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Cardiovascular Biology of the Incretin System

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Glucagon-like peptide-1 (GLP-1) is an incretin hormone that enhances glucose-stimulated insulin secretion and exerts direct and indirect actions on the cardiovascular system. GLP-1 and its related incretin hormone, glucose-dependent insulinotropic polypeptide, are rapidly inactivated by the enzyme dipeptidyl peptidase 4 (DPP-4), a key determinant of incretin bioactivity. Two classes of medications that enhance incretin action, GLP-1 receptor (GLP-1R) agonists and DPP-4 inhibitors, are used for the treatment of type 2 diabetes mellitus. We review herein the cardiovascular biology of GLP-1R agonists and DPP-4 inhibitors, including direct and indirect effects on cardiomyocytes, blood vessels, adipocytes, the control of blood pressure, and postprandial lipoprotein secretion. Both GLP-1R activation and DPP-4 inhibition exert multiple cardioprotective actions in preclinical models of cardiovascular dysfunction, and short-term studies in human subjects appear to demonstrate modest yet beneficial actions on cardiac function in subjects with ischemic heart disease. Incretin-based agents control body weight, improve glycemic control with a low risk of hypoglycemia, decrease blood pressure, inhibit the secretion of intestinal chylomicrons, and reduce inflammation in preclinical studies. Nevertheless, there is limited information on the cardiovascular actions of these agents in patients with diabetes and established cardiovascular disease. Hence, a more complete understanding of the cardiovascular risk to benefit ratio of incretin-based therapies will require completion of long-term cardiovascular outcome studies currently underway in patients with type 2 diabetes mellitus. (***Endocrine Reviews*** **33: 0000–0000, 2012)**

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Abbreviations: AMI, Acute myocardial infarction; AMPK, 5′-AMP-activated protein kinase; ApoB-48, apolipoprotein B-48; BNP, B-type (brain) natriuretic peptide; CD26, cluster of differentiation 26; CNS, central nervous system; CoA, coenzyme A; DBP, diastolic blood pressure; DPP-4, dipeptidyl peptidase-4; eNOS, endothelial nitric oxide synthase; FFA, free fatty acid; G-CSF, granulocyte colony-stimulating factor; GIP, glucose-dependent insulinotropic polypeptide; GIPR, GIP receptor; GLP-1, glucagon-like peptide-1; GLP-1R, GLP-1 receptor; GLUT4, glucose transporter 4; GPCR, G protein-coupled receptor; HCAEC, human coronary artery endothelial cells; HR, heart rate; HUVEC, human umbilical vein endothelial cells; icv, intracerebroventricular; LAD, left anterior descending; LDL, low-density lipoprotein; LV, left ventricular; LVDP, LV developed pressure; LVEF, LV ejection fraction; NPY, neuropeptide Y; PI3K, phosphatidylinositol-3 kinase; PKA, protein kinase A; PPARα, peroxisome proliferator-activated receptor-α; PYY, peptide YY; SBP, systolic blood pressure; SDF-1α, stromal cell-derived factor-1α; TAG, triacylglycerol; T2DM, type 2 diabetes mellitus; VLDL, very-low-density lipoprotein.

I. Introduction

Glucagon-like peptide-1 (GLP-1) is a peptide hormone that stimulates insulin and inhibits glucagon secretion in a glucose-dependent manner. GLP-1 also inhibits gastric emptying and reduces appetite, actions that contribute to improved glycemic control (Fig. 1). Several GLP-1 receptor (GLP-1R) agonists, and inhibitors of dipeptidyl peptidase-4 (DPP-4), the enzyme responsible for GLP-1 inactivation, have been developed as therapeutic agents for the treatment of type 2 diabetes mellitus (T2DM). Because cardiovascular complications represent the primary source of morbidity and mortality in diabetic subjects (1–3), we now review the potential mechanisms and current understanding of the cardiovascular consequences of augmenting GLP-1 action for the treatment of T2DM.

II. The Incretin Axis

Enteral glucose administration markedly potentiates insulin secretion to a much greater extent than the same level of plasma glucose achieved via parenteral glucose administration, a concept known as the incretin effect. The elucidation of the structure and function of glucose-dependent insulinotropic polypeptide (GIP) in the 1970s (4–6) established GIP as the first incretin hormone. More than a decade later, cloning of the proglucagon cDNA enabled identification of the GLP-1 sequence and subsequent characterization of the insulinotropic biological activity of GLP-1 (7–10). Together, GIP and GLP-1 are widely viewed as the predominant gut-derived hormones that promote glucose-dependent insulin secretion after meal ingestion.

A. Glucagon-like peptide-1

Although initial studies of GLP-1 assessed the biological activity of GLP-1 (1–37) and GLP-1 (1–36) amide, the realization that the first six amino acids of GLP-1 could be cleaved to generate GLP-1 (7–37) or GLP-1 (7–36)amide accelerated the discovery of multiple metabolic actions of GLP-1 *in vivo*. GLP-1 is synthesized in and secreted from enteroendocrine L cells distributed throughout the small and large intestine; however, the majority of intestinal GLP-1 content has been localized to the distal small bowel

Figure 1.

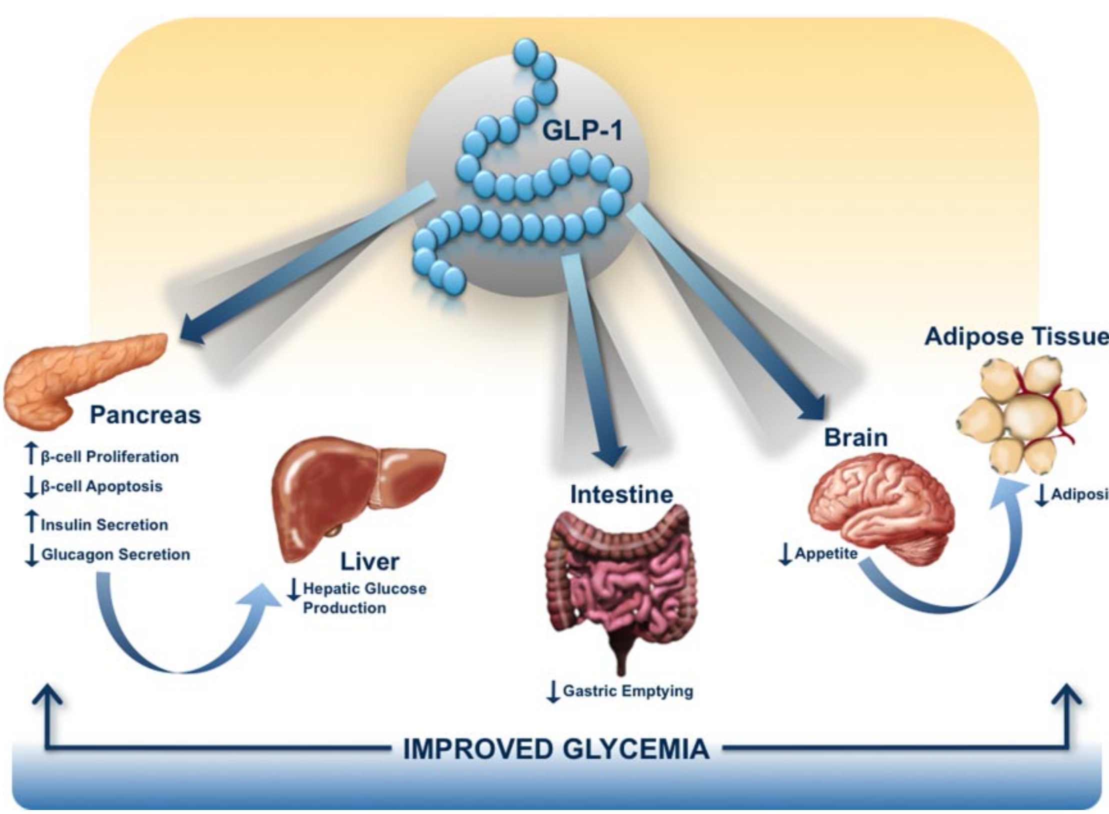

Figure 1. GLP-1 targets multiple organs to improve glucose control in T2DM. GLP-1 acts directly and indirectly on several peripheral tissues that contribute to lowering of blood glucose levels. These include potent effects on the pancreatic β-cell to stimulate insulin secretion, inhibition of α-cell glucagon secretion that reduces hepatic glucose production, a decrease in gastric motility, and a reduction in appetite that contributes to weight loss, reduced levels of adipocytokines, and decreased inflammation.

and colon. GLP-1 is also produced in the central nervous system (CNS), predominantly in the brainstem, and subsequently transported to a large number of regions within the CNS. GLP-1 is secreted from the gut at low basal levels even in the fasted state, and plasma levels of GLP-1 increase 2- to 3-fold after nutrient ingestion. The biology of GLP-1 synthesis and secretion and the multiple metabolic and extrapancreatic actions of GLP-1 and GIP have been extensively reviewed (11–15).

B. The GLP-1 receptor

A single G protein-coupled receptor (GPCR), with considerable amino acid homology to related class B family GPCR (16), transduces the majority of GLP-1 actions *in vivo* and represents the only specific high-affinity GLP-1R identified to date (17). The GLP-1R was originally identified in islet β-cells but is widely expressed in extrapancreatic tissues, including the lung, kidney, CNS, enteric and peripheral nervous system, lymphocytes, blood vessels, and heart (17, 18). Multiple actions of GLP-1 and structurally related GLP-1R agonists have been reported in cells and tissues that do not express the classical GLP-1R, hence there continues to be analysis of mechanisms and pathways capable of transducing actions of GLP-1 independent of the known GLP-1R (19).

C. GLP-1-mediated regulation of insulin and glucagon secretion

GLP-1 directly potentiates insulin secretion in a glucose-dependent manner, minimizing the risk of hypoglycemia in diabetic subjects treated with GLP-1R agonists. GLP-1 also induces glucose competence in previously unresponsive β-cells (20) and rapidly improves β-cell glucose sensitivity, thereby restoring insulin secretion toward normal levels in human patients with T2DM (21). GLP-1 also inhibits glucagon secretion in a glucose-dependent manner (22). Because the majority of α-cells do not express the GLP-1R, and GLP-1 inhibits glucagon secretion even in C-peptide-negative subjects with type 1 diabetes, the glucagonostatic effects of GLP-1 are likely indirect, mediated through somatostatin-dependent mechanisms (23).

D. Glucose-dependent insulinotropic polypeptide

GIP is a 42-amino-acid peptide synthesized in and secreted from enteroendocrine K cells localized to the proximal small bowel. Like GLP-1, GIP is secreted at low basal levels in the fasted state, and plasma levels of GIP increase within minutes of nutrient ingestion. Although the major action of GIP is the glucose-dependent stimulation of insulin secretion, GIP also promotes lipid uptake and expansion of adipocyte mass and exerts a number of extrapancreatic actions in the brain, bone, and adrenal gland, delineated predominantly in preclinical studies (24).

E. The GIP receptor

The GIP receptor (GIPR) is a member of the class B family of GPCR, and GIPR activation leads to cAMP generation and insulin secretion from islet β-cells. The GIPR is widely expressed in extrapancreatic tissues, including the gastrointestinal tract, adipose tissue, heart, pituitary, adrenal cortex, and multiple regions of the CNS (25). Disruption or attenuation of GIP action is associated with diminished weight gain, resistance to diet-induced obesity, and improved insulin sensitivity in preclinical studies (24, 26), whereas genetic variation within the human *Gipr* gene is linked to control of postprandial glucose and body weight (27, 28).

F. Dipeptidyl peptidase-4 and incretin degradation

DPP-4, originally described as a lymphocyte cell surface protein, cluster of differentiation 26 (CD26), is a membrane-spanning exopeptidase that cleaves dipeptides from the N terminus of proteins or peptides, immediately after a position 2 proline or alanine, although DPP-4 can also cleave peptides containing other position 2 residues (29, 30). DPP-4 exists in two molecular forms that both exhibit proteolytic activity: a membrane-spanning protein with a short intracellular tail and a circulating protein devoid of the membrane-spanning and intracellular regions. The biology of DPP-4 is highly complex, because both the membrane-spanning protein and the soluble circulating form exert multiple biological activities independent of the catalytic activity of the enzyme (31). The observation that DPP-4 cleaves both GLP-1 and GIP at the N terminus (32–34), followed by demonstrations that chemical inhibition or genetic inactivation of DPP-4 increases the circulating levels of intact GLP-1 and GIP (35, 36), firmly established DPP-4 as a major regulator of incretin degradation.

