Barragan *et al.*, 1994; Bojanowska and Stempniak, 2000). It appears increasingly likely that this effect may be mediated by its breakdown product, GLP-1(9–36), as accumulating evidence indicates that this metabolically inactive peptide may play an active beneficial role in the cardiovascular system (see later sections) (Nikolaidis *et al.*, 2005b; Ban *et al.*, 2008; Green *et al.*, 2008; Sonne *et al.*, 2008).

The precise mechanisms underlying the reported effects of GLP-1 on blood pressure and heart rate are yet to be fully established. It appears that they may be mediated via the GLP-1R as exendin(9–39) was found to prevent GLP-1 and exendin-4 induced increases in blood pressure and heart rate after both central and peripheral administration in the rat (Barragan *et al.*, 1996; 1999). Furthermore, gene-modified mice lacking the GLP-1R have been reported to exhibit a reduced resting heart rate compared to wild-type controls (Gros *et al.*, 2003), supporting a role for endogenous GLP-1 in cardiovascular control. However, in an experimental *in vivo* rat model, a mesenteric vasoconstriction in response to acute exendin-4 infusion was found to persist in the face of GLP-1R antagonism with exendin(9–39), suggesting that a component of this effect may occur independently of the classical GLP-1R (Gardiner *et al.*, 2006). The involvement of different downstream signalling pathways in the tachycardic and pressor effects of GLP-1 is also the subject of some debate. It was initially suggested that they occurred independently of

α- or β-adrenoceptor activation (Barragan *et al.*, 1994). However, subsequent studies concluded that these actions were largely mediated via β-adrenoceptor-dependent activation of the autonomic nervous system (Yamamoto *et al.*, 2002; Gardiner *et al.*, 2006), although it was later reported that β-adrenoceptor blockade with propranolol actually enhanced the pressor effect of GLP-1 (Gardiner *et al.*, 2008). Both central and peripheral administration of GLP-1 have been found to induce Fos-like immunoreactivity in autonomic regulatory sites, including medullary cathecholamine neurons in the area postrema, suggesting neuronal regulation of these cardiovascular actions of GLP-1 (Yamamoto *et al.*, 2002; 2003). However, recent studies have reported an autonomic-independent exendin-4-induced vasoconstriction which does not involve angiotensin II, vasopressin, neuropeptide Y, endothelin or vasoconstrictor prostanoids (Gardiner *et al.*, 2008), and that cholinergic activation may also be involved in GLP-1-induced increases in blood pressure and heart rate (Isbil-Buyukcoskun and Gulec, 2004).

In addition to the established effects of GLP-1 on blood pressure and heart rate in normal rodents, GLP-1 has also been demonstrated to exert beneficial effects in the pathological situation. Acute infusion of GLP-1 was found to restore blood pressure in hypovolaemic rats after experimental haemorrhage via stimulation of the neurohypophysial hormones, oxytocin and vasopressin (Bojanowska and Stempniak, 2002). In addition, chronic treatment with GLP-1 significantly attenuated the development of hypertension in Dahl salt-sensitive rats (Yu *et al.*, 2003), although these changes were attributed to its diuretic and natriuretic actions, rather than secondary to improvement in insulin resistance or direct central effects. Interestingly, chronic treatment of type 2 diabetic patients with the stable GLP-1 mimetic, exendin-4, and the GLP-1 analogue, liraglutide, have been reported to be associated with beneficial effects on both systolic and diastolic blood pressure without affecting heart rate, although it is likely that these changes occurred secondary to parallel improvements in several cardiovascular risk factors (Klonoff *et al.*, 2008; Garber *et al.*, 2009). As diabetic patients are frequently characterized by activation of the sympathetic nervous system and hypertension, which is an established risk factor for CVD (Bell, 2003), it is imperative that the precise nature of the sympathetic actions of GLP-1 and the mechanisms underlying these effects are fully understood.