III. Role of the GLP-1R in the Cardiovascular System

Elucidation of GLP-1 actions in the cardiovascular system has important implications for the treatment of subjects with T2DM. Studies in animals and humans have explored the biological actions of GLP-1 on the myocardium and the vasculature, as well as on cardiovascular risk factors that will be discussed below in *Section III*.

A. GLP-1R expression and signal transduction in the myocardium

Glp1r mRNA transcripts have been detected by RT-PCR in the rat and mouse heart (18, 37) and in the human heart using ribonuclease protection assays (38). Immunohistochemistry detected GLP-1R protein in mouse cardiomyocytes and endocardium, whereas Western blotting revealed GLP-1R protein expression in all chambers of the mouse heart (37). Furthermore, immunoreactive GLP-1R protein has been detected in sarcolemmal membranes from canine myocardium by Western blotting (39).

1. GLP-1R signaling in primary cultured cardiomyocytes and cardiomyocyte cell lines

Studies in cardiomyocytes have delineated signaling pathways transduced downstream of the cardiac GLP-1R (Fig. 2). Direct treatment of adult rat cardiomyocytes with native GLP-1 (10 nM) for 20 min increased cAMP levels, consistent with actions of GLP-1 in pancreatic β-cells (40). Surprisingly, the GLP-1-induced increase in intracellular cAMP was not coupled to an increase in intracellular Ca^{2+} and subsequent cardiomyocyte contractility as would be expected for a cAMP-generating agent in the heart (40). Whole-cell patch clamping of adult canine ventricular myocytes demonstrated a protein kinase A (PKA)-dependent increase in L-type Ca^{2+} channel current and action potential duration in response to 5 nM GLP-1 that peaked 15 min after treatment (41). In cultured neonatal mouse cardiomyocytes, treatment with exendin-4 (3 nM) for 20 min increased Akt and ERK phosphorylation (42), both well-characterized regulators of cardiomyocyte growth and glucose metabolism (43, 44).

GLP-1R activation in primary cultures of neonatal mouse cardiomyocytes is antiapoptotic, because pretreatment of cells with the GLP-1R agonist liraglutide for 1 h (10–1000 nM) reduced caspase-3 cleavage induced by TNFα (100 ng/ml for 24 h) (45). Furthermore, native GLP-1 (10 nM) reduced apoptosis in neonatal rat cardiomyocytes after 16 h hypoxia (92% N_2/5% CO_2/3% O_2)/4 h reoxygenation (95% CO_2/5% O_2) (46). These effects were prevented by coincubation with the phosphatidylinositol-3 kinase (PI3K) inhibitor LY294002 or the ERK inhibitor UO126.

GLP-1 (200 nM) also directly activated PI3K/Akt and ERK in HL-1 cardiomyocytes, reducing the extent of apo-

Figure 2.

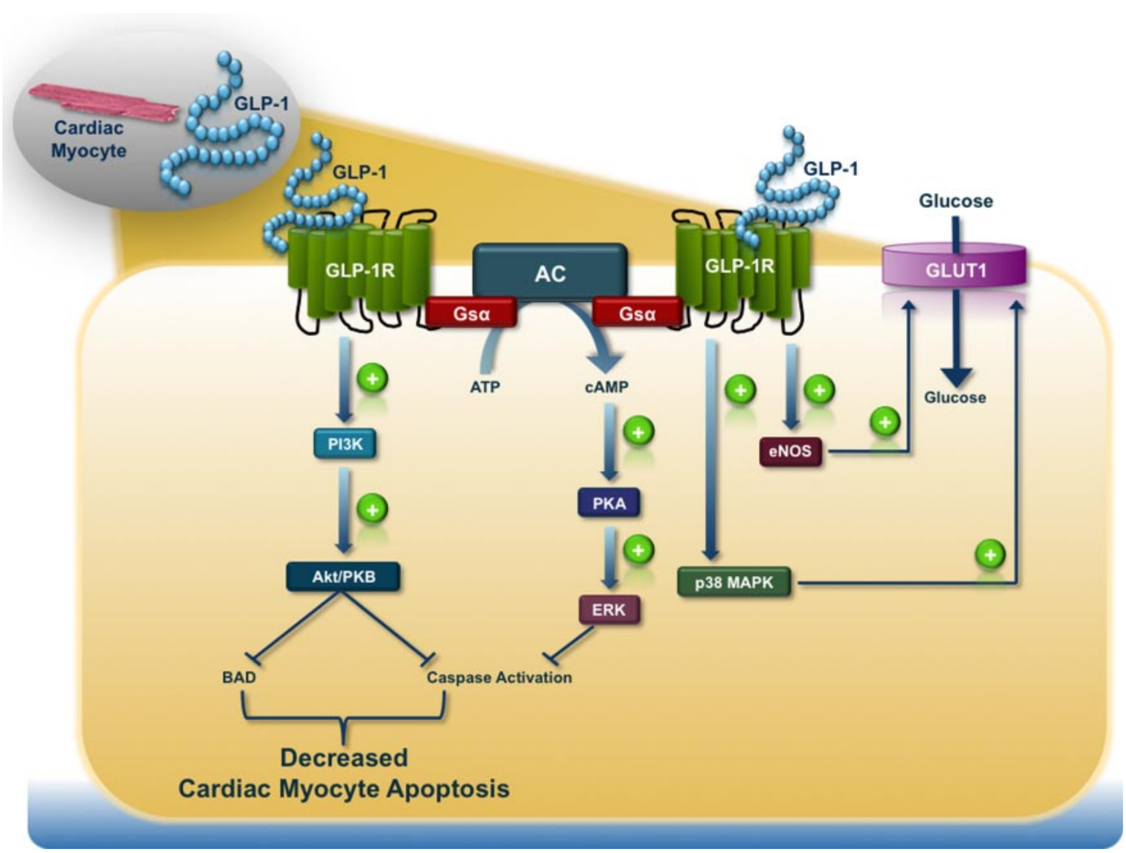

Figure 2. GLP-1R-dependent intracellular signal transduction pathways in the cardiomyocyte. The signaling pathways engaged downstream of the cardiomyocyte GLP-1R lead to a reduction in apoptosis and increase in glucose uptake independent of the classical insulin-dependent pathway. ROS, Reactive oxygen species. AC, Adenylate cyclase.

ptosis induced by staurosporine, palmitic acid, or ceramide; these cytoprotective effects were abolished by coincubation with UO126 or the PI3K inhibitor wortmannin (47). However, whether the GLP-1R is expressed in HL-1 cells remains uncertain.

2. GLP-1R activation in isolated nonischemic hearts and in conscious animals

The paradoxical observation that GLP-1 decreased contractility in primary cultured adult rat cardiomyocytes, despite increasing cAMP levels (40), has also been observed in isolated rat hearts, where addition of GLP-1 (0.5 nM) to the perfusate reduced left ventricular (LV) developed pressure (LVDP) (48). In contrast, 0.3 nM GLP-1 increased LVDP by approximately 20% at 20 min after treatment during Langendorff aerobic perfusion of isolated mouse hearts (37). GLP-1 increased myocardial glucose uptake during aerobic perfusion (37, 48), independent of insulin-stimulated Akt phosphorylation and glucose transporter 4 (GLUT4) translocation, in association with increased p38 MAPK activity, enhanced nitric oxide (NO) production, and increased GLUT1 protein levels at the sarcolemmal membrane (Fig. 2) (48).

Studies in conscious dogs demonstrate that 48 h continuous infusion of recombinant GLP-1 (1.5 pmol/kg · min) increased myocardial glucose uptake during a hyperinsulinemic-euglycemic clamp (49). This effect was inhibited by pretreatment with either SB 203580 (1 mg/kg iv) or nitro-L-arginine (30 mg/kg iv), suggesting essential roles for p38 MAPK and endothelial nitric oxide synthase (eNOS) as downstream targets of GLP-1 action (39).

3. Cardiovascular phenotype of Glp1r$^{-/-}$ *mice*

In vivo hemodynamic measurements with Millar catheters in hearts from anesthetized whole-body *Glp1r*$^{-/-}$ mice demonstrate normal basal LV function but reduced contractile function in response to ip insulin (3 U/kg) or iv adrenaline administration (1 μg/kg) (50). Histological analysis of the 5-month-old *Glp1r*$^{-/-}$ mouse heart on a CD1 genetic background revealed thicker ventricular walls, suggesting that endogenous GLP-1R activity may influence structural development of the heart. Interestingly, hearts from *Glp1r*$^{-/-}$ mice exhibited increased baseline LVDP (~25% increase) throughout the course of a 40-min Langendorff aerobic perfusion (37), findings consistent with previous observations of GLP-1 reducing contractility and LVDP in adult rat cardiomyocytes (40) and Langendorff aerobically perfused isolated rat hearts (48), respectively.

B. GLP-1R in the vasculature

An immunoreactive GLP-1R protein has been detected in human coronary artery endothelial cells (HCAEC) and human umbilical vein endothelial cells (HUVEC) (51, 52). GLP-1R protein expression has also been detected in mouse coronary endothelial and smooth muscle cells via immunohistochemistry (37).

1. GLP-1R activation in endothelial cells

A 5-h incubation with the GLP-1R agonist liraglutide (0.1–100 μg/ml) increased eNOS phosphorylation and NO production via a 5′-AMP-activated protein kinase (AMPK)-dependent pathway in HUVEC cultures (53). Similarly, 48 h incubation with either GLP-1 (100 nM) or exendin-4 (10 nM) increased Akt and eNOS phosphorylation, and subsequent NO production in HCAEC (54). GLP-1R activation also increased proliferation of HCAEC as determined via [^{3}H]thymidine incorporation into DNA; stimulation of cell proliferation was abrogated by coincubation with either N_ω-nitro-L-arginine methyl ester hydrochloride (L-NAME) or the Akt inhibitor IV (54). In contrast, 1 h treatment of HUVEC with 10 nM GLP-1 had no effect on Akt phosphorylation but increased PKA activity and cAMP response element-binding protein (CREB) phosphorylation (55). Hence, the endothelial cell is a direct target for GLP-1 action (Fig. 3).

Liraglutide (100 nM) prevented the increase in plasminogen activator inhibitor type-1 and vascular cell adhesion molecule-1 (VCAM-1) mRNA and protein expression in response to TNFα (10 ng/ml for 5 or 16 h) or hyperglycemia (10 mM for 48 h) in the C11 STH human umbilical vein endothelial cell line (56). GLP-1 (0.03 and 0.3 nM for 4 h) reduced reactive oxygen species and VCAM-1 mRNA expression in HUVEC after exposure to advanced glycation end products (100 μg/ml glycated BSA) (52). Using an *in vitro* model of vascular aging, treatment of HUVEC for 30 min with GLP-1 or exendin-4 before a 1-h incubation with 30 μM H_2O_2 reduced the number of senescent cells in an exendin(9–39)-sensitive manner (55). These effects were also dependent on PKA activity because they were abolished by pretreatment with 1 μM H89. Hence, GLP-1R activation exerts cytoprotective actions in endothelial cells.

2. GLP-1 and endothelial function in humans

Studies in nondiabetic normotensive volunteers demonstrated that GLP-1 (1.2 pmol/kg · min) infusion enhanced acetylcholine-induced (2–8 μg/100 ml) forearm blood flow measured via venous occlusion plethysmography, changes abolished by coadministration of the sulfonylurea glyburide but not glimepiride. In contrast, GLP-1 had no effect on blood flow induced via sodium nitro-

Figure 3.

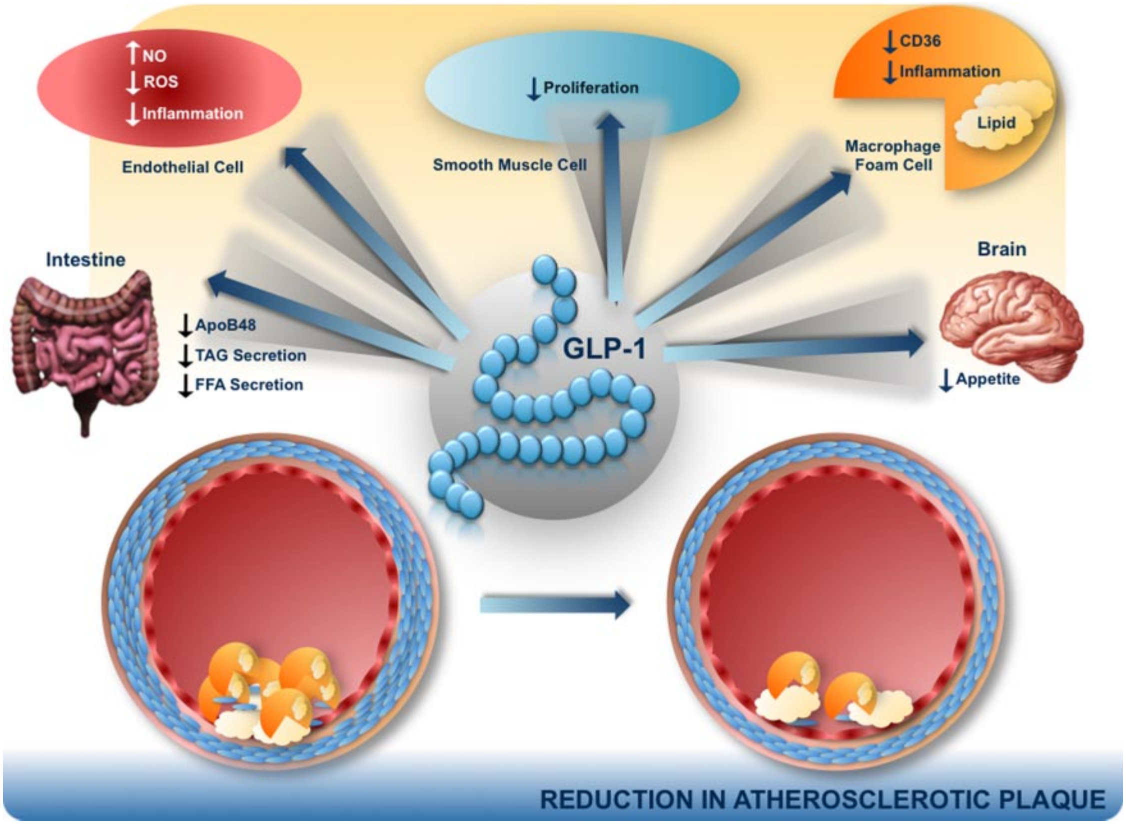

Figure 3. Antiatherosclerotic potential of GLP-1 action. The direct actions of GLP-1 on blood vessels and macrophages and on the regulation of plasma lipid profiles may impact the development and/or progression of atherosclerotic plaques.

prusside (0.5- 2 μg/100 ml) (57). Similarly, fasted subjects with T2DM and stable coronary artery disease (n = 12) exhibited improved endothelial function in response to GLP-1 infusion (2 pmol/kg · min), as indicated by an increase in flow-mediated vasodilation of the brachial artery, independent of changes in systolic blood pressure (SBP) and diastolic blood pressure (DBP) during a hyperinsulinemic clamp (51). A study of 16 subjects with T2DM and 12 nondiabetic controls demonstrated that infusion of 0.4 pmol/kg · min GLP-1 (plasma concentration of ~180 pg/ml) during a 2-h hyperglycemic clamp improved flow-mediated vasodilation in both groups. However, the GLP-1-mediated improvements in blood flow were considerably attenuated after a 2-month period of improved glycemic control (58). Whether the effects of native GLP-1 on blood flow are mimicked by degradation-resistant GLP-1R agonists remains unclear; GLP-1 but not exenatide produced vasodilation and increased cGMP release from isolated preconstricted blood vessels *ex vivo* (37), and exenatide had no effect on endothelial function in rat conduit arteries *ex vivo* after infusion with intralipid *in vivo*, whereas native GLP-1 and GLP-1 (9–36) both exerted vasodilatory actions in control experiments (59).

3. GLP-1-mediated control of heart rate (HR) and BP in animals

GLP-1R-dependent control of HR and BP is complex and species specific. Synthetic human GLP-1 (0.1–1000 ng) administered into the jugular vein of male rats acutely increased SBP and DBP and HR; BP and HR returned to basal levels by 25 min after GLP-1 administration (60). These effects were independent of catecholamines and adrenergic receptors, because pretreatment with either propranolol (1 mg/kg iv) or phentolamine (0.1 mg/kg iv) did not prevent the increases in HR and BP (60). GLP-1 also increased HR and BP in streptozotocin-induced diabetic rats, although plasma insulin levels were not measured (60). The increased BP and HR observed after administration of GLP-1 or exendin-4 in rats was dependent on GLP-1R signaling and abolished by iv infusion of exendin(9–39) (61). The GLP-1-stimulated increase in HR and BP in rodents involves dual pathways originating from both the CNS and periphery, with the neural pathway requiring intact vagus nerve transmission, because intracerebroventricular (icv) GLP-1 (100 ng) failed to increase HR and BP in vagotomized rats (62). Support for a role of the CNS in the GLP-1R-dependent control of HR and BP derives from telemetry studies of freely moving rats, whereby GLP-1R agonist-mediated increases in HR and BP were coupled to activation of autonomic control centers in the CNS (63). Administration of icv exendin-4 induced Fos-like immunoreactivity in neurons innervating sympathetic preganglionic neurons in the paraventricular hypothalamus, the arcuate nucleus, and the lateral hypothalamic area in the rat brain (63). Furthermore, double-labeling immunohistochemistry after icv exendin-4 detected induction of Fos-like and tyrosine hydroxylase immunoreactivity in catecholaminergic neurons in the nucleus of the solitary tract and locus coeruleus (63); icv exendin-4 also activated tyrosine hydroxylase transcription in adrenal medullary catecholamine neurons (63). Continuous infusion of the selective β_2-adrenoceptor antagonist ICI 118551 or the nonselective β-adrenoceptor antagonist propranolol abolished the effects of exendin-4 (250 ng/kg iv) on HR in rats (64). Furthermore, exendin-4 (250 ng/kg iv) was unable to increase HR in adrenalectomized rats (25). Taken together, considerable data suggest that GLP-1 engages the rodent sympathetic nervous system to modify HR and BP (63–65). Nevertheless, other investigators have reported that the vasoconstrictor properties of exendin-4 were independent of the autonomic nervous system, because the increase in mean arterial pressure after exendin-4 administration persisted despite continuous infusion with propranolol or the α-adrenoceptor antagonist phentolamine (24). Furthermore, iv GLP-1 (20 nmol) acutely increased HR over a 1-h period in adult male rats that had previously undergone either adrenalectomy or vagotomy (66).

GLP-1R activation in neurons that innervate cardiac vagal neurons in the nucleus ambiguous, resulted in diminished HR variability, and reduced parasympathetic modulation of the heart (67). GLP-1 may also increase BP in rodents through the vasopressin system, because an intraarterially injected vasopressin receptor antagonist, B-mercapto (10 μg/kg), abolished the rise in BP after 100 ng icv GLP-1 (68). In contrast to findings of increased BP in mice and rats after acute GLP-1R activation, GLP-1 (30–60 μmol/kg body weight · min) transiently increased HR yet had no effect on aortic BP in conscious calves (69). Hence, the mechanisms underlying the acute effects of GLP-1R agonists to increase HR and BP are complex, may involve both the sympathetic and parasympathetic nervous system, and appear species specific.

4. GLP-1-mediated control of HR and BP in humans

Studies of GLP-1 effects on BP in humans have yielded results quite different from those obtained acutely in rodents. Infusion of GLP-1 (2.4 pmol/kg · min for 10 min followed by 1.2 pmol/kg · min) for 65 min in 55 healthy human subjects (13 men and 42 women, mean age 31 yr) had no effect on SBP or DBP, plasma norepinephrine levels, or HR variability calculated at both low and high frequency, indices of cardiac sympathetic and parasympathetic activity, respectively (70). In contrast, acute sc injection of GLP-1 (80 nmol/ml) into the anterior abdominal wall transiently increased HR (64 ± 2 *vs.* 54 ± 2 beats/min at 40 min after injection) and BP (83 ± 5 *vs.* 77 ± 4 mm Hg at 20 min after injection) in 10 healthy human subjects; however, values returned to near baseline by approximately 50–60 min after injection (71). The effects of GLP-1R activation in patients with elevated BP also contrasts from those reported in animals, which will be described in detail in *Section IV.A.2*.

C. GLP-1 action and dyslipidemia

1. Studies of GLP-1 action on lipoprotein synthesis and secretion

Rats infused with GLP-1 (20 pmol/kg · min) via the jugular vein for 6 h exhibited a reduction in triacylglycerol (TAG) absorption, decreased intestinal lymph flow, and reduced intestinal apolipoprotein B-48 (ApoB-48) production (72). Furthermore, in studies of fructose-fed hamsters, the DPP-4 inhibitor sitagliptin (5 mg/kg via oral gavage for 2 or 3 wk) decreased fasting plasma TAG, predominantly in the very-low-density lipoprotein (VLDL) fraction, whereas both exendin-4 (24 nmol/kg ip) and sitagliptin (10 mg/kg via oral gavage) acutely decreased postprandial TAG and ApoB-48 levels in male C57BL/6J mice administered olive oil and Triton WR1339 after a 5 h fast (73). Exendin-4 also reduced TAG and ApoB-48 secretion when administered 1 h after the oral fat load, suggesting the effect on postprandial lipid metabolism was not related to delayed gastric emptying (73). Moreover, exendin-4 (0.1 nM) directly decreased ApoB-48 secretion in primary cultured hamster enterocytes, as assessed by reduced levels of ^{35}S-labeled ApoB-48 in the media over a 90-min time course, whereas *Glp1r*$^{-/-}$ mice exhibited enhanced appearance of plasma TAG after olive oil administration. These findings support a direct essential role for GLP-1 in the control of chylomicron secretion (Fig. 3) independent of changes in gastric emptying (73).

2. GLP-1 action, on the liver and dyslipidemia

Chronic administration of the DPP-4 inhibitor vildagliptin (1 mM in drinking water) for 8 wk reduced fasting plasma TAG and cholesterol levels in wild-type mice fed a high-fat diet for 14 wk (45% kcal from lard) but failed to lower plasma lipid levels in mice lacking both the *Glp1r* and *Gipr* (74). Vildagliptin also reduced hepatic expression of long-chain acyl coenzyme A (CoA) synthetase mRNA in wild-type but not in $Glp1r^{-/-}$:$Gipr^{-/-}$ mice, suggesting that DPP-4 inhibition may reduce plasma TAG levels via prevention of VLDL assembly (74). *Ob/ob* mice treated for 60 d with exendin-4 (10 μg/kg every 24 h for the first 14 d, followed by either 10 or 20 μg/kg every 12 h for the remaining 46 d) exhibited a marked reduction in hepatic lipid content as assessed by oil red O staining, associated with reduced steroyl CoA desaturase and sterol response element-binding protein-1c mRNA expression and increased peroxisome proliferator-activated receptor-α (PPARα) mRNA expression (75). Furthermore, daily liraglutide (200 μg/kg ip) treatment for 4 wk in mice fed a high-fat and high-fructose corn syrup diet for 8 wk reversed hepatic steatosis assessed by oil red O staining (76). This effect was associated with increased mRNA expression of acyl CoA oxidase and increased protein expression of long-chain acyl CoA dehydrogenase, suggesting an elevation in peroxisomal and mitochondrial fatty acid β-oxidation, respectively (76). In primary cultured human hepatocytes, 24 h treatment with 10 nM exendin-4 in the presence of 0.8 mM oleate, elaidate, or palmitate reduced lipid stores assessed by oil red O staining as well as caspase-3 cleavage and DNA condensation, resulting in improved cell survival (77). Evidence for reduced endoplasmic reticulum stress and increased macroautophagy was also observed, because exendin-4 increased protein expression of glucose-regulated protein 78, reduced expression of CCAAT-enhancer-binding protein homologous protein, and increased autophagic vacuole formation.