Effect of GLP-1 on vascular function

In addition to its established effects on blood pressure and heart rate, GLP-1 has also been reported to have a direct vasorelaxant action in isolated rat vessels, in pulmonary artery, femoral artery and aorta (Golpon *et al.*, 2001; Nystrom *et al.*, 2005; Green *et al.*, 2008), and in mouse mesenteric artery (Ban *et al.*, 2008). Interestingly, although the GLP-1 mimetic, exendin-4, has also been demonstrated to cause dose-dependent relaxation of isolated rat aorta, the magnitude of the response was markedly less than that observed with GLP-1 (Green *et al.*, 2008), and exendin-4 failed to produce vasodilatation in isolated mouse mesenteric artery (Ban *et al.*, 2008). Acute treatment with GLP-1 has also been demonstrated to increase endothelium-dependent blood flow in both the forearm of healthy non-diabetic human subjects (Basu *et al.*, 2007) and the brachial artery of type 2 diabetic patients with stable coronary artery disease (Nystrom *et al.*, 2004), although the latter study reported no significant effects of GLP-1 in normal healthy subjects. Importantly, it appears that the beneficial effects of GLP-1 on vascular function may also extend to the setting of CVD. Chronic treatment with both GLP-1 and exendin-4 was found to restore streptozotocin diabetes-induced impairment of endothelial function and vascular contraction in an experimental rat model (Ozyazgan *et al.*, 2005). Furthermore, continuous chronic infusion of GLP-1 in spontaneously hypertensive heart failure-prone rats has been reported to result in a significant increase in cardiac output in the absence of any changes in blood pressure, suggesting that GLP-1 may cause peripheral vasodilatation in this situation (Poornima *et al.*, 2008). However, in both of these studies, it was unclear whether the reported effects occurred via a direct action of GLP-1 or secondary to modulation of glucose metabolism. Interestingly, an *ex vivo* study conducted in isolated aortic rings indicated that GLP-1 significantly attenuated endothelial dysfunction in vessels from Dahl salt-sensitive rats (Yu *et al.*, 2003), suggesting that the beneficial vascular effects of GLP-1 may indeed occur directly and independently of its established insulinotropic actions.

It may appear rather disappointing that the reported vasodilatory effects of GLP-1 do not translate into potentially beneficial hypotensive effects *in vivo* due to the central tachycardic and pressor actions of GLP-1, which have been previously discussed (Yamamoto *et al.*, 2002; Gardiner *et al.*, 2006). However, it is conceivable that local vasodilatation of peripheral tissues combined with increases in blood pressure may still confer some benefit to diabetic patients by improving perfusion to compromised tissues, thus alleviating or preventing some of the associated microvascular complications. This possibility is an exciting development; however, it is clear that a delicate balance exists between the central and peripheral vascular effects of GLP-1, which is complex and warrants further investigation.

Although it is well established that GLP-1 exerts beneficial actions on vascular function, the underlying mechanisms are less clear. Some studies have suggested that the vasorelaxant actions of GLP-1 are both endothelium and nitric oxide dependent (Golpon *et al.*, 2001; Ban *et al.*, 2008), whereas others have indicated that these effects may occur via other pathways, such as activation of K_{ATP} channels and cyclic AMP (Nystrom *et al.*, 2005; Green *et al.*, 2008). It has also been suggested that the vascular actions of GLP-1 may be mediated via the classical GLP-1R, as they were found to be abolished in the presence of the established GLP-1R antagonist, exendin(9–39) (Nystrom *et al.*, 2005). However, it has recently been reported that both exendin(9–39) and metabolically inactive GLP-1(9–36) are themselves capable of causing vascular relaxation (Ban *et al.*, 2008; Green *et al.*, 2008). Indeed, relaxation responses to both native GLP-1 and GLP-1(9–36) were found to persist in gene-modified mice lacking the GLP-1R (Ban *et al.*, 2008), indicating that there may be more than one type of GLP-1R in the cardiovascular system or that GLP-1 is capable of exerting receptor-independent effects. Although the precise mechanisms of its vascular actions remain unclear, it appears that GLP-1 may be beneficial in

attenuating the endothelial dysfunction which underlies many of the cardiovascular complications of diabetes (Nystrom *et al.*, 2004).