Daily treatment of high-fat diet-fed mice (6 wk, 44% kcal from fat) for 4 wk with the GLP-1R agonist CNT0736 (0.1 or 1 mg/kg ip) reduced fasting VLDL production in a body weight-independent manner; however, exendin-4 administration (7.1 μg/kg ip) did not affect these parameters (78). Nevertheless, whether GLP-1 acts directly on the liver to control lipoprotein synthesis and secretion remains unclear because treatment of primary murine hepatocytes for 16 h with 40 nM exendin-4 had no effect on TAG or cholesterol synthesis/secretion, and *Glp1r* mRNA transcripts corresponding to the entire open reading frame of the GLP-1R were not detected in isolated hepatocytes (74). In contrast, a 142-bp *Glp1r* mRNA transcript and immunoreactive GLP-1R protein were detected in RNA from human liver biopsies and human hepatoma HepG2 cells via RT-PCR and Western blotting, respectively (79). Immunoreactive GLP-1R protein detected by Western blotting was also identified in both isolated human and rat hepatocytes, and exenatide directly increased mRNA expression of PPARγ and PPARα in human hepatocytes (75, 79, 80). Because data from multiple groups has yielded contrasting results on the presence of hepatic GLP-1R expression in rodent or human hepatocytes, the mechanism through which GLP-1 acts on the liver requires further elucidation (18, 74, 81, 82).

3. GLP-1 action in adipose tissue

Whether the classical GLP-1R is expressed in the adipocyte is uncertain. GLP-1 binding sites were detected in solubilized membranes from both human and rat adipose tissue using radioligand binding assays (83, 84), although others have been unable to detect *Glp1r* mRNA expression in adipose tissue from humans and rats (18, 38). Nevertheless, GLP-1 treatment of isolated rat adipocytes increased lipolysis (85) and exogenous GLP-1 exerted both lipolytic and lipogenic effects in human adipocytes (86). On the other hand, *in situ* microdialysis in nine healthy volunteers failed to demonstrate a lipolytic action of GLP-1 (87). Hence, whether GLP-1 exerts direct actions in adipose tissue depots through the GLP-1R remains unclear.

4. GLP-1 and dyslipidemia in human subjects

GLP-1 infusion (1.2 pmol/kg · min) for 6.5 h in 14 healthy volunteers inhibited the postprandial increase in plasma TAG and free fatty acid (FFA) levels after a 250-kcal solid test meal (88). Short-term studies in 12 subjects with T2DM demonstrated that addition of GLP-1 (sc injection of 25 nmol directly before meals) to insulin therapy for 5 d, followed by administration of GLP-1 alone for the last 2 d, decreased plasma concentrations of VLDL-TAG (1.30 ± 0.36 *vs.* 2.08 ± 1.11 mM) while increasing the size of low-density lipoprotein (LDL) cholesterol particles (mean diameter of 22.9 *vs.* 22.3 nm) (89). A study in 50 subjects with T2DM infused for 4 h with GLP-1 (1.2 pmol/kg · min) after an overnight 10-h fast revealed a reduction in plasma FFA levels (~0.25 mM decrease throughout the first 2 h) (90). A hyperglycemic clamp followed by a hyperinsulinemic-euglycemic clamp in 16 elderly patients with T2DM treated for 3 months with a continuous sc GLP-1 infusion (100 pmol/kg · h) also demonstrated decreased plasma FFA levels during the hyperinsulinemic portion of the clamp, which was associated with a significant increase in plasma insulin levels (91). A double-blind crossover study in 35 patients with recent-onset T2DM demonstrated that a single sc injection of exenatide (10 μg) markedly reduced postprandial levels of TAG and ApoB-48, as well as plasma remnant lipoprotein cholesterol, for up to 8 h in subjects fed a high-

Figure 4.

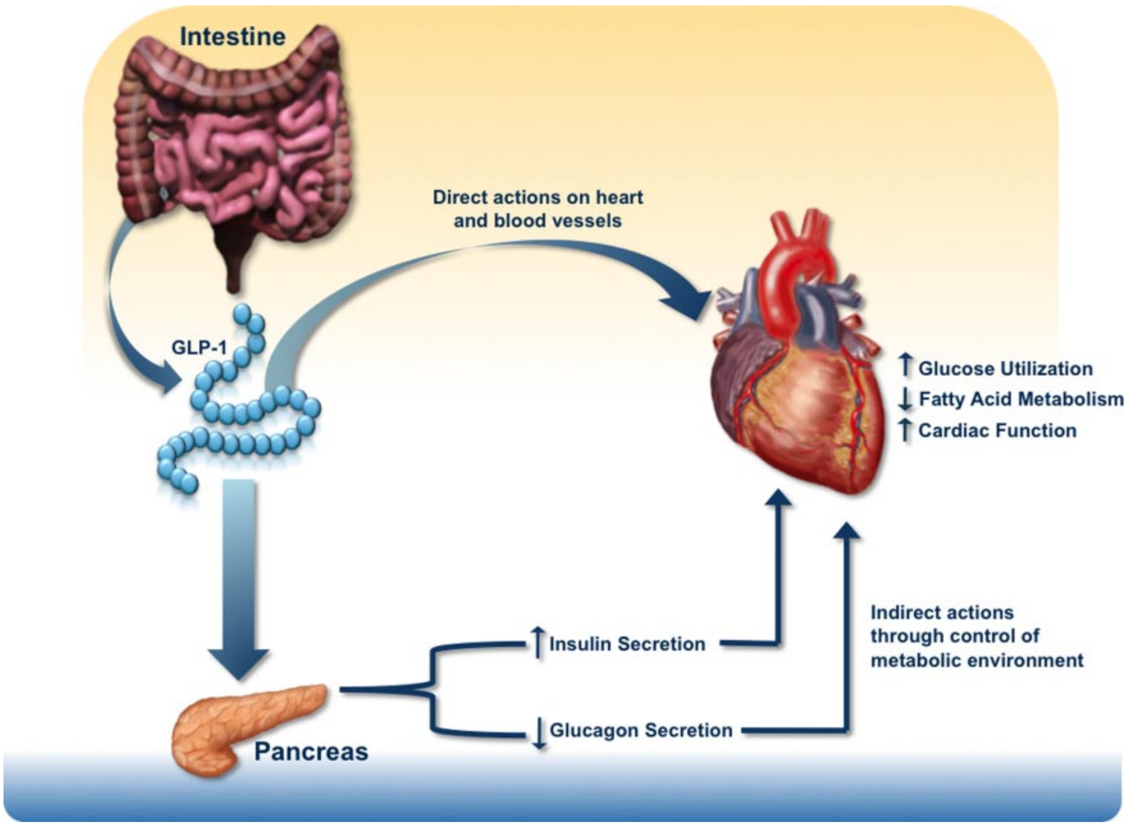

Figure 4. Indirect cardiac actions of GLP-1. In addition to direct actions on the cardiomyocyte, GLP-1 may also influence cardiac function indirectly through its actions on pancreatic islets to enhance glucose-dependent insulin secretion and inhibit glucagon secretion, thereby changing levels of islet hormones, glucose, and fatty acids, all of which may also directly impact the heart.

calorie (600 kcal/m^2 body surface area), fat-enriched (45%) breakfast meal after an overnight fast (92). These effects were already observed during the first 4 h after the breakfast meal, when no changes in plasma insulin were observed. Interpretation of studies demonstrating that acute GLP-1R activation lowers postprandial lipemia requires consideration of whether the findings are due in part to the acute effect of GLP-1R agonists to inhibit gastric emptying and gut motility, actions that are diminished with more sustained GLP-1R activation (93, 94).

DPP-4 inhibitors have also demonstrated favorable effects on postprandial dyslipidemia in T2DM. A 4-wk treatment with vildagliptin (50 mg twice daily) reduced postprandial elevations of plasma TAG, chylomicron-TAG, chylomicron-ApoB-48, and chylomicron-cholesterol for up to 8 h in response to a fat-rich test meal (1000 kcal, 72 g fat) after an overnight fast (95). Similarly, a double-blind crossover study in 36 subjects with T2DM treated with sitagliptin (100 mg/d taken during the morning meal) for 6 wk also demonstrated a reduction in postprandial elevations of plasma TAG, ApoB-48, and FFA levels for up to 8 h in response to a test meal (1003 kcal, 68.6 g fat) after a 12-h overnight fast (96). Because DPP-4 inhibitors reduce postprandial lipemia without inhibition of gastric emptying or weight loss, these findings are consistent with a role for local GLP-1 in the control of intestinal chylomicron secretion (73).

D. Direct *vs.* indirect effects of GLP-1R agonism on cardiac function

One of the challenges in understanding the effects of GLP-1 on the heart involves elucidation of the direct *vs.* indirect pleiotropic metabolic properties of GLP-1 (Figs. 1, 2, and 4). *Section III.D.1* will briefly highlight some of the key indirect actions/effectors of GLP-1 and their potential impact on cardiac function.

1. GLP-1 and insulin

Activation of the β-cell GLP-1R during hyperglycemia usually increases plasma insulin levels, leading to increased myocardial glucose uptake and glycogen synthesis and decreased fatty acid oxidation (Fig. 4) (97). Furthermore, increased insulin receptor signaling in the heart leads to activation of the PI3K/Akt pathway, increased eNOS activity, and inhibition of AMPK (98, 99). Insulin also inhibits lipolysis and reduces plasma FFA levels (100, 101), and this effect, coupled with the improvement in myocardial glucose metabolism, supports the rationale for evaluating glucose-insulin-potassium infusions in subjects with acute myocardial infarction (AMI) (102). Hence, whether the metabolic actions of GLP-1 on the heart *in vivo* reflect direct and/or indirect mechanisms requires careful consideration.

2. GLP-1, obesity, and adiponectin

GLP-1R activation in the hypothalamus reduces appetite and leads to weight loss (103–105). Obesity is a significant risk factor for the development of cardiovascular diseases, with every 1 kg/m^2 increment in body mass index resulting in a 5 and 7% increase in the risk of heart failure in men and women, respectively (106). Thus, weight loss associated with GLP-1R agonists may contribute to potential cardioprotective effects. Furthermore, weight loss is associated with increased plasma adiponectin levels, and adiponectin protects against both AMI and cardiac hypertrophy (107, 108). Indeed, the reduction in body mass in 9-month-old spontaneously hypertensive, and heart failure-prone rats infused ip with 1.5 pmol/kg · min GLP-1 for 3 months is associated with improved LV function, increased survival, and an approximately 50% increase in circulating adiponectin levels (109). Consistent with these findings, exendin-4 (2.5–5 nM) directly increased adiponectin mRNA expression in 3T3-L1 adipocytes, an effect blocked by the GLP-1R antagonist exendin (9–39) or the PKA inhibitor H89 (110). Weight loss arising from therapy with GLP-1R agonists is also associated with reductions in dyslipidemia (111). Hence, attribution of direct *vs.* indirect effects to the cardiovascular actions of GLP-1 may be difficult and requires careful analyses.

E. Cardiovascular biology of GLP-1 (9–36)

GLP-1 (9–36) is the primary GLP-1 metabolite *in vivo*, and circulating levels of GLP-1 (9–36) are greater than those of intact bioactive GLP-1 (34, 112). GLP-1 (9–36) binds to the classical GLP-1R with low affinity and at pharmacological doses acts as a weak competitive antagonist of the β-cell and gastrointestinal tract GLP-1R *in vivo*. However, iv administration of GLP-1 (9–36) in combination with glucose has no effect on insulin secretion, glucose elimination, or insulin-independent glucose disposal in either wild-type or *Glp1r*$^{-/-}$ mice (113). In contrast, GLP-1 (9–36) enhanced insulin-independent glucose clearance in anesthetized pigs (114). Similarly, infusion of GLP-1 (9–36) into healthy fasted humans in conjunction with a test meal significantly reduced postprandial glycemia, independently of changes in insulin or glucagon secretion or gastric emptying (115). The magnitude of these effects was minor in comparison with those of native GLP-1. Moreover, a separate study in healthy human subjects found that GLP-1 (9–36) infusion had no direct effect on glucose tolerance, insulin secretion or sensitivity, or GLP-1 action (116). Furthermore, simultaneous infusion of GLP-1 (9–36) with GLP-1 (7–36) did not alter the magnitude of GLP-1 (7–36)-stimulated insulin secretion (116). Intriguingly, infusion of GLP-1 (9–36) had no effect on insulin sensitivity in lean subjects but suppressed hepatic glucose production, in association with increased plasma insulin levels, in obese subjects (117). The complexity of GLP-1 (9–36) action is underscored by studies demonstrating that a secondary cleavage product derived from GLP-1 (9–36), GLP-1 (28–36), localizes to mitochondria and also reduces hepatic glucose production in hepatocytes (118). It is important to note that the actions of both GLP-1 (9–36) and GLP-1 (28–36) appear at pharmacological concentrations and are most prominent in stressed metabolic states such as insulin resistance (118, 119). Hence, further research is required to ascertain the biological relevance of GLP-1 (9–36) and GLP-1 (28–36) in the human endocrine and cardiovascular system.

1. GLP-1 (9–36) action in cardiomyocytes

GLP-1 (9–36) improved the viability of both *Glp1r*$^{+/+}$ and *Glp1r*$^{-/-}$ mouse neonatal cardiomyocytes during reoxygenation after 48 h hypoxia; preincubation with 0.3 nM GLP-1 (9–36) for 20 min before exposure to hydrogen peroxide for 7 h also improved cell viability (42). Unexpectedly, the actions of GLP-1 (9–36) were blocked by pretreatment with the classical GLP-1R antagonist exendin (9–39). In contrast, exendin-4 (3 nM) had no effect on cell viability in hypoxic *Glp1r*$^{-/-}$ neonatal myocytes subjected to reoxygenation or in cardiomyocytes treated with hydrogen peroxide (42).

2. GLP-1 (9–36) activity in the isolated heart and in conscious animals

A 48-h continuous infusion of GLP-1 (9–36) (1.5 pmol/kg · min) in conscious dogs with pacing-induced dilated cardiomyopathy significantly reduced LV end diastolic pressure, and increased LV contractility and myocardial glucose uptake during a hyperinsulinemic-euglycemic clamp (120). Consistent with the hypothesis that GLP-1 (9–36) exerts its actions through a separate receptor, pretreatment with native GLP-1 (0.3 nM) enhanced the recovery of LVDP during reperfusion after 30 min global ischemia in hearts from *Glp1r*$^{-/-}$ mice (37). Notably, prevention of the formation of GLP-1 (9–36) with the DPP-4 inhibitor sitagliptin prevented the recovery of LVDP during reperfusion in hearts from

$Glp1r^{-/-}$ mice. Interestingly, exendin-4 (5 nM) also modestly improved the recovery of LVDP during reperfusion after 30 min global ischemia in $Glp1r^{-/-}$ mice (37), adding further support for a second receptor capable of recognizing GLP-1R agonists in the heart. Related studies demonstrated that 0.03–3 nM GLP-1 (9–36) added to the perfusate for the first 15 min of a 120-min reperfusion period after a 45-min ischemic insult in isolated rat hearts resulted in a significant improvement in LVDP, although it did not reduce infarct size (121). However, follow-up studies from this same group could not reproduce these findings, demonstrating that native GLP-1, but not GLP-1 (9–36), was cardioprotective in isolated ischemic rat hearts *ex vivo* (122). Although pharmacological levels of GLP-1 (9–36) are cardioprotective, results using DPP-4 inhibitors, which markedly lower levels of GLP-1 (9–36), demonstrate significant cardioprotection after DPP-4 inhibition in both normal and diabetic rodent models and in short-term human studies (123–125).

IV. GLP-1 and Cardiovascular Disease

A. GLP-1 and hypertension

1. Animal studies

Although acute GLP-1R activation increases HR and BP in rodents, GLP-1 actions on endothelial cells, such as increased NO production (Fig. 3), would be predicted to be antihypertensive (54). Indeed, *db/db* mice chronically treated with exendin-4 (ip 20 nmol/kg twice daily) for 12 wk displayed a marked reduction in SBP; exendin-4 also attenuated the increase in SBP (~10 *vs.* ~25 mm Hg increase) in *db/db* mice provided with 2% salt in their drinking water for 2 wk (126). In male C57BL/6J mice infused with angiotensin II (1 μg/kg · min for 2 wk), twice-daily exendin-4 (20 nmol/kg ip) also reduced SBP (126). A 4-h treatment with exendin-4 (10 nM) prevented acute angiotensin II-induced ERK phosphorylation in kidney proximal tubular cells, raising the possibility that GLP-1R signaling directly modifies the actions of exogenous angiotensin II (126). A 7-d sc infusion of exendin-4 (1 μg/kg · d) via osmotic pumps also reversed corticosterone-induced increases in SBP and DBP in rats independent of changes in body weight and caloric intake (127). Hence, although acute GLP-1R activation transiently increases BP in preclinical studies, sustained GLP-1R activation reduces BP in different animal models.

2. Studies in subjects with diabetes and/or heart disease

Infusion of GLP-1 (0.7 pmol/kg · min) for 48 h in 15 nondiabetic human subjects with heart failure (New York Heart Association class II/III) resulted in small increases in HR (67 ± 2 *vs.* 65 ± 2 beats/min) and DBP (71 ± 2 *vs.* 68 ± 2 mm Hg) (128). Exenatide administered twice daily to diabetic subjects for 12 wk produced small (~2 beats/min) but nonsignificant changes in HR, and a trend toward a reduction in SBP, associated with a modest weight loss of 1.8 kg (129). In a 26-wk head-to-head study of liraglutide *vs.* sitagliptin in subjects with T2DM, HR increased with liraglutide (3.94 beats/min) but not with sitagliptin, and reductions in SBP and DBP were actually greater with sitagliptin compared with liraglutide (130). A greater reduction in DBP with sitagliptin was also observed after 1 yr of treatment in comparison with liraglutide (131), and BP reductions were also modestly greater with sitagliptin compared with exenatide once weekly in the Results from the Diabetes Therapy Utilization: Researching Changes in A1C, Weight and Other Factors Through Intervention with Exenatide Once Weekly (DURATION-4) study (132). Nevertheless, acute administration of the high-molecular-weight exendin-transferrin fusion protein in subjects with T2DM produced significant increases in HR and DBP (mean increase of 10 mm Hg) (133). Hence, further analysis of the effects of structurally distinct GLP-1R agonists on HR and BP in diabetic subjects is warranted.

Nevertheless, the majority of clinical trials investigating the antidiabetic actions of GLP-1R agonists have reported reductions in BP. Results from the DURATION-1 trial demonstrated that patients treated with exenatide continuously for 52 wk had a significant reduction in SBP (134). Approximately 50% of patients with a SBP of at least 130 mm Hg at baseline achieved a normal SBP by wk 52. Consistent with these findings, 314 overweight patients receiving exenatide 10 μg twice daily for 82 wk also experienced improvement in both SBP and DBP (135), and a retrospective analysis of 6280 patients reported a significant reduction in both SBP and DBP with exenatide therapy (111). Similarly, combination therapy with daily liraglutide (0.6, 1.2, or 1.8 mg)/metformin (2000 mg) for 16 wk in 928 Asian subjects with T2DM reduced SBP (>3 mm Hg decrease) in comparison with glimepiride (4 mg)/metformin (2000 mg) administration (136). A retrospective analysis of 110 patients with T2DM treated with liraglutide (0.6, 1.2, or 1.8 mg daily) for a mean duration of 7.5 months (range, 6 months to 1.1 yr) demonstrated a reduction in SBP (5 ± 2 mm Hg) in the first 3 months of treatment (137). In an analysis encompassing three randomized phase 3 trials totaling 2665 patients, 26 wk treatment with once-daily liraglutide (1.2 or 1.8 mg) in combination with metformin, glimepiride, or metformin and rosiglitazone reduced SBP by 2.29–6.71 mm Hg (138). Furthermore, a study of 268 obese nondiabetic patients who completed a 20-wk treatment with once-daily sc liraglutide (1.2, 1.8, 2.4, or 3.0 mg) followed by a nonblinded 2-yr extension (final dose of 3.0 mg) demonstrated a mean 4.6 mm Hg decrease in SBP (139). The improvement in BP in the majority of these studies was associated with reductions in body weight. Nevertheless, the reductions in SBP with liraglutide appear rapidly, often before significant weight loss is observed (136, 138).

B. GLP-1 action in experimental models of atherosclerosis

Both direct and indirect actions of GLP-1 may contribute to the potential reduction of atherogenesis (Fig. 3). GLP-1R has been localized to mouse aortic smooth muscle and endothelial cells, as well as monocytes and macrophages, using immunocytochemistry and Western blotting (140). Continuous infusion of exendin-4 (300 pmol/kg · d or 24 nmol/kg · d) in nondiabetic C57BL/6 and *ApoE*$^{-/-}$ mice reduced monocyte adhesion to aortic endothelial cells at 24 d, associated with a reduction in atherosclerotic lesion size after 28 d treatment. Furthermore, treatment for 1 h with exendin-4 (0.03–3 nM) reduced levels of mRNA transcripts for the inflammatory markers monocyte chemoattractant protein-1 and TNFα in response to 1 h lipopolysaccharide (1 μg/kg) in cultured peritoneal macrophages harvested from mice 3 d after injection of 3% thioglycolate (140). Continuous infusion of exendin-4 (24 nmol/kg · d) for 4 wk in C57BL/6 mice also reduced neointimal formation in response to endothelial denudation of the femoral artery (141). Intriguingly, 12 h pretreatment with 10 nM exendin-4 reduced platelet-derived growth factor-induced (25 ng/ml) bromodeoxyuridine incorporation into DNA of primary cultured mouse aortic smooth muscle cells (141). Nagashima *et al.* (142) reported that continuous GLP-1 or GIP administration (4 wk osmotic mini-pump infusion of 2.2 nmol/kg · d) prevented atherosclerotic lesion development in *ApoE*$^{-/-}$ mice, which may involve reduced foam cell formation in macrophages, because peritoneal macrophages harvested from exendin-4-treated mice exhibited reduced CD36 protein expression and decreased cholesterol ester accumulation after 18 h exposure to 10 μg/ml oxidized LDL. Despite these intriguing findings in animals, data on the long-term effects of incretin-based therapy on atherosclerosis-associated outcomes in diabetic humans is not yet available.

C. GLP-1R activation in ischemic heart disease

1. Animal studies

Multiple preclinical studies have demonstrated cardioprotective effects of native GLP-1 and GLP-1R agonists in experimental models of ischemic heart disease (37, 45, 48, 143, 144). Both GLP-1 and exendin-4 improved recovery of LVDP in isolated perfused rat and mouse hearts during reperfusion after ischemia (37, 48, 121). Similarly, iv infusion of 4.8 pmol/kg · min GLP-1 decreased infarct size after 30 min ischemia induced by temporary occlusion of the left anterior descending (LAD) coronary artery in rats (143), and exendin-4 (10 μg iv and sc 5 min before the onset of reperfusion) decreased infarct size and improved LV systolic function 72 h following 75 min LAD coronary artery occlusion in pigs (145). In contrast, recombinant GLP-1 administered via the jugular vein at 3 pmol/kg · min 15 min before the onset of ischemia failed to reduce infarct size in a pig model of ischemia secondary to 60 min left circumflex coronary artery occlusion (146). Furthermore, liraglutide (10 μg/kg) administered to pigs for 3 d before LAD coronary artery occlusion did not reduce infarct size or improve LV function (147). The GLP-1R agonist albiglutide, injected sc for 3 d at 3 or 10 mg/kg · d, reduced infarct size assessed 24 h after 30 min temporary LAD coronary artery occlusion in normoglycemic rats (148). The benefit of albiglutide was attributed to improved cardiac energetics, because *in vivo* ^{13}C nuclear magnetic resonance studies demonstrated decreased fatty acid oxidation and increased glucose oxidation rates.

A GLP-1-transferrin protein also limited infarct size after 30 min LAD coronary artery occlusion in rabbits, whether given sc at a dose of 10 mg/kg 12 h before the onset of ischemia or iv at the onset of ischemia (149). Furthermore, liraglutide administration (75 μg/kg ip twice daily) for 1 wk before LAD occlusion improved survival and cardiac output assessed 4 wk after permanent occlusion of the LAD coronary artery in both nondiabetic and diabetic male mice (45). Interestingly, 30 nM liraglutide administered in coronary arteries at the onset of reperfusion did not protect against reperfusion injury in isolated perfused mouse hearts after 30 min of global ischemia, but liraglutide did improve recovery of LVDP during reperfusion if injected into the mouse *in vivo* before ischemia/reperfusion *ex vivo*. This observation suggests that liraglutide may achieve cardioprotection in part through mechanisms requiring the heart to receive its normal neural, humoral, and vascular input. Furthermore, the observations that GLP-1R activation does not universally produce cardioprotection in preclinical studies highlights the importance of future studies designed to understand the precise biological mechanisms and cellular sites of action for different GLP-1R agonists in the cardiovascular system.