Cardiac actions of GLP-1

In addition to its effects on systemic haemodynamics and vascular function, GLP-1 also appears to play an important role in the heart. Gene-modified mice lacking a functional GLP-1R demonstrate diastolic dysfunction, increased left ventricular (LV) wall thickness and impaired cardiac reserve compared to wild-type controls, suggesting that GLP-1 may play an essential role in the control of normal cardiac structure and function (Gros *et al.*, 2003). Furthermore, studies in both isolated perfused rat hearts and cardiac myocytes suggest that exogenous GLP-1 may inhibit myocardial contractility under basal conditions, despite being associated with elevated intracellular cyclic AMP (Vila Petroff *et al.*, 2001; Zhao *et al.*, 2006). This effect was found to be mediated via the GLP-1R, as it was completely reversed in the presence of exendin(9–39), and occurred secondary to decreased myofilament Ca^{2+} responsiveness resulting from intracellular acidification (Vila Petroff *et al.*, 2001). It is interesting to note that GLP-1-induced increases in intracellular cyclic AMP appear to have a negative inotropic action, whereas β-adrenoceptor stimulation is known to cause opposite contractile effects, also secondary to elevated cyclic AMP production (Lohse *et al.*, 2003). This striking observation may indicate specific compartmentalization of cyclic AMP/protein kinase A pathways between β-adrenergic and GLP-1 signalling, which could have significant implications for modulation of other important processes within the cardiac myocyte by GLP-1.

GLP-1 and cardiac ischaemia

The majority of studies on the potential beneficial role of GLP-1 in CVD have focused on its actions in the ischaemic heart and its apparent ability to protect cardiac myocytes from ischaemic damage. Several different groups using various experimental models have reported that acute GLP-1 treatment exerts beneficial effects after ischaemia and successful reperfusion. Most of the studies to date have employed models of *ex vivo* isolated rodent Langendorff heart perfusion with short periods of ischaemia (30–45 min) and reperfusion (30–120 min), and have universally demonstrated that both GLP-1 and exendin-4 significantly reduce infarct size and enhance the recovery of contractile function after transient coronary artery occlusion (Bose *et al.*, 2005a,b; Zhao *et al.*, 2006; Ban *et al.*, 2008; Sonne *et al.*, 2008). Importantly, similar findings have also been reported *in vivo*. Acute treatment with GLP-1 (in the presence of the DPP-4 inhibitor, valine pyrrolidide) after a short period of ischaemia (30 min) in the rat was found to significantly protect against infarct development after a 2 h reperfusion (Bose *et al.*, 2005a). In an experimental canine model, infusion of GLP-1 during a period of 24 h reperfusion after brief coronary artery occlusion (10 min) was also demonstrated to result in significant improvement in LV regional wall motion and relaxation, although it had no effect on systemic haemodynamics or global systolic function (Nikolaidis *et al.*, 2005a). However, a recent study employing an *in vivo* porcine model of ischaemia–reperfusion, found that extended treatment with exendin-4 during a 3 day period after 75 min ischaemia significantly decreased infarct size and improved recovery of both systolic and diastolic function (Timmers *et al.*, 2009). In contrast, an earlier study using the same experimental preparation found that treatment with native GLP-1 over a much shorter period of reperfusion had no effect on infarct size, although it did significantly decrease interstitial levels of pyruvate and lactate (Kavianipour *et al.*, 2003).

Interestingly, a recent study has indicated that GLP-1 may also confer beneficial effects on the ischaemic heart in the setting of diabetes. Pretreatment of both normoglycaemic and streptozotocin-induced diabetic mice with the GLP-1 analogue, liraglutide, for a period of 7 days was found to significantly decrease infarct size and the incidence of cardiac rupture after chronic myocardial infarction (MI) (Noyan-Ashraf *et al.*, 2009). This was associated with improvements in survival and cardiac output, although these most likely occurred secondary to the reported benefits on early post-MI remodelling. More interesting was the finding that chronic treatment with liraglutide was found to confer cardioprotection and survival advantages over and above those of the insulin sensitizer, metformin, despite equivalent effects on glycaemic control. Importantly, it appears that these experimental findings may also extend to the clinical situation. In a small non-randomized study of predominantly non-diabetic patients with acute MI undergoing primary percutaneous coronary intervention, short-term infusion of GLP-1 for 72 h was demonstrated to significantly improve LV ejection fraction, and both global and regional wall motion (Nikolaidis *et al.*, 2004b). In addition, a randomized study conducted in a similar number of patients with preserved LV function undergoing coronary artery bypass grafting, found that GLP-1 treatment for 12 h pre- and 36 h post-surgery improved glycaemic control while lowering the requirement for high-dose insulin or inotropes (Sokos *et al.*, 2007). Although further more detailed and chronic studies are clearly required to confirm these initial observations, this nonetheless indicates that GLP-1 may hold potential therapeutic benefit for the treatment of ischaemic heart disease.

The precise mechanisms underlying the beneficial effects of GLP-1 in cardiac ischaemia have yet to be established. However, several experimental studies indicate that they occur independently of effects on glucose metabolism and may involve activation of cyclic GMP/cyclic AMP-dependent pathways and pro-survival kinases such as PI3K, Akt, glycogen synthase kinase-3β, p70s6 kinase, ERK1/2 and p38 MAPK (Bose *et al.*, 2005a; 2007; Zhao *et al.*, 2006; Ban *et al.*, 2008; Xie *et al.*, 2008; Noyan-Ashraf *et al.*, 2009). It has also been suggested that GLP-1 may exert its protective effects on the ischaemic myocardium, at least partly, via beneficial actions on cardiomyocyte apoptosis, oxidative stress and endogenous antioxidant defence mechanisms (Bose *et al.*, 2005a; Xie *et al.*, 2008; Noyan-Ashraf *et al.*, 2009; Timmers *et al.*, 2009). The role of the GLP-1R in the ischaemic heart appears to be particularly interesting. It has been reported that the protective effects of both GLP-1 and exendin-4 against *ex vivo* ischaemia–reperfusion injury, and the GLP-1

analogue, liraglutide, against MI-induced cardiomyocyte apoptosis, are completely abolished by the established GLP-1R antagonist, exendin(9–39) (Bose *et al.*, 2005a; Sonne *et al.*, 2008; Noyan-Ashraf *et al.*, 2009), suggesting that the early remodelling changes that occur after ischaemia are mediated exclusively via the GLP-1R. However, a couple of recent studies suggest that GLP-1 may improve functional recovery in the ischaemic heart via mechanisms independent of the established GLP-1R, which may involve its 'inactive' metabolite GLP-1(9–36). The beneficial effect of both exendin-4 and GLP-1 on cardiac contractile function after experimental ischaemia–reperfusion injury observed in wild-type mice was found to be both resistant to exendin(9–39) and to persist in gene-modified mice lacking a functional GLP-1R (Ban *et al.*, 2008; Sonne *et al.*, 2008). Furthermore, acute treatment with GLP-1(9–36) upon reperfusion (but not before the onset of ischaemia) resulted in an improvement in functional recovery, which occurred independently of the GLP-1R (Ban *et al.*, 2008; Sonne *et al.*, 2008). Interestingly, the beneficial effects of GLP-1 on cardiac functional recovery observed in hearts from GLP-1R mice were abolished by the DPP-4 inhibitor, sitagliptin, suggesting that these effects were mediated by its breakdown product, GLP-1(9–36) (Ban *et al.*, 2008). Taken together, these experiments not only provide compelling evidence for the existence of receptor-independent pathways and/or an unidentified GLP-1R within the heart, but also suggest possible divergence of the mechanisms underlying GLP-1 effects on the ischaemic myocardium. This brings forward the intriguing possibility of selective therapeutic targeting of different aspects of the ischaemic phenotype, although significant further research is clearly required before this may become a reality. It also raises the important question as to whether, in the context of beneficial effects of GLP-1 on the cardiovascular system, it may actually be better not to inhibit DPP-4. In this regard, it is interesting to note that the potential use of DPP-4 inhibitors, such as sitagliptin, as a therapeutic strategy to augment endogenous GLP-1 in CVD remains unexplored.