2. Studies in subjects with ischemic heart disease

The observation that a 72-h infusion of GLP-1 (1.5 pmol/kg · min) initiated approximately 3.5 h after angioplasty within approximately 6.5 h from symptom onset in patients with AMI enhanced LV ejection fraction (LVEF) (29 ± 2 *vs.* 39 ± 2%) and infarct zone-related regional wall motion engendered considerable interest in the cardioprotective actions of GLP-1 in humans (150). However, this report was a single-center nonrandomized pilot study in a small number of patients (n = 10). Nevertheless, subsequent studies have confirmed that GLP-1 may be cardioprotective. An iv infusion of GLP-1 (1.2 pmol/kg · min) 30 min before dobutamine stress echocardiography and continuing for 30 min into recovery in 14 patients (four with T2DM) with known coronary artery disease, protected against LV dysfunction, and mitigated postischemic myocardial stunning (151). These same investigators demonstrated a reduction in LV dysfunction and myocardial stunning during dual inflation coronary balloon occlusion in 20 nondiabetic patients with single-vessel disease in the LAD coronary artery, in whom GLP-1 was infused at 1.2 pmol/kg · min after completion of the first balloon occlusion (152). A larger randomized, double-blind, placebo-controlled trial investigating the effects of a 6-h exenatide infusion initiated 15 min before onset of reperfusion in 172 patients undergoing primary angioplasty to treat ST-segment elevation MI also demonstrated a reduction in the ischemic area at risk (153). Exenatide was infused to achieve a target plasma concentration from 0.03–0.3 nM (mean concentration 0.177 ± 0.069 nM) and reduced reperfusion injury in these patients as determined by an increase in myocardial salvage index and decrease in infarct size relative to the ischemic area at risk assessed by cardiac magnetic resonance at approximately 90 d after infusion. However, mortality and LV contractility were not different in patients receiving exenatide (153). Although accumulating data on GLP-1 action and cardiovascular function in humans with ischemic heart disease appears promising with respect to safety and potential benefit, much larger double-blinded randomized trials are necessary to determine whether GLP-1R agonists are cardioprotective in a wide range of subjects with T2DM.

D. GLP-1R agonists in heart failure

1. Preclinical models of heart failure

Studies in animals illustrate that GLP-1R activation may produce beneficial effects on the failing heart (49, 154). In a dog model of 28-d rapid pacing-induced heart failure, a 48-h infusion of GLP-1 (1.5 pmol/kg · min) exerted insulinomimetic properties on the heart, increasing glucose uptake during a hyperinsulinemic-euglycemic clamp (49). Furthermore, GLP-1 decreased HR and increased LV systolic function, and decreased plasma levels of norepinephrine and glucagon. In the spontaneously hypertensive and heart failure-prone rat, a 3-month ip infusion of 1.5 pmol/kg · min GLP-1 improved survival and preserved LV contractile function, an effect associated with reduced cardiomyocyte apoptosis (109). Similarly, liraglutide administered for 1 wk (75 μg/kg ip twice daily) to mice before permanent occlusion of the LAD coronary artery reduced cardiac hypertrophy, decreased LV structural remodeling, and improved cardiac output 4 wk after induction of ischemia and LV dysfunction (45). In a rat model of chronic heart failure, an 11-wk sc infusion of GLP-1 (2.5 or 25 pmol/kg · min) or the exenatide analog AC3174 (1.7 or 5 pmol/kg · min) 2 wk after permanent LAD coronary artery occlusion significantly enhanced LV function (increased LVEF and fractional shortening), while also reducing adverse LV remodeling (decreased LV end systolic and diastolic dimensions) and improving survival (155). Taken together, the available data support a beneficial role for both native GLP-1 and GLP-1R agonists in preclinical models of heart failure.

2. Studies in human subjects with heart failure

Initial trials in humans demonstrated salutary effects of GLP-1 in subjects with heart failure; a 5-wk infusion of GLP-1 (2.5 pmol/kg · min) in 12 patients with New York Heart Association class III/IV heart failure improved LVEF, oxygen consumption, and 6-min walk test scores (154). However, this was a single-center nonrandomized trial, whose results require replication. A 48-h infusion of GLP-1 (0.7 pmol/kg · min) in 15 humans with congestive heart failure but without diabetes produced no beneficial effects on LV function and actually resulted in minor increases in HR (2 beats/min) and DBP (3 mm Hg) (128). Although this particular study was randomized and double-blinded, a brief duration of GLP-1 infusion may be insufficient to increase function in a decompensated failing heart.

V. DPP-4 Inhibition and Cardiovascular Function

This section will discuss the cardiovascular biology of DPP-4 and actions of DPP-4 inhibitors in the regulation of BP, the development of atherosclerosis, the setting of AMI, and the failing heart, illustrating differences between DPP-4 inhibitors and GLP-1R agonists where appropriate.

A. DPP-4 expression in the cardiovascular system

DPP-4 is a widely expressed enzyme and has been localized to smooth muscle and endothelial cells in different species (156, 157). Short-term exposure to high glucose induces DPP-4 activity in microvascular endothelial cells (158). Although the precise biological role of DPP-4 in the cardiomyocyte, endothelial, or coronary smooth muscle cell requires further study, DPP-4 is also a circulating protein, and thus DPP-4 activity in the systemic and coronary circulation may influence intact levels of GLP-1 and other vasoactive DPP-4 substrates reaching the myocardium

Figure 5.

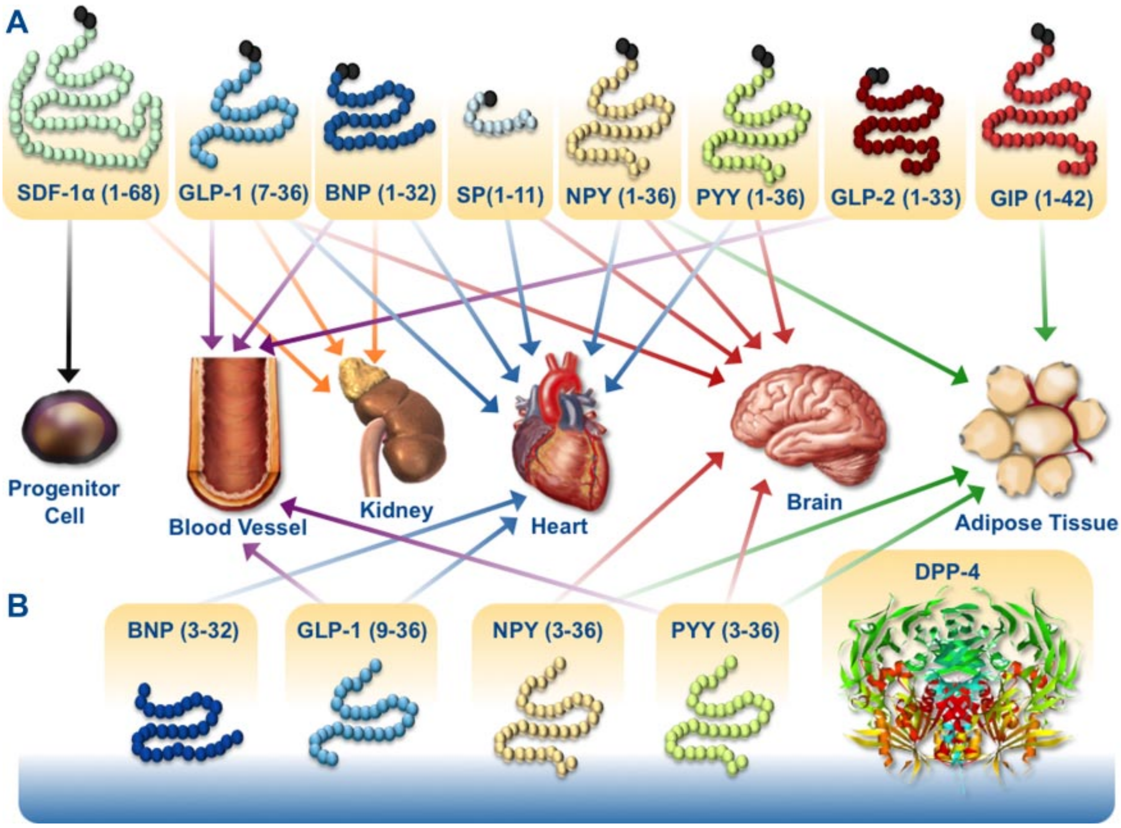

Figure 5. DPP-4 substrates that directly or indirectly regulate cardiovascular function. Multiple DPP-4 substrates have been identified that act on multiple peripheral tissues that influence the cardiovascular system. For a summary of these direct effects on target tissues, refer to Table 1. SP, Substance P.

and vasculature (Fig. 5). The soluble form of DPP-4 may also interact with the mannose 6-phosphate receptor on human endothelial cells, promoting endothelial cell transmigration of T cells (159).

B. Potential cardioactive DPP-4 substrates

Although GLP-1 is classically viewed as the primary DPP-4 substrate capable of influencing cardiovascular function, DPP-4 cleaves multiple peptides, many of which also have direct actions on the heart and blood vessels (Table 1 and Fig. 5), as discussed below in *Section V.B.1*.

1. Glucose-dependent insulinotropic polypeptide

The GIPR has been detected by immunohistochemistry in both rat atrial and ventricular tissue (25). Although the biological function, if any, of GIP in the heart is unknown, studies in rodents have linked GIPR activation to increased adipogenesis, enhanced adipokine expression, and obesity (160–163). Hence, DPP-4-inhibition and the subsequent increase in plasma levels of intact bioactive GIP may have potential direct or indirect effects on the heart. The GIPR is also expressed in endothelial cells (164), and GIPR activation promotes endothelial cell proliferation in an endothelin-1-dependent manner in HUVEC cultures (165). The observation that GIP activates different signal transduction pathways in endothelial cells isolated from the hepatic artery *vs.* the portal vein further highlights the need for more investigation of the actions of GIP in different vascular beds (166). GIPR protein has also been detected in mouse aortic smooth muscle cells and peritoneal macrophages extracted from $ApoE^{-/-}$ mice (142). Moreover, administration of native GIP (25 nmol/kg · d) via continuous infusion with osmotic mini-pumps prevented atherosclerotic lesion development and macrophage infiltration in the aortic wall of $ApoE^{-/-}$ mice (142). Because modulation of GIPR activity is being pursued for the treatment of diabetes and perhaps obesity (24), and DPP-4 inhibitors prevent the degradation of biologically active levels of intact GIP, a greater understanding of GIP action in the cardiovascular system seems prudent.

2. Stromal cell-derived factor-1α (SDF-1α)

SDF-1α is a 7.97-kDa chemokine secreted from multiple cell types that promotes homing of endothelial pro-

TABLE 1. DPP-4 cardioactive substrates and their effects on peripheral tissues that influence the cardiovascular system

Peptide	Target tissue	Effect	Ref.
GLP-1 (7-36)	Heart	Increased cardiac function, glucose uptake, decreased contractility, apoptosis	42, 48, 49, 120
	Blood vessel	Increased NO production, decreased inflammation	37, 46, 47, 52–54, 56
	Brain	Decreased appetite	103–105
GLP-1 (9-36)	Heart	Increased cardiac function, glucose uptake, decreased apoptosis	37, 42, 120
	Blood vessel	Increased vasodilation	37
GIP (1-42)	Adipose tissue	Increased lipogenesis and adipogenesis	160–163
GLP-2 (1-33)	Blood vessel	Increased blood flow, HR, and BP	192
SDF-1 (1-68)	Progenitor cell	Increased homing of progenitor cells to ischemic myocardium, increased angiogenesis	167–171
BNP (1-32)	Heart	Decreased LV remodeling	189
	Blood vessel	Increased vasodilation	224
	Kidney	Increased natriuresis	224
BNP (3-32)	Kidney	Increased natriuresis	224
SP (1-11)	Heart	Decreased chronotropy and inotropy	196
	Brain	Altered cardiac adrenergic tone	225
NPY (1-36)	Heart	Increased $[Ca^{2+}]_i$ current	179, 180
	Brain	Increased appetite	226
	Adipose tissue	Increased adipocyte differentiation, decreased lipolysis	227, 228
NPY (3-36)	Adipose tissue	Increased lipogenesis	183
	Blood vessel	Increased angiogenesis	182
PYY (1-36)	Blood vessel	Increased collateral blood flow	229, 230
	Adipose tissue	Decreased lipolysis	

*SP, Substance P.

genitor cells to sites of cellular injury. It plays an important role in myelopoiesis and development of the embryonic heart, as well as in the homing of hematopoietic stem cells and neural progenitors during embryonic development (167, 168). SDF-1 is essential for the migration of endogenous and transplanted stem cells in rodents and humans and promotes healing of injured blood vessels and myocardium (169–171). For example, SDF-1 is secreted from endothelial cells in ischemic tissue in response to activation of hypoxia-inducible factor-1, promoting migration and homing of C-X-C chemokine receptor type 4-positive progenitor cells to ischemic tissue (172). Because SDF-1 is subject to inactivation via either DPP-4- or matrix metalloproteinase-mediated cleavage (169, 173), DPP-4 inhibitors have been used to enhance SDF-1 activity and increase stem cell number in both preclinical and clinical studies of cardiovascular injury (169, 174, 175).

3. Neuropeptide Y (NPY)

NPY is a 36-amino-acid neuropeptide with potent orexigenic properties, increasing appetite and food intake. Cleavage of NPY (1–36) to NPY (3–36) by DPP-4 changes the receptor preference and biological activity of the NPY system (176). NPY receptors have been detected in cardiomyocytes and blood vessels (177, 178). Hence, inhibition of DPP-4-mediated NPY cleavage may have a number of effects on the myocardium, including modulation of ion currents (179, 180) and induction of local coronary artery vasoconstriction (181). Y also exerts potent angiogenic actions mediated by both Y1 and Y2 receptors, and generation of NPY (3–36) by DPP-4 enhances the angiogenic activity of NPY via the Y2 receptor (182). Furthermore activation of the Y2 receptor stimulates fat angiogenesis, macrophage infiltration, and the proliferation and differentiation of adipocytes, promoting abdominal obesity and a metabolic syndrome-like condition in preclinical studies (183). Because both NPY and NPY (3–36) appear to influence the cardiovascular system via different NPY receptor subtypes, DPP-4-mediated control of the ratio of NPY (1–36) to (3–36) may have potential implications for regulation of blood flow, BP, cardiomyocyte signal transduction, adiposity, and inflammation, (184).

4. Peptide YY (PYY)

PYY is a 36-amino-acid peptide released from L cells located predominantly in the ileum and colon. PYY and its DPP-4-mediated cleavage product PYY (3–36) are both agonists for NPY receptors. Preventing DPP-4-mediated PYY cleavage has been shown to enhance angiotensin II-induced vasoconstriction of isolated perfused kidneys from spontaneously hypertensive rats (185), hence the potential effects of PYY on cardiac function require further study.

5. B-type (brain) natriuretic peptide (BNP)

BNP is a 32-amino-acid peptide secreted from the ventricle in response to increased myocyte stretch and/or volume overload (186, 187) and is used as a diagnostic marker for acute heart failure. BNP is cleaved by both purified human DPP-4 and endogenous DPP-4 present in human plasma (188). BNP binds to natriuretic peptide receptors that are linked to activation of guanylyl cyclase and subsequent cGMP production, inducing arterial and venous vasodilation. Furthermore, direct intramyocardial injection of a human BNP-expressing adenovirus before permanent occlusion of the LAD coronary artery or after infusion of angiotensin II improves LV function and reduces adverse LV remodeling in rats (189). Recombinant human BNP, nesiritide, has been investigated for the management of acute decompensated heart failure; however, the Acute Study of Clinical Effectiveness of Nesiritide in Decompensated Heart Failure Trial suggests that nesiritide does not improve clinical outcomes (190). Both BNP (1–32) and BNP (3–32) produce natriuretic and vasodilatory actions, hence the extent to which reduction of DPP-4 activity influences BP and LV function through modulation of BNP in diabetic subjects requires further study.

6. Glucagon-like peptide-2

GLP-2 is a 33-amino-acid DPP-4-sensitive peptide (191) coencoded together with glucagon and GLP-1 in the proglucagon gene. GLP-2 administration in humans increases mesenteric artery blood flow, which may result in a compensatory increase in HR and cardiac output (192). An immunoreactive GLP-2 receptor protein has been identified in rat heart ventricles via Western blotting, and GLP-2 treatment of Langendorff aerobic-perfused rat hearts increased contractility at 10^{-12} M concentrations but decreased contractility at escalating concentrations (10^{-10} to 10^{-7} M) (193). In contrast, Northern blot and RT-PCR analyses failed to detect the GLP-2R in mouse and rat heart, respectively (194), hence the mechanisms through which GLP-2 regulates cardiovascular function require additional scrutiny.

7. Other cardioactive DPP-4 substrates

DPP-4 cleaves substance P (195), and exogenous substance P administration exerts negative chronotropic and inotropic effects in Langendorff perfused guinea pig hearts (196). Substance P may also modulate adrenergic activity in the heart. Bradykinin is cleaved in part by DPP-4 and predominantly by aminopeptidase P. Whether DPP-4 inhibition significantly modulates bradykinin activity, thereby potentially contributing to the pathophysiology of angiotensin-converting enzyme inhibitor-associated angioedema, requires further study (197). Because plasma levels of various DPP-4 substrates are often low and difficult to quantify, delineating the contributions of different substrates to changes observed in cardiovascular biology pursuant to DPP-4 inhibition remains challenging.

C. DPP-4 in hypertension

1. DPP-4 inhibition and BP control in animals

Analysis of 20-wk-old Zucker diabetic fatty rats orally gavaged with sitagliptin (10 mg/kg · d) for 6 wk revealed significant reductions in SBP (~25 mm Hg decrease) and DBP (~18 mm Hg decrease) (198). Furthermore, 5-wk-old male spontaneously hypertensive rats treated with sitagliptin (40 mg/kg by gavage) for 8 d demonstrated significant reductions in both SBP (~26 mm Hg decrease) and DBP (~16 mm Hg decrease), although no effect on BP was observed in 14-wk-old rats (199). The mechanisms through which DPP-4 inhibition lowered BP in these studies remain unclear.

2. DPP-4 inhibition and BP control in humans

Sitagliptin (50 or 100 mg twice daily for 5 d) reduced both SBP and DBP in 19 nondiabetic patients with mild to moderate hypertension (200). Administration of 50 mg sitagliptin every other day for 6 months in 17 hypertensive Japanese patients with T2DM resulted in a significant reduction in SBP evident after only 1 month of treatment (201). Patients treated with sitagliptin (100 mg/d) for 26 wk in the DURATION-2 or DURATION-4 trials also demonstrated small reductions in SBP independent of changes in body weight (132, 202). Similarly, sitagliptin-treated patients experienced a small reduction in SBP and DBP over 26 wk on a background of metformin therapy (130).

D. DPP-4 in atherosclerosis

Limited information is available on the effects of DPP-4 inhibitors in the development of atherosclerosis. Treatment of high-fat-fed LDL receptor-deficient mice with alogliptin (40 mg/kg · d) for 12 wk resulted in reduction of atherosclerosis (203). Furthermore, alogliptin reduced levels of plasma cholesterol, TAG, SBP (~5 mm Hg decrease), adiposity, proinflammatory $CD11b^+/CD11c^+$ adipose tissue macrophages, atherosclerotic plaque area, plaque collagen content, and proinflammatory $CD11b^+/CD206^+$ macrophages in the plaque (203).

E. DPP-4 in ischemic heart disease

Studies in rats treated with valine pyrrolidide (20 mg/kg) demonstrated that DPP-4 inhibition had no impact on infarct size after LAD coronary artery occlusion *in vivo* or ischemia/reperfusion *ex vivo* (143). Similarly, acute DPP-4 inhibition with sitagliptin *ex vivo* conferred no benefit against ischemia/reperfusion injury in perfused Langendorff mouse hearts (123). In contrast, acute administration of sitagliptin *in vivo* to mice improved the recovery of LVDP in hearts subsequently subjected to *ex vivo* ischemia/reperfusion. Furthermore, normoglycemic $Dpp4^{-/-}$ mice exhibited significantly improved survival and reduced infarct size after permanent LAD coronary artery occlusion *in vivo*. Moreover, diabetic mice treated with sitagliptin for 12 wk exhibited improved survival after LAD coronary artery ligation, and 7 d of sitagliptin administration (250 mg/kg · d) induced a cardioprotective gene expression profile in murine heart tissue (123). Pretreatment with sitagliptin for 3 or 14 d in nondiabetic mice or rats also reduced infarct size after induction of experimental ischemia through mechanisms sensitive to PKA inhibition (124). Consistent with these findings, pretreatment of normoglycemic rats for several days with linagliptin reduced infarct size induced by 30 min transient ischemia, without significant changes in parameters of ventricular performance (204). Administration of the DPP-4 inhibitor PFK275–055 (10 mg/kg · d) for 4 wk to obese nondiabetic insulin-resistant Wistar rats decreased infarct size but, interestingly, had no impact on aortic output or coronary flow (205). Taken together, the majority of preclinical studies demonstrate cardioprotection after genetic or pharmacological reduction of DPP-4 activity in young rodents.

DPP-4 inhibition likely results in cardioprotection through both GLP-1R-dependent and -independent mechanisms and may protect against ischemic injury in mice by increasing angiogenesis and subsequent blood supply to the ischemic myocardium (169). Considerable evidence supports a role for SDF-1α, a potent chemoattractant of stem/progenitor cells, as a cardioactive DPP-4 substrate. Treatment of wild-type mice with the DPP-4 inhibitor diprotin A (70 μg/kg twice daily) and granulocyte colony-stimulating factor (G-CSF, 100 μg/kg · d ip) for up to 6 d immediately after permanent LAD coronary artery occlusion improved cardiovascular outcomes at 30 d after MI (169). These included decreased infarct size, LV wall thinning, end diastolic volume, and enhanced survival, LVEF, and neovascularization as indicated via increased $CD31^+$ capillaries at the infarct border zone. Identical findings of improved survival, cardiac function, and ventricular remodeling were observed in G-CSF-treated $Dpp4^{-/-}$ mice (169). Furthermore, inhibition of the SDF-1/C-X-C chemokine receptor type 4 axis using the antagonist AMD3100 substantially attenuated the benefits of diprotin A/G-CSF administration in the murine LAD coronary artery occlusion model (206). Follow-up studies demonstrated that combined diprotin A and G-CSF (100 μg/kg · d ip) also improved outcomes after LAD coronary artery occlusion in mice with cardiac-specific overexpression of cyclin D2 (α myosin heavy chain-cycD2) (207).

Treatment of rats with a bioengineered matrix metalloproteinase and DPP-4-resistant SDF-1 led to a significant improvement in angiogenesis and ventricular function after a 3-h ischemic insult induced via LAD coronary artery occlusion (208). The Sitagliptin Plus Granulocyte-Colony Stimulating Factor in Patients Suffering from Acute Myocardial Infarction (SITAGRAMI) trial in humans is examining the feasibility and potential clinical utility of DPP-4 inhibition and G-CSF administration to improve myocardial function in patients pursuant to an AMI and revascularization (175).

F. DPP-4 in heart failure and cardiomyopathy

There is limited information on whether DPP-4 inhibition modifies ventricular function in the failing heart. Administration of sitagliptin, 30 mg/kg, once daily for 3 wk to normoglycemic pigs with pacing-induced heart failure, resulted in reduced HR, increased stroke volume, and preservation of glomerular filtration rate (209). In contrast, administration of vildagliptin to nondiabetic rats either before or after LAD ligation and development of ischemic cardiomyopathy had no beneficial effects on parameters of LV function or cardiac gene expression (210). Hence, whether DPP-4 inhibition directly impacts the development or progression of heart failure in animals or humans independent of its actions to reduce infarct size requires further investigation.