GLP-1 and heart failure

Although most of the research to date concerning the potential therapeutic application of GLP-1 in CVD has focused on cardiac ischaemia, several recent experimental and clinical studies have also reported favourable functional effects of GLP-1 in failing hearts. Short-term infusion with recombinant GLP-1 over 48 h has been demonstrated to significantly improve LV systolic and diastolic function, and increase myocardial insulin sensitivity and glucose uptake in a canine model of rapid pacing-induced dilated cardiomyopathy (Nikolaidis *et al.*, 2004a). Interestingly, GLP-1(9–36) was found to exert similar beneficial effects to native GLP-1 in this model (Nikolaidis *et al.*, 2005b), supporting the growing suggestion that the metabolically inactive form of GLP-1 may play an active role in the cardiovascular system. Furthermore, spontaneously hypertensive heart failure-prone rats (characterized by obesity, insulin resistance, hypertension and dilated cardiomyopathy), treated chronically with GLP-1 from 9 months of age (when they begin to progress to advanced heart failure and death) exhibited preserved cardiac contractile function, increased myocardial glucose uptake, improved survival and a significant reduction in cardiac myocyte apoptosis (Poornima *et al.*, 2008). Although this study also reported GLP-1 to stimulate myocardial glucose uptake in the failing myocardium, it was unclear whether its beneficial effects on contractile function occurred due to a direct cardiac action or secondary to its established insulinotropic effects.

Importantly, these experimental data are supported by preliminary clinical studies indicating that GLP-1 may also improve LV contractile function in patients with chronic heart failure. An early investigation conducted in a small number of type 2 diabetic patients with chronic heart failure found that short-term GLP-1 infusion for 3 days tended to improve both systolic and diastolic function, although these changes did not reach statistical significance (Thrainsdottir *et al.*, 2004). However, longer-term GLP-1 treatment (5 weeks) in both diabetic and normoglycaemic chronic heart failure patients (New York Heart Association class III and IV) was reported to significantly improve LV ejection fraction, myocardial oxygen consumption and functional status, whereas no effect of GLP-1 was observed in patients with normal cardiac function (Sokos *et al.*, 2006). Although these preliminary clinical studies provide some encouragement for the potential use of GLP-1 in the treatment of heart failure, it is clear that significant further research is required to confirm these initial observations, investigate the underlying mechanisms and explore possible interactions with current heart failure therapies.

Conclusions

There is no doubt that GLP-1-related drug compounds have proven efficacy in the treatment of hyperglycaemia associated with type 2 diabetes. However, it now appears that these agents also exert beneficial effects on the cardiovascular system, which may open up additional therapeutic avenues for the use of GLP-1 compounds in the treatment of CVD in both normal and diabetic patients. However, at present the precise nature and mechanisms underlying these potentially beneficial actions remain largely unknown and appear to be complex. For example, recent research has supported the existence of GLP-1 pathways independent of the classical GLP-1R and/or the existence of multiple GLP-1Rs within the cardiovascular system, and several studies have suggested that the metabolically inactive form of GLP-1, GLP-1(9–36), may also play a significant cardiovascular role. Although preliminary short-term clinical studies with GLP-1 have been encouraging, it is clear that further basic mechanistic research together with longer-term clinical investigations and meta-analyses are required before any potential therapeutic benefits may be realized.

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Conflicts of interest

The authors state that they have no conflicts of interest.

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