Studies in 6-wk-old *db/db* mice treated with sitagliptin (16 mg/kg) for 4 wk had multiple metabolic effects on the myocardium, including reductions in AMPK and acetyl CoA carboxylase phosphorylation and decreased CD36 protein expression at the sarcolemmal membrane, suggesting that DPP-4 inhibition reduces myocardial fatty acid uptake and subsequent fatty acid metabolism (211). Sitagliptin did not improve systolic function in *db/db* mice but did reduce myocardial fibrosis and improved the LV relaxation constant, indicative of improved diastolic function. Furthermore, *db/db* mice treated with sitagliptin also demonstrated reduced myocardial p53 expression, suggestive of reduced cardiomyocyte apoptosis, although whether this reduction would prevent death of cardiomyocytes with prolonged aging and the development of heart failure in this animal model was not determined.

VI. Clinical Trials

A. GLP-1R agonists and cardiovascular outcomes

The majority of GLP-1R agonists are undergoing assessment in large, multicenter clinical trials of cardiovascular outcomes (Table 2) (www.clinicaltrials.gov) (212). The cardiovascular safety of the GLP-1R agonist exenatide is being studied in the Exenatide Study of Cardiovascular Event Lowering Trial (EXSCEL), a double-blind randomized trial investigating the time to first confirmed cardiovascular event in patients treated with once-weekly exenatide (2 mg). Liraglutide is being assessed in the Li-

TABLE 2. GLP-1R agonist and DPP-4 inhibitor cardiovascular outcomes trials

Drug	Study	Dose	Primary outcome	No. of patients
GLP-1R agonists				
Exenatide	Exenatide Study of Cardiovascular Event Lowering Trial (EXSCEL): a trial to evaluate cardiovascular outcomes after treatment with exenatide once weekly in patients with T2DM	2.0 mg injected sc once weekly	Time to first confirmed cardiovascular event	~9,500
Liraglutide	Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results—A Long-Term Evaluation (LEADER)	Maximum dose 1.8 mg/d injected sc	Time from randomization to first occurrence of nonfatal MI, nonfatal stroke, or cardiovascular death	~8,750
Lixasenatide	Evaluation of Cardiovascular Outcomes in Patients with Type 2 Diabetes After Acute Coronary Syndrome During Treatment With AVE0010 (Lixisenatide) (ELIXA)	20 μg in 0.2 ml once a day injection 1 h before breakfast	Time to the first occurrence of nonfatal MI, nonfatal stroke, hospitalization for unstable angina, or cardiovascular death	~6,000
Dulaglutide	Researching Cardiovascular Events with a Weekly Incretin in Diabetes (REWIND)	1.5 mg injected sc once weekly	Time from randomization to first occurrence of nonfatal MI, nonfatal stroke, or cardiovascular death	~9,600
DPP-4 inhibitors				
Vildagliptin	Effect of Vildagliptin on Left Ventricular Function in Patients with Type 2 Diabetes and Congestive Heart Failure	50 mg twice daily	LV function as determined via changes in ejection fraction	~490
Sitagliptin	Sitagliptin Cardiovascular Outcome Study (0431–082 AM1) (TECOS)	50 or 100 mg/d oral tablet	Time to first confirmed cardiovascular event (nonfatal MI, nonfatal stroke, or hospitalization for unstable angina)	~14,000
Alogliptin	Cardiovascular Outcomes Study of Alogliptin in Subjects with Type 2 Diabetes and Acute Coronary Syndrome (EXAMINE)	6.25 or 12.5 or 25 mg/d oral tablet	Time from randomization to the first occurrence of a primary major adverse cardiac event (nonfatal MI, nonfatal stroke, or cardiovascular death)	~5,400
Saxagliptin	Does Saxagliptin Reduce the Risk of Cardiovascular Events When Used Alone or Added to Other Diabetes Medications (SAVOR-TIMI 53)	2.5 or 5 mg/d oral tablet	Time to first confirmed cardiovascular event (nonfatal MI, nonfatal ischemic stroke, or cardiovascular death)	~16,500
Linagliptin	CAROLINA: Cardiovascular Outcome Study of Linagliptin Versus Glimepiride in Patients With Type 2 Diabetes	5 mg/d oral tablet	Time to the first occurrence of nonfatal MI, nonfatal stroke, hospitalization for unstable angina, or cardiovascular death	~6,000

raglutide Effect and Action in Diabetes (LEADER) trial, a double-blind randomized clinical trial investigating the effects of once-daily liraglutide (maximum dose up to 1.8 mg) with a primary outcomes measure of time from randomization to first occurrence of cardiovascular death, nonfatal MI, or nonfatal stroke. Furthermore, the GLP-1R agonist lixisenatide is undergoing scrutiny in the Evaluation of Cardiovascular Outcomes in Patients with Type 2 Diabetes after Acute Coronary Syndrome during Treatment with Lixisenatide (ELIXA) trial, a double-blind randomized trial investigating whether lixisenatide can reduce cardiovascular mortality compared with placebo in type 2 diabetic patients who have recently experienced an acute coronary event. Dulaglutide is currently recruiting for the Researching Cardiovascular Events with a Weekly Incretin in Diabetes (REWIND) trial, which will determine the effect of once-weekly dulaglutide (1.5 mg) on major cardiovascular events in patients with T2DM. Patient enrollment in these trials ranges from 6,000–10,000.

B. DPP-4 inhibitors and cardiovascular outcomes

The majority of DPP-4 inhibitors are also being assessed in large, multicenter clinical trials for cardiovascular outcomes (Table 2) (www.clinicaltrials.gov). The cardiovascular safety of sitagliptin is being evaluated in The Sitagliptin Cardiovascular Outcome Study (TECOS), which is investigating the time to first confirmed cardiovascular event in type 2 diabetic patients being treated with once-daily sitagliptin (50 or 100 mg). The consequences of vildagliptin therapy are being assessed in the

Effect of Vildagliptin on Left Ventricular Function in Patients with Type 2 Diabetes and Congestive Heart Failure study, an ongoing trial evaluating vildagliptin's effect on LVEF in subjects with T2DM and congestive heart failure having a LVEF below 40%. Saxagliptin is under ongoing evaluation in the Does Saxagliptin Reduce the Risk of Cardiovascular Events When Used Alone or Added to Other Diabetes Medications (SAVOR-TIMI 53) study to determine whether once-daily saxagliptin (2.5 or 5 mg) affects cardiovascular mortality or nonfatal AMI or nonfatal ischemic stroke. An initial meta-analysis encompassing eight phase II/III trials of T2DM patients treated with saxagliptin suggested a possible reduction of cardiovascular events with saxagliptin; however, the event rate was extremely low, with only 40 events from a total of 4607 enrolled subjects (213). The Cardiovascular Outcomes Study of Alogliptin in Subjects with Type 2 Diabetes and Acute Coronary Syndrome (EXAMINE) is evaluating whether once daily alogliptin (6.25, 12.5, or 25 mg) affects the time from randomization to the occurrence of primary major adverse cardiac events (cardiovascular death, nonfatal MI, and nonfatal stroke). Linagliptin is being evaluated in the Cardiovascular Outcome Study of Linagliptin *Vs.* Glimepiride in Patients with Type 2 Diabetes (CAROLINA). This study compares daily linagliptin (5 mg) *vs.* glimepiride with regards to long-term impact on cardiovascular morbidity and mortality in patients with T2DM at elevated risk for cardiovascular disease. Patient enrollment in these trials ranges from 500 to as high as 16,500.

VII. Future Directions

Although considerable preclinical evidence supports a cardioprotective role for GLP-1R agonists and DPP-4 inhibitors, the majority of these studies are carried out in young healthy animals, without extensive atherosclerosis, diabetes, or preexisting heart disease. Whereas short-term studies of GLP-1R agonists and DPP-4 inhibitors demonstrate modest improvements in cardiac function under conditions of ischemia, there are no long-term data on whether these agents truly modify event rates in high-risk patients. Retrospective reviews of healthcare claims databases suggest potential benefits of GLP-1R agonists in the reduction of cardiovascular events and cardiovascular-related hospitalization (214); however, these studies are nonrandomized, the events are not always verified, and firm conclusions cannot be drawn from retrospective studies. The putative reduction in cardiovascular events observed in clinical trials of DPP-4 inhibitors awaits confirmation in larger ongoing cardiovascular outcome studies (215).

A. Potential pitfalls of incretin-based therapy

1. GLP-1 and pancreatitis

Case reports have linked GLP-1R agonists to the development of pancreatitis in human subjects; however, several healthcare database analyses of T2DM patients treated with multiple antidiabetic agents have failed to confirm an increased rate of pancreatitis in subjects exposed to incretin-based therapy (216).

2. GLP-1 and cancer

Sustained administration of GLP-1R agonists stimulates calcitonin secretion and promotes the development of C-cell hyperplasia and medullary thyroid cancer in rats and to a lesser extent in mice (217). In contrast, human C cells express a much lower level of GLP-1R protein, and follow-up of thousands of subjects treated with liraglutide for diabetes or obesity for several years has not detected an increase in plasma calcitonin in exposed subjects (218). Nevertheless, because GLP-1R agonists and DPP-4 inhibitors are still relatively new agents, and the rates of many cancers are increased in subjects with T2DM, long-term follow-up is required to ascertain whether these agents modify the risk of developing specific malignancies.

3. GLP-1 and hypoglycemia

GLP-1 lowers glucose and increases plasma insulin levels in most subjects with T2DM, with low rates of treatment-associated hypoglycemia reflecting the glucose-dependent mechanism of GLP-1R signaling in the β-cell. Because intensive glucose lowering reduces the number of infarction-related events, yet increases death due to cardiovascular causes (219), it will be important for future studies to carefully consider and better understand the potential risk to benefit ratio of different antidiabetic agents that lower glucose through different mechanisms and with varying rates of treatment-associated hypoglycemia.

4. GLP-1 and HR

The available data suggest that 26 wk to 1 yr of therapy with GLP-1R agonists is frequently associated with a mean increase in HR of 2–4 beats/min, while simultaneously reducing body weight and BP. Because increased HR above 85 beats/min has been associated with increased rates of death and increased cardiovascular event rates, more information is required on the mechanisms through which GLP-1R agonists affect HR and whether the increase in HR persists with longer durations of therapy.

5. Obesity paradox and heart failure

Although obesity is a significant risk factor for the development of cardiovascular diseases such as heart failure

(106), retrospective studies suggest that obese patients with clinically significant weight loss experience a greater risk of mortality (220, 221). Because GLP-1R agonists frequently decrease body weight in humans, obese patients with heart failure may represent a subset of individuals where therapy with GLP-1R agonists requires more careful scrutiny. On the other hand, a recent retrospective trial observed that the obesity paradox in heart failure did not apply in patients with diabetes (222). Furthermore, because DPP-4 inhibitors do not induce clinically significant weight loss (223), they may be associated with less risk in obese patients with heart failure. More rigorous work in this area is necessary to better understand the obesity paradox in heart failure, because the majority of findings to date are derived from retrospective observational studies.

B. Novel avenues of research

Although ongoing research in human studies will be focused on determining the safety and long-term consequences of GLP-1R agonists and DPP-4 inhibitors on cardiovascular safety, the mechanisms and cell types mediating the potential cardioprotective effects of GLP-1R activation remain elusive. Hence, more precise elucidation of the consequences of GLP-1R activation in the cardiac myocyte or vasculature is imperative. It will also be important for future studies to measure the levels of potential cardioactive DPP-4 substrates in human subjects and tissues, because changes in these peptides may play an important role in determining cardiovascular outcomes in subjects treated with DPP-4 inhibitors. Finally, although the majority of insights into the cardiovascular biology of incretin hormones to date have been derived from studies in diabetic subjects, more research is required to determine whether GLP-1R activation or DPP-4 inhibition represents a useful therapeutic strategy in selected nondiabetic subjects with different types of cardiovascular disease.

C. Summary

Preclinical studies illustrate multiple cardioprotective actions of GLP-1R agonists and DPP-4 inhibitors, and short-term studies of GLP-1R agonists or DPP-4 inhibitors in human subjects with heart disease have not revealed evidence for adverse effects on cardiac function. Nevertheless, the safety of incretin-based therapies in older subjects with a long duration of T2DM and established cardiovascular disease remains unknown. Although many of the actions of GLP-1R agonists and DPP-4 inhibitors on cardiovascular risk factors, blood vessels, and cardiomyocytes might be predicted to reduce cardiovascular risk, the results of multiple ongoing cardiovascular outcome studies will ultimately determine the place of incretin-based therapies in the treatment paradigm of T2DM.

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